

Research Article**Cryptococcal Meningitis Encountered in Patients with HIV in A Tertiary Care Hospital, Mangalore- A Series of Three Cases****Dr. Jutang Babat Ain Tiewsoh¹, Dr. Beena Antony², Dr. Rekha B³**¹Post Graduate Resident, Department of Microbiology, Father Muller Medical College, Mangalore.²Professor, Department of Microbiology, Father Muller Medical College, Mangalore.³Professor, Department of Microbiology, Father Muller Medical College, Mangalore.***Corresponding author**

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Abstract: Cryptococcus neoformans is an encapsulated yeast which can infect the central nervous system due to its ability to synthesise melanin from catecholamines. Cryptococcal meningitis is a common opportunistic infection in HIV infected individuals and an AIDS defining illness. Mortality rate is high, if treatment is delayed. This prospective observational study in a tertiary care hospital, during August 2013 to June 2015 includes patient's demographic data, clinical symptoms, physical findings, laboratory parameters; Cerebrospinal fluid examination findings, other opportunistic infection, co-infections, treatment, hospital stay and outcome were recorded. Three cases presented with Cryptococcal meningitis. They were all males between 35-50 years of age, married and known cases of HIV with two cases on ART. Headache and vomiting were the common presenting symptoms in Cryptococcus meningitis was diagnosed by india ink wet mount, Cryptococcal antigen test and by culture. Two of the cases had other opportunistic infection with low CD4 count. Two patients improved on treatment with Amphotericin B while the other left against medical advice. HIV infected individuals presenting with meningitis symptoms should be suspected for Cryptococcal meningitis. In addition to india ink preparation, Cryptococcal Antigen test should also be included as part of the diagnostic schedule. Low CD4 count was observed in Cryptococcal meningitis but early diagnosis and treatment shows good outcome.

Keywords: AIDS Acquired Immunodeficiency Syndrome, ART Antiretroviral therapy, CD4 CD4 T lymphocyte cell, CDC The Centers for Disease Control and Prevention, CM Cryptococcal meningitis, HIV Human Immunodeficiency Virus, WHO World Health Organization

INTRODUCTION

Cryptococcus neoformans is encapsulated yeast which is found in the environment and most commonly in pigeon droppings. It infects individuals who are immuno-compromised such as HIV, cancer patients, diabetics, patients on corticosteroid therapy, transplant patients, renal failure, lung diseases, lymphoma and immunologic diseases [1, 2]. It enters the human host mainly by inhalation and remains dormant in the lungs until the immune system of the individual decreases resulting in its reactivation and dissemination to the central nervous system and other sites. It has been proposed that its ability to invade the CNS is due to its ability to synthesize melanin from catecholamines [3].

Cryptococcus meningitis in HIV/AIDS occurs worldwide with South East Asia being next only to the Sub-Saharan region, where Cryptococcus meningitis is

the most common cause of meningitis and also the leading cause of death in HIV/AIDS patients, killing more people than tuberculosis in the area [4]. According to official estimate in 2011, the adult prevalence of HIV in India was 0.27%, with nearly 21 lakh people living with HIV [5].

It usually infects HIV infected individuals who have a CD4 count of < 100 cells/μl and is also an AIDS defining illness according to the CDC guidelines and WHO clinical staging of HIV/AIDS [6, 7, 8]. The presentation of this infection maybe acute, sub-acute or chronic meningitis or meningo-encephalitis and without treatment it is known to be fatal. Hence, early diagnosis and treatment are required to prevent fatality of cases.

The treatment of Cryptococcus meningitis is by Amphotericin B with flucytosine or fluconazole for induction therapy and by fluconazole alone as the

consolidation and maintenance therapy [9]. With the availability of anti-retroviral therapy, opportunistic infections like Cryptococcus and others have decreased in recent years but in the developing countries the mortality is between 13-40% as compared to the developed countries where it is 10-30% [10].

Studies related to Cryptococcal meningitis is lacking from in and around Mangalore. Hence, the present study is being carried out to investigate the clinico-microbiological analysis of cryptococcal meningitis in patients with HIV in a tertiary care hospital in Mangalore.

MATERIALS AND METHODS

This prospective observational study was carried out at Father Muller Medical College, Mangalore, a tertiary care hospital in South India. The patients attending our hospital from August 2013 to June 2015, who were newly diagnosed and known cases of HIV infection admitted in our hospital, were included in the study.

The patient's demographic data which includes age, sex, marital status and address were recorded. Prior infection with HIV and history of any previous admission with any AIDS defining illness and whether on ART treatment was also recorded. The presenting clinical and physical symptoms on admission, cerebrospinal fluid examination findings, pathological laboratory parameters which includes haemoglobin, leucocyte differential count, leucocyte count total, platelet count and biochemical blood parameters like serum urea, creatinine, uric acid, serum electrolytes, liver function test, other opportunistic and co-infections like Tuberculosis, candidiasis, etc. were looked for according to presenting symptoms. Treatment that was given, the total number of days of hospital stay and the outcome were recorded.

A case of Cryptococcal meningitis was defined by the analysis of the cerebrospinal fluid which usually shows mildly raised protein, normal or slightly low glucose with an increased white cell count (5-100 cells predominantly mononuclear lymphocytes)

Confirmation of Cryptococcus infection is identified in the CSF or Central nervous system tissue by positive culture or histopathology [11].

Probable diagnosis is said to be in the presence of [11]:

- Compatible clinical syndrome that includes fever and one or more of the following signs of meningitis: headache, altered mental status, neck stiffness, photophobia, seizures or focal deficits.

- Positive serum cryptococcal antigen

Specific antifungal therapy initiated and recommended

- Possible diagnosis is said to be when [11]:
- Compatible clinical syndrome that includes fever and one or more of the following signs of meningitis: headache, altered mental status, neck stiffness, photophobia, seizures or focal deficits.
- Specific antifungal therapy initiated and recommended

Acute or subacute infection is a term used for patients presenting with symptoms of duration less than a month and diagnosed as cryptococcal meningitis.

RESULTS

During the study period from August 2013 to June 2015, a total number of 232 patients who were newly diagnosed and known cases of HIV were admitted in our hospital setup. Three cases of Cryptococcus meningitis were diagnosed. All the 3 cases were males who were married, between the age group of 35 to 50 years and known cases of HIV infection with two of them on ART treatment. Two of the cases were previously admitted 7 and 9 months back with diagnosis of HIV with tuberculosis and oral candidiasis with one of the cases co-infected with malaria also.

In the 3 cases diagnosed with cryptococcal meningitis, the common presenting symptom in all the cases was headache and vomiting, headache duration on admission ranged between 5 days to a month and vomiting between 3 days to a month. Fever, neck rigidity/stiffness and raised intracranial tension were present in two of the cases. The other presenting symptoms include nausea, seizure, night sweats, loose stools, bi-lateral papilledema, rash, right lateral rectus palsy, pleural effusion and pallor.

The CSF analysis of the three cases showed presence of cells with the predominating cells being lymphocytes, also the CSF protein, CSF LDH and CSF Adenosine deaminase were elevated whereas CSF glucose and chloride were reduced. CSF amylase which was also done showed normal values. Gram stain showed predominance of lymphocytes with two cases showing gram positive budding yeast cells with capsule resembling Cryptococcus. In one of the cases where india ink was reported negative, Cryptococcal antigen test was sent which came positive for a dilution of 1:6 but Cryptococcus was cultured in all the three cases. CSF AFB staining which was sent was negative in all the three cases.

Table 1: Clinical presentation of the three cases

	Case 1	Case 2	Case 3
Age(years)	48	45	36
On ART treatment	Yes	Yes	No
Headache	+ (5days)	+ (1 month)	+ (1 month)
Vomiting	+(3days)	+ (1 month)	+ (1 month)
Fever	-	+ (1 month)	+ (1 month)
Neck rigidity/stiffness	-	+	+
Raised intracranial tension	-	+	+
Nausea	+(3days)	-	-
Seizure	+	-	-
Loose stools	-	-	+
Night sweats	-	-	+
Bi lateral papilledema	-	+	-
Rash	+	-	-
Right lateral rectus palsy	-	-	+
Pleural effusion	-	+	-

Table 2: CSF analysis of the three cases

	Normal values	Case 1	Case 2	Case 3
Total cell count (cells/cumm)		120	234	30
Differential count		80%lymphocytes; 20 % neutrophils	95% lymphocytes; 5% neutrophils	100% lymphocytes
CSF Protein	15-45mg/dl	196.8	82.3	48.9
CSF Glucose	40-70mg/dl	20	34	37
CSF Chloride	120-130 mEq/L	105	121.3	119.5
CSF Amylase	< 45 IU/L	1.1	2.1	1.6
CSF LDH	0 IU/L	40	39	26
CSF Adenosine deaminase	< 10 IU/L	14	10	15
India ink		Negative	+	+
Cryptococcus antigen		+ (dilution 1:6)	-	-
Culture		+	+	+



Fig.1: Colonies of Cryptococcus on Sabouraud Dextrose Agar, yeast-like highly mucoid, cream to buffed coloured colonies seen.

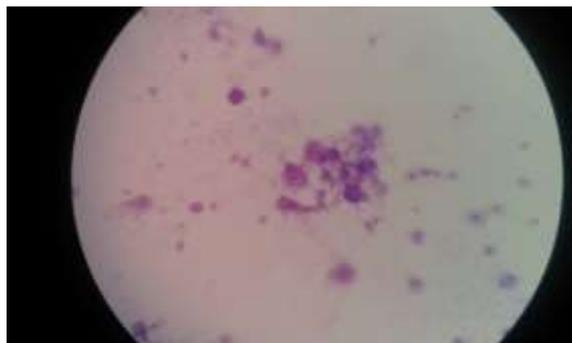


Fig. 2: Gram stain showing few lymphocytes, No pus cells, numerous large round Gram positive budding yeast with capsule resembling Cryptococcus

With respect to the blood parameters it was seen that all had anaemia with two cases having mild anaemia and the other of moderate type. The total leucocyte count was decreased in one whereas normal in the other two. ESR was increased in two cases and normal in one. Platelet count was normal in all though on the lower side of normalcy. CD4 count was found to be less than 100 in two cases whereas in one case a CD4 count of 377 was recorded 4 months before the present admission.

It was also found that Serum Sodium and Serum Chloride were decreased and serum Urea was

within normal limits in all the cases. In one there was a decreased in Serum uric acid and the other a decreased in Serum Creatinine. Serum potassium level is increased in one, decreased in one and normal in one. The liver function test of the cases showed normal values of S. total protein, S. albumin, Un-conjugated bilirubin and SGPT but there was an increased in S. globulin and S. Alkaline phosphatase. There was also an increased in SGOT in two cases; while an increased in S. total bilirubin and conjugated bilirubin in one. There was a slight decreased in S. Albumin/Globulin ratio in one.

Table 3: Blood parameters, renal function test, serum electrolytes and liver function test.

	Normal	Case 1	Case 2	Case3
Haemoglobin	14-18	13.4	8.7	11.1
TLC	4000-11000	5900	2400	4600
ESR	<10 mm/ !sthr	5	84	40
Platelet counts	150000-500000	195000	160000	220000
CD4		55	29	377(4 months prior)
S. Urea	10-50	10	21	35
S. Uric acid	3.4-7.0	1.94		7.86
S. Creatinine	0.7-1.3	0.71	0.60	1.22
S. Sodium	136-145	134	132	130
S. Potassium	3.5-5.1	4.45	3.14	5.33
S. Chloride	98 – 107	93.9	94.5	91.1
S. Total protein	6.4-8.3	7.75	7.91	8.12
S. Albumin	3.5-5.2	4.19	3.49	4.02
S. Globulin	2.3-3.5	3.6	4.4	4.1
S. A/G ratio	1- 1.5	1.2	0.8	1.0
S. Total bilirubin	.1-1.2	1.86	0.27	0.33
Conjugated bilirubin	Upto 0.2	1.49	0.11	0.15
Unconjugated bilirubin	0.2-0.8	0.37	0.2	0.18
SGOT	<35 IU/L	79	45	13
SGPT	< 45 IU/L	23	24	14
S. Alkaline Phosphatase	40-129 IU/L	195	297	127

In two cases, other opportunistic infections were also diagnosed with tuberculosis on treatment in

one case and history of TB who had completed DOTS treatment 2 months back in the other, oral candidiasis,

CMV retinitis, Viridans Streptococci in blood and miliaracrysalina as a skin infection also occurred. CT scan of the brain which was performed in two of the cases was found to be normal in one whereas in the other showed mild diffuse leptomenigeal enhancement suggestive of meningitis and features of raised intracranial tension. The abdomino-pelvic sonography which was asked for in two of the patients was reported

as hemangioma in one and abdominal lymphadenopathy in the other. The hospital stay of the patients was from 2 to 26 days with two patients cured on discharged while one left against medical advice. In both the cured cases, Amphotericin B was given for a period of 14 days followed by tablet fluconazole which was asked to be continued for another 8 weeks.

Table 4: The different co-infections, radiological investigations, treatment, with hospital stay and outcome

	Case 1	Case 2	Case 3
Co-infections	Tuberculosis DOTS treatment completed	Tuberculosis on DOTS treatment	-
	CMV retinitis	Oral candidiasis	
Blood culture	Viridians Streptococci	-	-
Skin infection	Miliaracrysalina	-	-
CT Scan brain	-	Normal	Impression of Meningitis, raised ICT
USG abdomen	-	Hemangioma	Abdominal lymphadenopathy
Treatment	Amphotericin B Fluconazole Linezolid	Amphotericin B Candid mouth paint	Fluconazole
Hospital stay	26 days	19 days	2 days
Outcome	Improved	Improved	Left Against Medical Advice

DISCUSSION

In our study, the three cases of Cryptococcus meningitis were in the age group of 35- 50 years and all were males. This is similar to the NACO publication regarding the National level statistics in the year 2012 [5]. The presentation was acute in one case and chronic in the other two, where the presentation was more than 4 weeks. Immune reconstitution inflammatory syndrome (IRIS) is a spectrum of clinical signs and symptoms resulting in inflammatory response associated with the immune system. Clinical events occurring within the first 6 months of ART therapy is suggestive of IRIS [12, 13, 14]. In this study, all the three cases were known cases of HIV infection with two of them on ART, but both were treated for more than 6 months with ART with one being treated for 6 years and the other for 9 months, hence no case of IRIS was encountered in our study.

The common presenting symptoms on admission were headache, vomiting and fever which are similar to earlier reports [10, 15, 22]. Neck rigidity/stiffness and raised intracranial tension was, observed in two cases where others reported about 30% [23]. Seizure was seen in one case where other studies observed 25% in HIV infected individuals [23]. Cranial involvement was prominent in one case where there was right lateral rectus palsy indicating that the 6th cranial nerve (abducens) was affected which may have resulted from the increased intracranial pressure. Bilateral papilledema was also noted in one case which

is a common finding of Cryptococcal meningitis in the immune-competent individuals [24].

The cerebrospinal fluid (CSF) analysis usually reveals lymphocytic pleocytosis with raised protein and low sugar levels, where it was also noted in our study. Microbiological diagnosis of cryptococcal meningitis is usually done by India Ink which had shown positivity in AIDS patients in a range of 70-90%, in our study india ink was reported negative in one in which due to high suspicion, a cryptococcal antigen test was performed which came positive [10, 25]. Studies have shown that cryptococcal antigen test have a high sensitivity and specificity, with false positive results of < 0.4% [10, 25]. Gram stain which was also performed showed predominance of lymphocytes with presence of Cryptococcus. Culture was positive in all the 3 cases which is the confirmation test of cryptococcal infection. The liver function test showed increased in serum globulin which is common in these cases. The mild hyponatremia present in the 3 patients is regarded as a bad prognostic marker in many studies [26, 27].

The CD4+ T cell count are the best indicator of the stage, progression of the disease and is the main criteria for staging the disease according to the CDC guidelines and for initiation of ART treatment [6, 7, 8]. In this study, the CD4 count was less than 100 in the two cases where CD4 count was available during presentation.

Cryptococcal meningitis was an AIDS defining illness in one case and other opportunistic infections like tuberculosis, oral candidiasis and CMV retinitis also occurred in these cases, which is common as the immune system is decreased. The two cases were treated with amphotericin B and fluconazole for a period of 14 days as induction therapy and followed with fluconazole as the consolidation and maintenance therapy in accordance with the WHO document. Where the outcome was excellent as in majority of the studies [9].

CONCLUSION

In this study, cryptococcal meningitis presented as both acute and chronic infection. It also showed that there was failure or non-compliance of ART regime which resulted in the occurrence of cryptococcal meningitis along with other opportunistic infections. The microbiological diagnosis should not depend on India ink alone but cryptococcal antigen test, culture along with CSF analysis should always be performed in suspected cases to rule out infection. With early diagnosis and initiation of treatment, the outcome has been observed to be promising.

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