A Study of Clearance of Infection in HIV-Associated Cryptococcal Meningitis after Amphotericin-Based Induction and Fluconazole-Based Consolidation Therapy

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Int J Recent Surg Med Sci 2021;7:23–33.

Abstract

Introduction India has third largest human immunodeficiency virus (HIV) population in the world. Average HIV prevalence was 0.22% (range, 0.16–0.30%) in 2017, and Manipur is one of the five states with the highest prevalence of HIV. Cryptococcal meningitis being one of the acquired immunodeficiency syndrome (AIDS)-defining illnesses is the second most common cause of opportunistic neuro-infection and usually occurs in advanced HIV disease when the cluster of differentiation 4 glycoprotein (CD4+) count is usually less than 100 cells/μL. Treatment includes amphotericin-B induction therapy for 2 weeks followed by fluconazole consolidation therapy for 8 weeks as per National AIDS Control Organisation guidelines. There is not much data on how much infection is cleared off after induction and consolidation treatment. So, this study was conducted to know the clearance of *Cryptococci* in cerebrospinal fluid (CSF) after induction and consolidation treatment in people living with HIV (PLHIV)-associated cryptococcal meningitis.

Objective This work aimed to study the persistence of cryptococcal meningitis after amphotericin-based 2 weeks of induction therapy and 8 weeks of consolidation therapy with fluconazole and to evaluate the association between CD4 count and clearance rate of cryptococcal infection.

Materials and Methods The study was conducted in Department of Medicine, Regional Institute of Medical Sciences, Imphal, from 2016 to 2018. Fifty-one patients above 18 years of age diagnosed as cryptococcal meningitis with HIV were included and treated with amphotericin for 2 weeks and fluconazole for 10 weeks. CSF analysis was done at 2nd and 10th weeks to study the clearance of infection.

Results At 2nd week of induction therapy, out of 51 patients, 28 (54.9%) got cleared of infection, 18 (35.3%) had persistent infection, and 5 (9.2%) patients had either died or discontinued treatment. At 10th week of consolidation therapy, 36 (70.5%) patients

Keywords
- CSF analysis
- fluconazole consolidation
- amphotericin induction
- cryptococcal meningitis
- HIV

published online
Feburary 10, 2021

DOI https://doi.org/10.1055/s-0041-1723920
ISSN 2455-7420.

Thieme Medical and Scientific Publishers Pvt. Ltd. A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

got cleared of cryptococcal infection, 2 (4%) patients were having persistent infection, and 5 (9.8%) patients died, while 8 (15.7%) patients were lost in follow-up. However, after excluding mortality and lost in follow-up cases, from analysis in final outcome, 94% (34 out of 36) patients showed response to this regimen.

Conclusion The present study showed that cryptococcal meningitis in PLHIV responded to amphotericin-based induction therapy with 60% clearance of infection followed by fluconazole-based consolidation therapy with 94% of clearance of infection. However, there is still need for good antifungal regimen that could clear infection in induction phase with less side effects.

Introduction

India has third largest human immunodeficiency virus (HIV) population in the world with average prevalence of 0.22% (range, 0.16–0.30%) in 2017.[1] Cryptococcal meningitis is the second most common cause of opportunistic neuro-infection occurring when the cluster of differentiation 4 glycoprotein (CD4+) count is usually less than 100 cells/µL.[2,3] Treatment includes induction therapy with amphotericin-B (0.7–1 mg/kg/day) for 2 weeks and consolidation therapy with fluconazole (400 mg once daily [OD]) for 8 weeks as per National AIDS Control Organisation (NACO) guidelines.[4] Estimated death from acquired immunodeficiency syndrome (AIDS)-related illness in India is 130,000.[5] The five states with the highest prevalence (Manipur, Mizoram, Nagaland, Andhra Pradesh, and Karnataka) are in the east or south of the country.[6]

Cryptococcal meningitis has emerged as a leading cause of morbidity and mortality in patients with AIDS. Among the HIV seropositive subjects, cryptococcal meningitis is the second most common cause of opportunistic neuro-infection and usually occurs in advanced HIV disease. In India, the incidence of cryptococcal meningitis is 3% per year (120,000 cases) with mortality rate of 20 to 50%.[7,8]

Cryptococcal meningitis is the second most common form of fungal meningitis and is caused by *Cryptococcus neoformans*.[9] In those with HIV infection, cryptococcal infection occurs in the advanced stages of the disease when the CD4+ count is usually less than 100 cells/µL.[3]

C. neoformans is an encapsulated hetero-basidiomycetous fungus. Traditionally, *C. neoformans* is classified into two varieties and five serotypes (A, B, C, D, AD) based on capsule structure.[10] Serotypes A and D and AD hybrids are globally responsible for 98% of all cryptococcal infections in patients with AIDS. Serotypes B and C predominantly affect immune-competent individuals but have also been recently reported in patients with AIDS.[11]

The fungus enters the human body through inhalation into the lungs. Though this pulmonary infection is usually asymptomatic, the organism may disseminate to other organs depending on the immune status of the individual. The cerebrospinal fluid (CSF) is an ideal site for infection as it lacks complements and immunoglobins.[12]

Cryptococcal meningitis commonly presents as chronic or subacute meningitis, but rarely has a rapid course. For the diagnosis of cryptococcal meningitis, lumbar puncture (LP) with manometry is the diagnostic procedure of choice. CSF pressure may be elevated in some patients.[13]

CSF analysis usually reveals lymphocytic pleocytosis with raised protein and low sugar levels. India ink stain shows fungus in >50% of the cases of cryptococcal meningitis in HIV-negative cases and in >90% of patients with AIDS.[14] A positive fungal culture is the gold standard of diagnosis of cryptococcal infection and CSF samples show fungal growth in all the cases.[15]

The CSF and serum sample should be evaluated for cryptococcal antigen assay that is positive in almost all cases except very early in the disease or in those with very high titers due to prozone effect and in certain patients with cryptococcomas.[16] The method used for antigen detection are latex agglutination test and enzyme immunoassay and are >90% sensitive and specific. Cryptococcal antigen titers usually decrease with treatment but it can remain at low titers for long periods even after effective therapy. Neuroimaging is done to rule out any space occupying lesions like cryptococcoma and hydrocephalus.[17]

Treatment

According to NACO guidelines, treatment process of cryptococcal meningitis is as follows.[4]

- Induction: Amphotericin-B (0.7 mg/kg/d) ± 5-flucytosine 25 mg/kg four times a day (q.i.d.) × 14 days.
- Consolidation: Fluconazole is given 400 mg/day for 8 to 10 weeks or until the CSF becomes sterile.
- Maintenance: Maintenance therapy is given with fluconazole at the dose of 200 mg daily lifelong or until the CD4+ count remains above 350 cells/mm^3.
- Then initiation of highly active antiretroviral therapy (HAART) is to be done after consolidation.[18]

To the best of our knowledge there is not much data on how much infection is cleared off after induction and consolidation treatment. So, this study was conducted to know the clearance of *Cryptococci* in CSF after induction and consolidation treatment in patients with HIV-associated cryptococcal meningitis.

Aims and Objectives

This study aimed to:

- Study the persistence of cryptococcal infection in patients with HIV following induction therapy with amphotericin-B IV (0.7–1 mg/kg/day) ± flucytosine 100 mg q.i.d. oral for 2 weeks and consolidation therapy with fluconazole (400 mg OD) for 8 weeks, and maintenance by oral fluconazole 200 mg OD till CD4 reaches ≥350 cells/mm^3.
- Evaluate the efficacy of current recommended amphotericin-B and fluconazole therapy and find the association between CD4 count and clearance rate of cryptococcal infection.

Materials and Methods

An observational cohort study was conducted in Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, for a period of 2 years from September 2016 to August 2018. Fifty-one patients above 18 years of age diagnosed as cryptococcal meningitis with HIV and admitted in medicine ward were included. We excluded patients not willing to participate in the study, pregnancy cases, lactating mothers, cancer patients, cases on immunotherapy, and those diagnosed to be meningitis of other etiologies. Study was conducted after clearance from research ethics board of RIMS. Patients were explained about the procedure and purpose of the study, and written informed consents were taken.

Working Definition

- **HIV infection:** Diagnosis of HIV infection will be done as per the NACO guidelines, by Enzyme Linked Immunosorbent Assay (COMB AIDS) screening, and confirmed by western blot (MeriScreen HIV 1–2 WB) immune concentration flow through, HIV 1–2 Trispot test kit (AIDSCAN) with principle of Immuno-filtration, and nucleic acid test/polymerase chain reaction test.

Cryptococcal meningitis: The Centers for Disease Control and Prevention, USA, defines cryptococcal meningitis as infection of meninges caused by fungus *C. neoformans* and diagnosed by CSF culture for *Cryptococci*, and CSF cryptococcal antigen detection.

Baseline characteristic data were recorded, including age, sex, and duration of HIV from period of diagnosis. All cases were sent for routine investigation as per NACO guidelines, with the emphasis given for CD4 count. Viral load was not included in the study. To confirm the cryptococcal infection, the following was done: routine CSF analysis done at 2 weeks and 8 weeks of treatment by LP (CSF protein, sugar, total cell count [TCC], differential cell count, opportunistic infections), Cryptococcal Antigen Latex Agglutination System (CALAS [CSF and serum]), India ink preparation (IIP) from CSF, and CSF culture for *Cryptococci*. Rest other investigations were done whenever necessary, which included computed tomography (CT) scan/magnetic resonance imaging (MRI) brain, chest X-ray, sputum examination for acid-fast bacillus, yeast cell of *Cryptococcus*, fine needle aspiration cytology of lymph gland, or skin scrapping from any lesion and stained by Giemsa stain for the diagnosis of the fungus.

Study Tools

CSF Culture for *Cryptococci*

CSF culture for cryptococci is done by centrifuging CSF sample at 1,000 × g for 15 minutes, and then CSF is divided into sediment and supernatant. Sediment is directly inoculated into Sabouraud dextrose agar or Bird seed agar and incubated for 3 to 10 days in room temperature. Colonies of milky white color usually grows within 3 to 10 days. CSF culture is 100% reliable.

CSF Cryptococcal Antigen

Supernatant of centrifuged CSF sample is used for this test. Test was done by CALAS developed by Meridian Bioscience, Inc. (USA), which is 100% specific and 97% sensitive. It is a qualitative and semiquantitative test system that detects capsular polysaccharide antigens of *C. neoformans* in serum and CSF and is proven to be superior to India ink mount. CALAS utilizes latex particles coated with anticryptococcal globulin (Detection Latex) and reacts with the cryptococcal polysaccharide antigen causing a visible agglutination. The gradation of the reaction strengths are as follows:

- Negative (–): A homogenous suspension of particles with no visible clumping.
- 1+: Fine granulation against a milky background.
- 2+: Small but definite clumps against a slightly cloudy background.
- 3+: Large and small clumps against a clear background.
- 4+: Large clumps against a very clear background.

IIP for *Cryptococci*

This test is done by putting one drop of CSF sediment and one drop of India ink on a glass slide, covered with coverslip, and observed under microscope. This test may detect the yeast cells from CSF in 40 to 60% of cases.

Outcome Measures

After 2 weeks of induction therapy (amphotericin-B) and 8 weeks of consolidation therapy (fluconazole), we did CSF culture for *Cryptococci*, CALAS, and CSF IIP for *Cryptococci* to evaluate the persistence of infection.

Sample Size

Sample size was determined based on the following formula:

$$n = 4PQ/L^2$$

Where, n = sample size, P = prevalence, Q = 100-P, and L = absolute allowable error (taken as 5% with 95% confidence interval). Considering the prevalence of cryptococcal meningitis in HIV patients to be 2.79% as per study conducted by Baradkar et al,[19] the sample size comes around 44.

Statistical Analysis

Descriptive and inferential statistical analysis has been performed in the present study. Results on continuous measurements are presented on mean (standard deviation [Min-Max]) and expressed in number (%). Student *t*-test

(two-tailed, independent), Leven's test for homogeneity of variance, and Chi-square/Fisher Exact test were used, and *p*-value <0.005 was considered significant.

Results

A total 51 patients of cryptococcal meningitis with HIV infection >18 years fulfilling inclusion criteria were enrolled and started on treatment with amphotericin-based induction therapy for 2 weeks followed by fluconazole-based consolidation therapy for 8 weeks (►**Fig. 1**). Accordingly, CSF analysis was done following induction and consolidation therapy.

Baseline characteristics of the study subjects are shown in ►**Table 1**. The mean age of patient was 39.02 ± 10.51 years and most of them were in the age group of 21 to 49 years (54.9%) followed by 41 to 60 (39.2%). There were 2 patients in the age group 18 to 20 years and only 1 >60 years of age. Majority of them were males (33, 65%) while females were 18 (35.3%).

In the present study, majority of 17 (33.3%) patients were newly detected with HIV infection. Duration of HIV within 2 years was in 12 (23.5%) patients, 2 to 5 years in 15 (29.3%), and >10 years in 4 (7.8%). Majority of the CD4 count distribution fell in 51 to 100 range, 23 (45.1%), while 5 (9.8%) patients had less than 20, 18 (35.3%) patients had CD4 count in the range of 21 to 50, 3 (5.9%) in 100 to 150 range, and 2 (3.9%) in 150 to 200 range.

In our study, previous history of (h/o) cryptococcal meningitis was present in 15 (30.4%) patients, out of which relapse occurred within 1 year in 10 (19.6%) patients and. within 1 to 2 years in 3 (6%) patients. There was no h/o cryptococcal meningitis in 36 (70%) patients. Headache was the most common presenting complaint in 50 (98%), followed by fever in 38 (74.5%), vomiting in 34 (66.7%), altered sensorium in 18 (35.3%), bowel and bladder involvement in 3 (5.9%), focal neurological deficits (FNDs, mainly cranial nerve palsy) in 6 (11.8%), and seizure was seen in 10 (19.6%) patients. Past h/o tuberculosis was present in 10 (19.6%) and oral candidiasis in 20 (39.2%) patients. Out of 51 patients, CT/MRI-brain study was available for 40 patients, of which majority, 37.5%, showed meningeal enhancement, while hydrocephalous was in 12.5%. Majority of baseline hematological and biochemical parameters were in normal range except mild anemia. Venereal disease research laboratory (test) was negative in all patients (►**Table 2**).

On diagnostic CSF analysis, CSF protein was elevated in all patients. It was below 100 in 51% of patients, in the range 100 to 150 in 26 (35.3%), and more than 150 in 7 (13.7%) patients. CSF sugar was decreased in majority of patients; it was below 30 mg/dL in 12 (23.5%), 30 to 40 mg/dL in 30 (58.8%), and more than 40 mg/dL or normal in 9 (17.6%) patients. CSF TCC showed below 20 cells/mm^3 in 25 (49%), 20 to 80 cells/mm^3 in 12 (23.5%), and more than 80 cells/mm^3 in 14 (27.5%) patients (►**Table 3**).

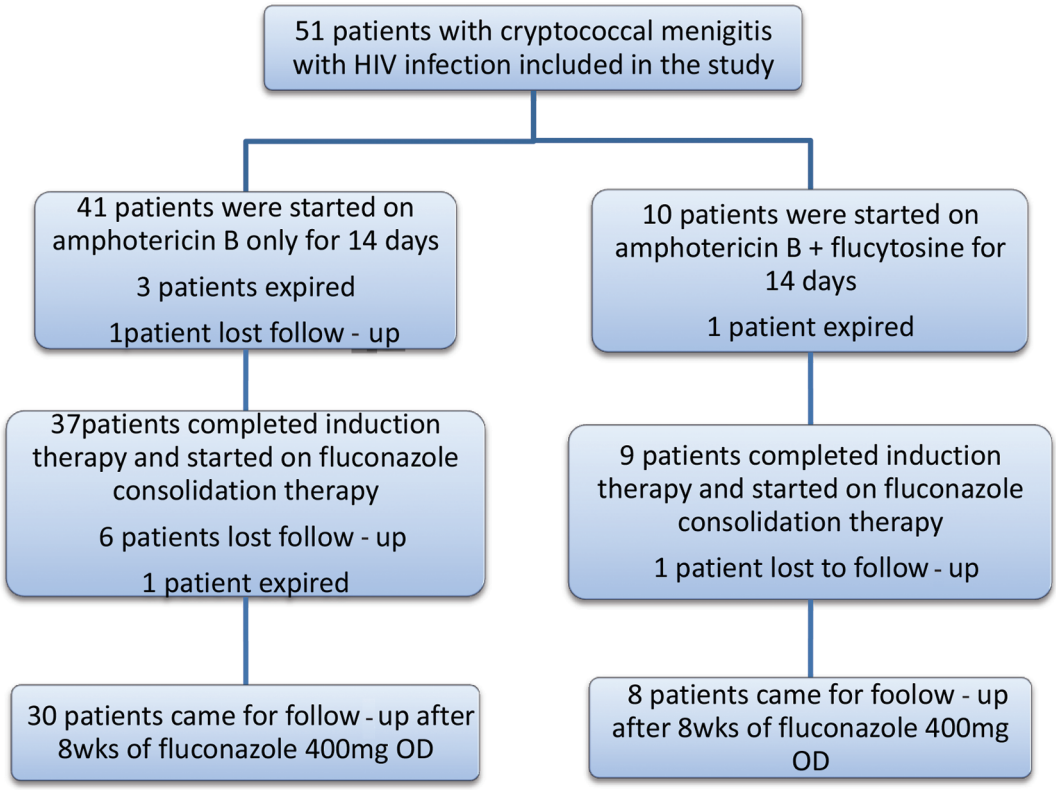

Fig. 1 Flowchart of study subjects. HIV, human immunodeficiency virus; OD, once daily.

Table 1 Baseline characteristics of the study subjects

Parameters	Results: *n* = 51
Age in years: median (range)	40 (18–65)
Gender	
Male	33 (65%)
Female	18 (35.3%)
Duration of HIV	
0 newly detected	17 (33.3%)
1–2 years	12 (23.5%)
2–5 years	15 (29.4%)
5–10 years	3 (5.9%)
>10 years	4 (7.8%)
CD4 count	
1–20	5 (9.8%)
21–50	18 (35.3%)
51–100	23 (45.1%)
100–150	3 (5.9%)
150–200	2 (3.9%)
H/o Cryptococcal meningitis	
No	36 (70.6%)
<1 year	10 (19.6%)
1–2 years	3 (6%)
2–5 years	1 (2%)
>5 years	1 (2%)
Presenting complaint	
Seizure	10 (19.6%)
Headache	50 (98%)
Fever	38 (74.5%)
Vomiting	34 (66.7%)
Altered sensorium	18 (35.3%)
Bowel and bladder disturbance	3 (5.9%)
Focal neurological deficits	6 (11.8%)
Past history	
H/o tuberculosis	10 (19.6%)
Oral candidiasis	20 (39.2%)
Diabetes mellitus	1 (2%)
Hypertension	2 (3.9%)
Brain imaging	
Meningeal enhancement	15 (37.5%)
Hydrocephalus	5 (12.5%)
Cryptococcoma	2 (5%)
Pseudo-cysts	4 (10%)
Cerebral atrophy	8 (20%)
Normal	14 (35%)

Abbreviations: CD4, cluster of differentiation 4 (glycoprotein); H/o, history of.

Table 2 Baseline characteristics of hematological and biochemical parameters

Parameters	Mean ± SD	Range
Hemoglobin (g/dL)	10.30 ± 1.75	8–14
Total leukocyte count	5,300 ± 2,620	2,000–16,000
Platelet count in thousands	160 ± 63	48–320
Seraum creatinine (mg/dL)	1.14 ± 0.39	0.6–1.4
Sodium (mEq/L)	134.82 ± 5.99	130–145
Potassium (mEq/L)	3.5 ± 0.48	3–5
Random blood sugar (mg/dL)	110 ± 30.41	76–132
Serum total protein (g/dL)	7.06 ± 0.32	5.1–7.4
Serum albumin (g/dL)	3.3 ± 0.25	3–4.1
Serum bilirubin (mg/dL)	1.1 ± 0.21	0.6–1.2
HBV infection present	3 (5.9%)	
HCV infection present	6 (11.8%)	
VDRL positive	0	
CD4 count	58.08 ± 32.98	2–180

Abbreviations: CD4, cluster of differentiation 4 (glycoprotein); HBV, hepatitis B virus; HCV, hepatitis C virus; SD, standard deviation; VDRL, venereal disease research laboratory (test).
Note: Majority of baseline hematological and biochemical parameters were in normal range except mild anemia. VDRL was negative in all patients.

On diagnostic CSF analysis, IIP was positive in 94.1% of patients and negative in 5.9% of patients. CSF cryptococcal antigen by CALAS test was positive in all patients and it was of grade 1+ in 13.7%, grade 2+ in 84.3%, and grade 3+ in 2% of patients. CSF fungal culture for *Cryptococcus*, a gold standard test for cryptococcal meningitis diagnosis, was positive in 94.1% and it was negative 5.9% of patients (►**Table 3**).

At the end of induction therapy, 46 patients underwent CSF analysis, which showed IIP was positive in 30 (65.3%) and negative in 16 (34.7%) patients. CSF CALAS was negative in 1 (2.2%), grade 1+ in 26 (56.6%), grade 2+ in 17 (36.9%), and grade 3+ in 2 (4.3%) patients. And it showed gradual decrease in CALAS titer (grade) compared with diagnostic CALAS test. CSF culture was positive in 18 (39.1%) patients and negative in 28 (60.1%) patients at the end of 2 weeks of treatment with induction therapy (►**Table 4**). Out of 41 patients being treated with amphotericin 0.7 mg/kg/d for 2 weeks, 16 (39.1%) patients were having persistent infection, 3 (7.3%) patients died, 21 (51.2%) patients showed clearance of cryptococcal infection, and 1 (2.4%) patient was lost in follow-up. Out of 10 patients being treated with amphotericin-B 0.7 mg/kg/day and flucytosine 100 mg/kg/day, only 2 (20%) had persistence of infection while 7 (70%) patients showed clearance of infection and 1 (10%) patient died. Hence, outcome is better with amphotericin-B and flucytosine combination therapy, though not statistically significant ($p = 0.578$; ►**Table 5**).

At the end of consolidation therapy, IIP was positive in 2 (5.3%) and negative in 36 (94.7%) patients. CSF CALAS test was negative in 10 (26.3%) and positive (1+) in 28 (73.6%), that is, in 73.6% of cases CALAS was persistently positive in low

Table 3 Distribution of diagnostic CSF analysis parameters

	Gender		Total (n = 51)	p-Value
	Male (n = 33)	Female (n = 18)		
CSF protein1				
• <100	20 (60.6%)	6 (33.3%)	26 (51%)	0.087+
• 100–150	8 (24.2%)	10 (55.6%)	18 (35.3%)	
• >150	5 (15.2%)	2 (11.1%)	7 (13.7%)	
CSF Sugar1				
• <30	7 (21.2%)	5 (27.8%)	12 (23.5%)	0.919
• 30–40	20 (60.6%)	10 (55.6%)	30 (58.8%)	
• >40	6 (18.2%)	3 (16.7%)	9 (17.6%)	
CSF total cell count1				
• <20	16 (48.5%)	9 (50%)	25 (49%)	0.327
• 20–80	6 (18.2%)	6 (33.3%)	12 (23.5%)	
• >80	11 (33.3%)	3 (16.7%)	14 (27.5%)	
IIP positive	30 (90.9%)	18 (100%)	48 (94.1%)	1.000
IIP negative	3 (9.1%)	0 (0%)	3 (5.9%)	
CSF CALAS				
1+	3 (9.1%)	4 (22.2%)	7 (13.7%)	0.391
2+	29 (87.9%)	14 (77.8%)	43 (84.3%)	
3+	1 (3%)	0 (0%)	1 (2%)	
CSF culture				
1 Positive	31 (93.9%)	17 (94.4%)	48 (94.1%)	0.857
2 Negative	2 (6.1%)	1 (5.6%)	3 (5.9%)	
Total	33 (100%)	18 (100%)	51 (100%)	

Abbreviations: CSF, cerebrospinal fluid; CALAS, Cryptococcal Antigen Latex Agglutination System; IIP, Indian ink preparation.
Note: On diagnostic CSF analysis, majority showed elevated CSF protein, reduced CSF sugar, and increased CSF total cell count.

Table 4 CSF analysis after induction therapy

		Treatment given		Total (n = 46) (%)	p-Value Chi-squared/Fisher's Exact
		Amphotericin (n = 37) (%)	Amphotericin + flucytosine (n = 9) (%)		
CSFIIP 2	Positive	22 (59.4)	8 (88.8)	30 (65.3)	0.096 Df = 1
	Negative	15 (40.6)	1 (2.8)	16 (34.7)	
Total		37 (100)	9 (100)	41 (100)	
CSF CALAS 2	Negative	1 (2.7)	0	1 (2.2)	0.516
	1+	19 (51.3)	7 (77.7)	26 (56.6)	
	2+	15 (40.5)	2 (22.3)	17 (36.9)	
	3+	2 (5.5)	0	2 (4.3)	
Total		37 (100)	9 (100)	41 (100)	
CSF culture 2	Positive	16 (43.2)	2 (22.3)	18 (39.1)	0.211
	Negative	21 (56.8)	7 (77.7)	28 (60.9)	
Total		37 (100)	9 (100)	46 (100)	

Abbreviations: CSF, cerebrospinal fluid; CALAS, Cryptococcal Antigen Latex Agglutination System; IIP, Indian ink preparation.
Note: CSF Culture was positive in 18 (39.1%) of patients and negative in 28 (60.1%) patients at the end of 2wks of treatment with induction therapy.

Table 5 Correlation of induction treatment with outcome after induction therapy

		Treatment given		Total (n = 51) (%)
		Amphotericin (n = 41) (%)	Amphotericin + flucytosine (n = 10) (%)	
Outcome after 15 days of treatment	Persistent infection	16 (39.1%)	2 (20%)	18 (35.3%)
	Death	3 (7.3%)	1 (10%)	4 (7.8%)
	Clearance of infection	21 (51.2%)	7 (70%)	28 (55%)
	Lost follow-up	1 (2.4%)	0 (0%)	1 (1.9%)
Total		41 (100%	10 (100%)	51 (100%)

Note: There is trend toward better outcome with amphotericin-B and flucytosine combination therapy though there is no statistically significant difference (p = 0.578).

Table 6 CSF analysis after consolidation therapy

	Gender		Total (n = 38) (%)	p-Value
	Male (n = 25) (%)	Female (n = 13) (%)		
IIP3 after consolidation therapy				
• Positive	1 (4%)	1 (7.6%)	2 (5.3%)	0.4
• Negative	24 (96%)	12 (92.4%)	36 (94.7%)	
CSF, CALAS3 after consolidation therapy				
• Negative	5 (20%)	5 (38.4%)	10 (26.3%)	0.363
• 1+	20 (80%)	8 (61.6%)	28 (73.6%)	
CSF, culture 3 after consolidation therapy				
• Positive	1 (4%)	1 (7.6%)	2 (5.3%)	0.494
• Negative	24 (96%)	12 (92.4%)	36 (94.7%)	

Abbreviations: CSF, cerebrospinal fluid; CALAS, Cryptococcal Antigen Latex Agglutination System; IIP, Indian ink preparation.
Note: CSF fungal culture was positive in only 2 (5.3%) patients after consolidation therapy, who were later on started on amphotericin induction therapy again for 2 more weeks till CSF was sterile.

titers. CSF fungal culture was positive in 2 (5.3%) and sterile or negative in 34 (94.7%) patients (►**Table 6**). These two patients with persistent infection were started on amphotericin induction therapy again for 2 more weeks till CSF was sterile. Thus, out of 46 patients, 2 (4.4%) had persistent infection, 1 patient died, 36 (78.2%) patients got cleared of infection, and 7 (15.2%) lost in follow-up.

After 10 weeks of treatment (2 weeks induction + 8 weeks consolidation), final outcome was as follows: out of 51 patients, 2 (4%) patients were having persistent infection, 5 (9.8%) patients died, and 8 (15.7%) patients were lost in follow-up. Only 36 (70.5%) patients got cleared of intracranial cryptococcal infection (►**Fig. 2**). At the end of consolidation therapy, all patients with persistent infection had CD4 count <50. All these patients died because of having CD4 count <100 due to cryptococcal meningitis. Majority patients who got cleared of infection were having CD4 count in the range of 50 to 200 (►**Fig. 3**).

Among the side effects, allergic reactions like fever, chills, and itching occurred mostly at the start of treatment seen in 11 patients of induction group, which was managed by steroids and antihistamines. Thrombophlebitis occurred in 14 (12 + 2) patients and they were treated with intravenous (i.v.) fluids, proper care of i.v. lines, and antibiotics. Hypokalemia occurred in 35 (30 + 5) patients and it was treated with potassium supplements and frequent electrolyte monitoring. In seven patients, elevation of creatinine was treated with hydration. In two patients, conventional amphotericin was replaced by liposomal amphotericin. Anemia occurred in four patients and hyponatremia in six patients during induction therapy and managed accordingly (►**Table 7**).

Discussion

Cryptococcal meningitis is the leading cause of mortality and morbidity in people living with HIV (PLHIV), and early clearance of infection in the form of sterile CSF after induction therapy is associated with favorable outcome.[6] The main focus of the present study is to know the persistence of *Cryptococcus* after induction therapy with amphotericin-B ±

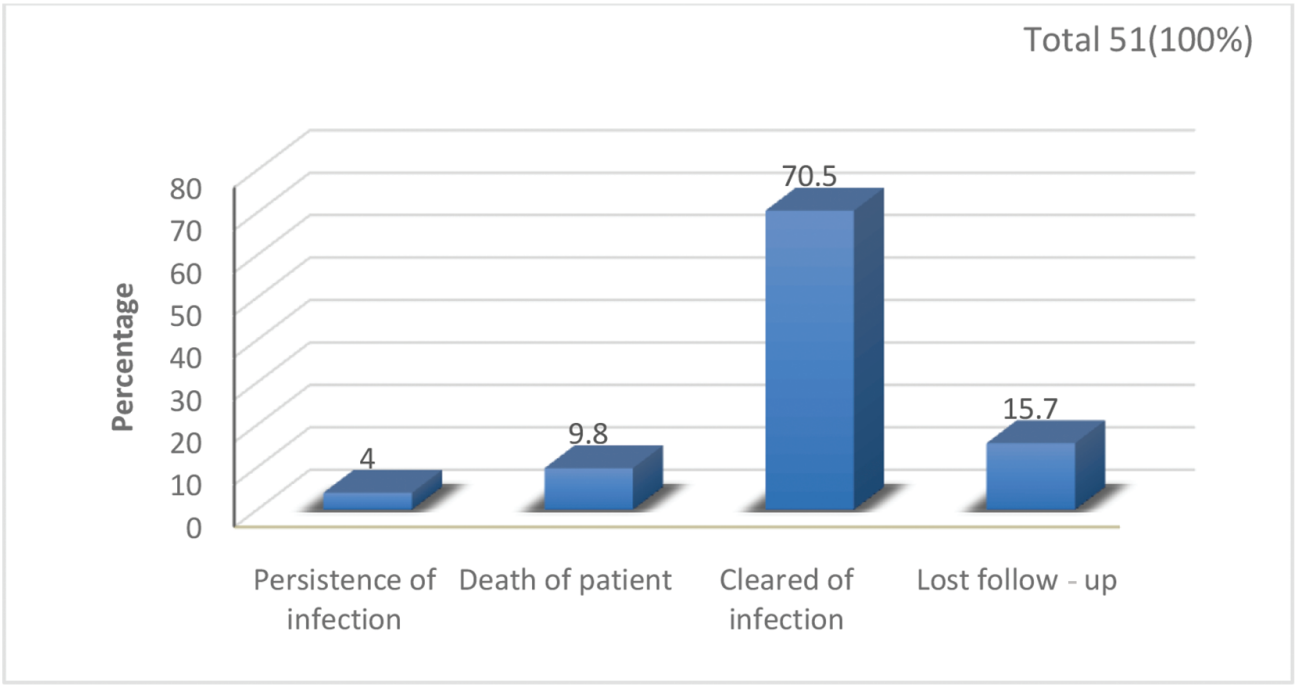

Fig. 2 Final outcome after 10 weeks of treatment (2 weeks of induction + 8 weeks of consolidation). Out of 51 patients included in the study, 2 (4%) patients were having persistent infection and 36 (70.5%) patients got cleared of intracranial cryptococcal infection.

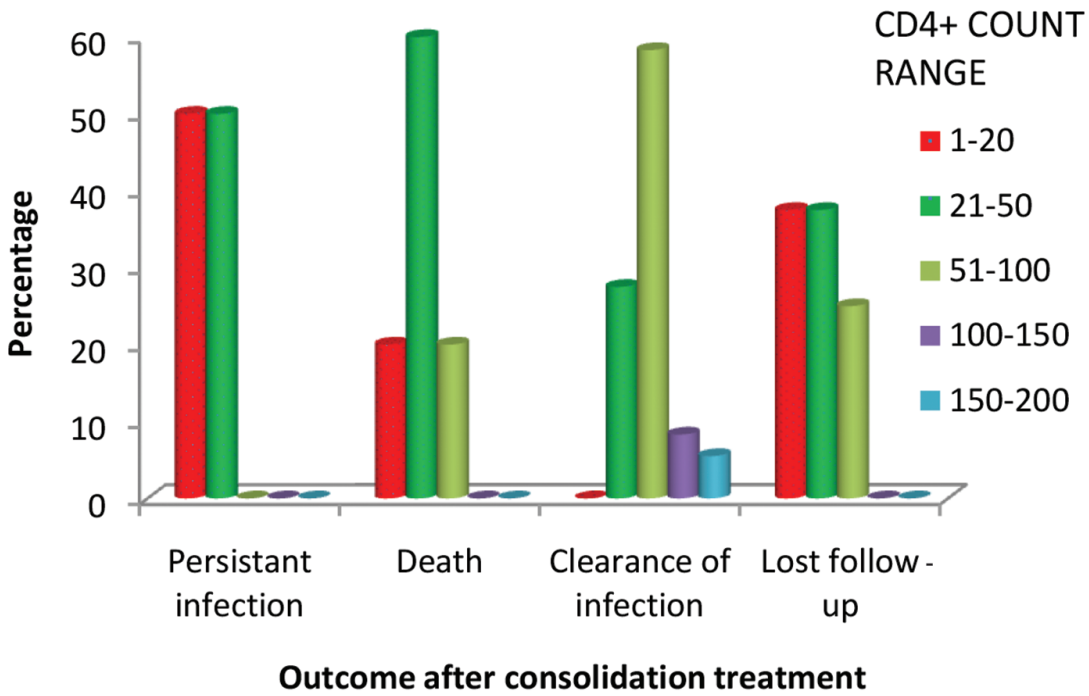

Fig. 3 CD4+ count range according to final outcome. At the end of consolidation therapy, majority patients who got cleared of infection were having CD4 count in the range of 50 to 200 and all patients with persistent infection had CD4 count <50. All patients died because of cryptococcal meningitis who were having CD4 count <100.

Table 7 Adverse reactions of drugs in treatment groups

Adverse reactions	Induction therapy (2 weeks)		Total	Consolidation therapy (8 weeks)
	Amphotericin: *n* = 41	Amphotericin + flucytosine: *n* = 10		Fluconazole: *n* = 38
Allergic reactions like fever, chills	10	1	11	0
Thrombophlebitis	12	2	14	0
Hypokalemia	30	5	35	0
Elevated creatinine	5	2	7	1
Anemia	4	0	4	3
Hyponatremia	5	1	6	1

Note: Among the side-effects, allergic reactions like fever, chills, and itching occurred mostly at the start of treatment, which was managed by steroids and antihistamines. Other side-effects include deranged creatinine, dyselectrolytemia, anemia, and thrombophlebitis.

flucytosine for 2 weeks and consolidation therapy with fluconazole 400 mg for 8 weeks.

A total of 51 PLHIV with cryptococcal meningitis were included in the study. The mean age of patient was 39.02 ± 10.51 years and most of them were in the age group of 25 to 49 years. Majority of patients were male 64.7% (33) and females constituted 35.3% (18).

In our study, headache was the commonest presenting complaint (98% of the patients), followed by fever (74.5%), vomiting (66.3%), altered sensorium (35.3%), seizures (19.6%), and FND (11.5%). The findings are in comparison with the study conducted by Satishchandra et al.[20] Another study conducted by Kumar et al[21] showed headache in 96%, seizure in 33%, altered sensorium in 33%, FND in 14%, fever in 66%, and vomiting in 77% of the patients.

The CD4 count is the best indicator of the immediate state of immunologic competence and also the strongest predictor of HIV-related complications. Cryptococcal infection was the commonest opportunistic infection and a major cause of death in PLHIV with CD4 count <100 cells/mL in the pre-HAART era.[9]

In our study, we did CSF analysis for the three times: first one diagnostic, second at the end of 2 weeks of induction therapy, and lastly at the end of 8 weeks of consolidation therapy. The diagnostic CSF analysis showed elevated CSF protein with a mean of 116 ± 48.14, low CSF sugar levels with mean being 34.73 ± 8.67, and elevated TCC with mean 57.61 ± 75.35 cells/m^3. In subsequent CSF analysis, after induction and after consolidation therapy, the mean protein was 94.16 ± 40.97 and 51.15 ± 35.75, respectively, which was gradually decreasing and statistically significant (p = 0.001). A similar study conducted by Huang et al[22] showed more decrease in CSF protein level from baseline, in good antifungal response group as compared with poor antifungal response group. Analysis using generalized linear mixed model showed that after antifungal treatment, CSF protein concentration of the CR-group decreased at a rate of 1.8 mg/L/day.

In the present study, diagnostic CSF analysis showed lymphocyte predominance in all patients. TCC was <20 cells/m^3 in 25 (49%) and >100 cells/m^3 in 14 (27.5%) patients. In a study conducted by Satishchandra et al,[20] 52% patient had TCC <20 cells/m^3 with lymphocyte predominance in all patients. In a study conducted by Kapila et al,[23] 72% of patients had cells <20 cells/m^3. This low cell count in CSF indicates poor inflammatory response to *Cryptococcus* in PLHIV due to impaired cell-mediated immunity.

In our study, diagnostic IIP was positive in 48 (94.1%) patients. CALAS test was positive in all 51 (100%) patients: it was grade 1+ in 7 (13.7%) patients, 2+ in 43 (84.3%) patients, though 3+ in only 1 patient. Diagnostic CSF culture was positive in 48 (94.1%) and was negative in 3 (5.9%) patients. In two separate studies conducted by Patel et al[24] and Kumar et al,[21] CSF IIP was positive in 96% and 85%, respectively, while CALAS was positive in all patients in both studies. Patel et al[24] showed 100% culture positivity versus 90% culture positivity in the Kumar et al[21] study.

Untreated cryptococcal meningitis is uniformly fatal. A large randomized controlled trial undertaken by Mycoses Study Group by van der Horst et al[25] established the combination of amphotericin-B deoxycholate (0.7 mg/kg/day) and flucytosine (100 mg/kg/day) as a standard of care for the treatment of cryptococcal meningitis in AIDS patients. The findings of the study were further substantiated by another study conducted by Brouwer et al.[26]

In our study, after 2 weeks of induction therapy, 28 (55%) patients achieved sterile CSF and 18 (35.3%) were having persistent cryptococcal meningitis. CSF cultures were negative in 70% (7 out of 10) of the patients who received amphotericin-B with flucytosine and in 51% (21 out of 41) of those who received amphotericin-B alone.

Even with treatment, cryptococcal meningitis has high mortality; and the reasons given in various studies include late presentation with low CD4 count, immune reconstitution inflammatory syndrome, uncontrolled raised intracranial pressure, FND, and amphotericin-B toxicity. A high mortality rate of 33% at week 4 was observed in KwaZulu-Natal by Lightowler et al.[27] In another study, the mortality was 14% at 2 weeks and 22% at 10 weeks.[20] Also, a study from North India

by Kumar et al[21] reported a 7.5% mortality. Mortality in our study was 9.8%; however, actual mortality may be higher as in the present study 8 (15%) patients were lost in follow-up. Death of the patient may be one of the reasons for the lost follow-up.

In our study, after excluding mortality and lost follow-up, among patients from analysis after induction therapy, 60.9% (28 out of 46) showed clearance of infection. In the amphotericin with flucytosine treatment group, 77% (7 out of 9) of the patients, and in amphotericin alone group, 56.7% (21 out of 37) of the patients showed culture negativity at the end of 2 weeks. Though there is no statistically significant difference, there is trend toward better outcome with amphotericin-B and flucytosine combination therapy. After consolidation therapy with fluconazole, 94.7% (36 out of 38) of patients showed negative culture. However, this study has limitations, like loss to follow-up of some patients in consolidation therapy and some patients not willing to undergo repeated LP during follow-up. There is no randomization in induction therapy and the sample size of amphotericin + flucytosine group was very small.

This study showed that only amphotericin-based regime in induction therapy has 50% of treatment failure. Suggesting adding flucytosine to induction therapy and extending the induction therapy till culture is sterile, gives good outcome in the long term.

Conclusion

The present study showed that cryptococcal meningitis in HIV patients responded to amphotericin-based induction therapy with 60% clearance of infection followed by fluconazole-based consolidation therapy with 94% of clearance of infection. Adding flucytosine in induction therapy showed increased clearance of infection, and patients with baseline CD4 count more than 50 showed good outcome. However, there is still need for good antifungal regimen that could clear infection in induction phase with less side effects.

Conflict of Interest

None declared.

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