**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BONE MARROW INVASION OF CRYPTOCOCCUS IN HIV INFECTED PATIENTS WITH ASYMPTOMATIC MENINGITIS: A CASE REPORT

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Received 19th July 2020; Revised 18th Aug. 2020; Accepted 25th Sept. 2020; Available online 1st June 2021

<https://doi.org/10.31032/IJBPAS/2021/10.6.5506>

ABSTRACT

Background:

Cryptococcal infection is the most common opportunistic fungal infection was seen in HIV patients. It is the second leading cause of mortality in AIDS (Acquired Immuno deficiency Syndrome) patients worldwide. The most common cryptococcal infection in patients with CD4+ T Lymphocytes count below 100 cells/ μ l. This case report presents the disseminated cryptococcosis with bone marrow involvement and asymptomatic meningitis, highlighting the role of bone marrow aspirate and biopsy study in HIV infected patients

Case Presentation:

A 42 year old HIV infected male patient with a CD4+ T Lymphocyte count of 25 cells/ μ l who was admitted to the emergency department of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow-226010, India, with fever and asthenia. During this period, the diagnostic evaluation of the bone marrow aspirate culture seen in encapsulated yeast cells. In the bone

marrow aspirate stained by Mucicarmin stain showed multinucleated giant cells rounded with yeast like organisms.

The patient underwent a CSF examination to investigate meningitis, patient had no signs of meningeal irritation and all neurological examinations were normal. The CSF culture is seen in *Cryptococcus sp.* The patient was treated with Amphotericine B plus Fluconazole therapy according to guidelines. However the patient developed acute kidney injury stage 3 and the treatment was switched for fluconazole monotherapy. The symptoms disappeared and CSF culture for fungi was negative for 2 weeks of treatment.

Conclusion:

Bone marrow infection caused by *Cryptococcus neoformans* is a rare presentation of cryptococcosis. The clinical symptom to diagnose the HIV infected patients with fever and cytopenias (when CD4+ T Lymphocytes count below 100 cells/ μ l).

Keywords: *Cryptococcus neoformans*, opportunistic infection, Cryptococcal meningitis, AIDS (Acquired immune deficiency syndrome), ART (Antiretroviral treatment), Bone marrow

INTRODUCTION

Cryptococcal meningitis, caused by the *Cryptococcus neoformans*, a ubiquitous environmental fungus, is a fatal HIV-related infection [1].

It is one of the leading causes of opportunistic infection and mortality amongst persons with advanced HIV in the developing world [2-4].

India and Southeast Asia have a substantial burden of cryptococcal meningitis cases (estimated incidence of 120,000 cases or 3% per year) with high mortality of 20%–50% [4, 5].

Amongst hospitalised HIV-infected patients in India with suspected meningitis and CD4

counts <100 cells/ μ l [3], up to 50% have cryptococcal meningitis [4, 5].

Most cryptococcal meningitis occurs in antiretroviral treatment (ART)-naïve people [6]. However, the unmasking of cryptococcal meningitis within the 1st week of ART initiation (also known as ART-associated cryptococcal meningitis) is also common.

In turn, HIV- infected patients with severe immunodeficiency commonly present fever accompanied with cytopenias. The hematological abnormalities can be due to HIV infection itself or non-infectious and infectious causes. The most frequent opportunistic infections that cause pancytopenia are histoplasmosis and

mycobacteriosis [7]. In severely immunosuppressed patients, *Cryptococcus neoformans* can infect virtually any human organ system, including the bone marrow, which is a rare presentation of cryptococcosis.

Here, we report a case of cryptococcal meningitis in an invasive disease of bone marrow.

CASE HISTORY

A 42 yr old HIV –infected man farmer was admitted to emergency Dept. of RMLIMS, Lucknow, referring progressive asthenia for 2 weeks with difficulty on performing basic daily routine activities, fever, headache, anorexia and disturbance of consciousness from a day before admission. During this time, he reported a weight loss of 4 kg. In the last 24 hrs, he had vomiting with nausea in small volumes. The patient denied other symptoms like cough, abdominal pain and diarrhea. The HIV infection was diagnosed 10 yrs ago and his current antiretroviral therapy showed CD4+ T lymphocytes counts were 25 (2%) and CD8+ T lymphocytes counts were 510 (37.3%) cells/ μ l respectively and the viral load was 251,189 (5.4 Log₁₀). He was also using a trimethoprim- sulfamethoxazole double strength tablet for *Pneumocystis jirovecii* prophylaxis.

The patient had pulmonary tuberculosis 10 years ago; his AIDS (Acquired Immunodeficiency Syndrome) defining illness. In addition he had several previous hospitalizations for abdominal pain with chronic diarrhea. He was born in Bihar City, rural area of Sahibganj, India, an endemic region for Kala-azar (Visceral leishmaniasis). On admission, his body temperature was 38.8°C; his other vital signs were normal. His physical examination; body weight of 38 kg and body mass index of 13.9kg/m². There were no signs of meningeal irritation. Neurological examination were normal (Papilledema, optic atrophy, Peripheral seventh CN palsy, Palsy of CNs III, IV, VI, Myelopathy, Ataxia, Cranial nerve examination. Laboratory data showed 9 gm/dl; white blood cells counts of (7000/ μ l) and platelet count (122x10⁹/L) but red blood cells count was decreased (220/ μ l), serum C-reactive protein levels (0.30mg/dl) and procalcitonin levels (0.11ng/mL). The serum creatinine was 0.059 mmol/L and Serum urea was 3.19mmol/L. Normal levels of β -D-glucan were observed (5.0pg/mL). The alanine and aspartate aminotransferases levels were 258 IU/L and 50 IU/L. Chest X-ray was normal. An abdominal ultrasonography showed cholelithiasis. Lumbar puncture yielded an opening CSF

pressure of 110 mm H₂O and pleocytosis (85 mononuclear cells and 219 polymorphonuclear cells/ μ l). The CSF glucose level was 59 mg/dl depleted and protein level (198mg/dl) was elevated.

Blood culture was negative for bacteria and fungi. The serological tests were negative for Hepatitis B & C, Kala-azar. Tuberculin (PPD, Montoux test) was non-reactive. VDRL testing was negative too. Due to megaloblastic anemia pancytopenia, the patient underwent for a bone marrow aspiration and bone marrow biopsy. The direct examination (KOH 10% preparation) presented a non-specific hypocellularity and was negative for fungi, mycobacteria and hemoparasites. After 5 days bone marrow aspirate culture on India ink preparation seen in encapsulated budding yeast cells like structure *Cryptococcus sp.*(*Crptococcus neoformans*). Then, the patient sustained to investigate meningitis goes through Lumbar puncture. The Cerebrospinal Fluid (CSF) had no turbidity, a clear fluid like appearance, an opening pressure of 150 mm H₂O, a cell count of 3 leukocytes, protein of 125mg/dl and glucose of 45 mg/dl.

The bone marrow biopsy discussed 3 cellular lineages:

- 1) Hypocellular bone marrow for age
- 2) Megakaryocyte increase in numbers
- 3) Megakaryocytes with mild dysplasia (hypolobated nuclei)

In the mucicarmine staining loosely formed granuloma composed of multinucleated giant cells with rounded yeast like organism with *Cryptococcus sp.* were observed (**Figure 1**).

The patient was treated with amphotericine B deoxycholate at 1 mg/kg and combination of 800 mg fluconazole daily given intravenously. However, Cryptococcal antigen was detected in CSF (titres, 1:16) on one week treatment. But patient developed acute kidney injury stage 3 [8].

Serum creatinine and urea levels were 0.195mmol/L and 12mmol/L, respectively. Doctor decides the treatment of Fluconazole at 1200 mg daily switched of the 14 day induction phase. The patient was managing for acute kidney injury, serum creatinine and urea levels were within normal range after six weeks. CSF cultures for fungi were negative for two weeks of treatment. The patient was on continuous therapy at the time of this report.

Figure 1: Mucicarmine stain in bone marrow seen in *Cryptococcus neoformans*

DISCUSSION

The most frequent clinical manifestation of *Cryptococcus* infection in HIV-infected patients is subacute meningitis. **Vechi *et al.*** [9], described a lumbar puncture defend the diagnosis of *Cryptococcus neoformans* in bone marrow aspirate culture, although the patient did not indicated clinical evidences of CNS involvement. The finding of encapsulated yeasts in CSF was accompanying by subclinical or latent form of Cryptococcal meningitis [9]. Another study reported two cases of asymptomatic Cryptococcal meningitis. One case was diabetic and hospitalized in cryptococcal pneumonia. Neurological examination and blood culture of fungi was negative, meningeal irritation was absent. His Lumbar puncture examination of CSF, found in numerous encapsulated yeast as

Cryptococcus neoformans [10]. **Smilack *et al.*** 1975, [11] studied at 21 year old man with Hodgkin's lymphoma with pleural effusion of cryptococcal etiology. He had not shown in neurological involvement, but *Cryptococcus neoformans* yeast cells seen by CSF fungal culture [11].

Butler *et al.* found Cryptococcal Antigen (CrAg) testing was valuable tool in the early diagnosis of cryptococcal infection at risk patients [12]. Since 2011, WHO has recommended routine CrAg testing in antiretroviral- naïve HIV infected adult patients with CD4+T lymphocyte count below 100 cells/ μ l, by either lateral flow assay, followed by a targeted pre-emptive fluconazole therapy among CrAg- positive patients [13]. In the study by **Chammard *et al.***, 2018, [14], proposed if india ink is positive (sensitivity =86%), instead of

waiting for culture reports, we can use CrAg testing is rapid and timely diagnosis of Cryptococcal meningitis [14]. In our study, the finding of *C. neoformans* in bone marrow aspirate to investigate the CNS before the symptom appears. The patient's opening pressure of CSF is 150mmH₂O. The pathophysiology related to the signs and symptoms of CM is not detected. According to WHO, routine examination of cryptococcal Antigen Latex agglutination or Lateral Flow Assay resulted in anti retroviral naïve HIV infected adult patients with CD4+ T lymphocytes count below 100 cells/ μ l; followed by a targeted Fluconazole therapy for all Cryptococcal Antigen positive patients [13].

We also did in Cryptococcal Antigen Lateral Flow Assay testing in blood and urine for early diagnosis of cryptococcal infection, it would be advantageous for early and timely diagnosis of meningeal involvement [15]. Our study observed headache, confusion, decreased alertness and nausea clinical sign and symptoms might be increased in intracranial pressure (ICP= 150mm H₂O). We studied Bone marrow aspirate investigate in the *Cryptococcus neoformans* in CNS. Patient's neuroimaging seen in atrophy of the brain parenchyma. In the study by Graybill et al. 2000, [16] Intracranial pressure (ICP)

were high, headache was the most common symptom in Cryptococcal meningitis. Two main reasons to increase the intracranial pressure of meningitis [16].

1. CSF reabsorption at the arachnoid granulations.
2. Direct cryptococcal infiltration.

In another study, in HIV infected patients extra neural sites are found in disseminated cryptococcosis (condition like Diabetes mellitus, Systemic Lupus Erythematosus etc.) [17-20] and bone marrow involvement [18, 20, 21]. Bone marrow infection in *Cryptococcus neoformans* is asymptomatic in HIV –infected patients. It can be subtle of bone marrow and nonspecific symptoms like fever, anorexia, weight loss and pancytopenia [22]. In our study direct examination of bone marrow aspirate, budding encapsulated yeast cells like structure seen. In biopsy study seen in granulomas with spherical encapsulated yeast seen. In other study Vechi et al. 2019, [9] observed most important opportunistic cause of pancytopenia in AIDS patients with visceral leishmaniasis (Kala azar) [9]. In Cryptococcosis, the treatment consist of an induction phase with an Amphotericine B (1mg/kg) combination of Fluconazole (800mg) daily. Our patient developed acute renal failure after a week of treatment. Doctor decides the treatment of Fluconazole

1200mg daily, according to guidelines [23]. The patient had a good response to the treatment. CSF cultures of fungi at two weeks were negative. The symptoms disappeared completely with recovery of leukocytes and platelets count.

CONCLUSION

Bone marrow infection caused by *Cryptococcus neoformans* is uncommon. The etiology included with HIV infected patient with fever and cytopenias, (when CD4 count below 100 cells/ μ l). This case present the disseminated cryptococcosis with bone marrow involvement and asymptomatic meningitis, highlighting the role of bone marrow aspirate and biopsy study in HIV infected patients for rapid and early diagnosis and appropriate treatment of opportunistic infections.

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