ORIGINAL RESEARCH PAPER

General Medicine

PRELIMINARY INVESTIGATION INTO THE EFFECT OF AMPHOTERICIN B WITH FLUCONAZOLE IN CRYPTOCOCCAL MENINGITIS PATIENT WITH AMPHOTERICIN B AS CONTROL.

KEY WORDS: CNS (Central nervous system), C. Antigen (Cryptococcal antigen), C.Meningitis (Cryptococcal Meningitis), HIV (Human immunodeficiency virus), AIDS (Acquired immunodeficiency syndrome), AmB (Amphotericin B), CSF (Cerebrospinal fluid), FCZ (Fluconazole)

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ABSTRACT

BACKGROUND: HIV infection is a global pandemic with cases reported from virtually every country. According to UNAIDS, 36.7 million people around the world are currently suffering from HIV/AIDS. 2.1 million children are living with HIV.Globally , it is estimated that approximately 957,900 cases of cryptococcal meningoencephalitis occur each year, resulting in more than 600,000 deaths.

MATERIALS AND METHODS:A randomized control study comprising of 84 cases in the age group of >12 years old admitted in tertiary care centre with HIV infection having cryptococcal meningitis diagnosed by CSF study were randomised into 2 groups and were given inj Amphotericin B and Amphotericin B plus fluconazole and were compared.

RESULTS: Study shows that amphotericine B + fluconazole was the most rapidly fungicidal combination in CSF, compared with amphotericine B alone.

INTRODUCTION

According to UNAIDS, 36.7 million people around the world are currently suffering from HIV/AIDS. 2.1 million children are living with HIV. Most of these children were infected by their HIV positive mother's during pregnancy, childbirth or breastfeeding. Approximately 70% of the people lving with their HIV globally were aware of there HIV status in 2016. The remaining 30% (over 11 million people) still needs access to HIV testing services. By June 2017, 20.9 million HIV infected people were accessing antiretroviral therapy(ART) globally, up from 15.8 million people in june 2015, 7.5 million in 2010 and less than 1 million in 2000. 1 million people suffering from AIDS died due to AIDS related illness summing it up to total of 35 million since the start of epidemic.(1)

The most predominant opportunistic infection (OI) among AIDS patients is tuberculosis, indicating a potential future high spread of the HIV TB co infection. In india as the epidemic is advancing it is also maturing. The common OI seen are tuberculosis(pulmonary and extrapulmonary), cryptococcosis, pneumocystis carini, toxoplasmosis, cryptosporidium diarrhea, cytomegalovirus infections, penicilium marneffel infections(a fungal infection in northeast part of the country) and progressive multifocal leukoencephalopathy. In addition there is rising number of HIV related malignancies such as lymphoma and cervical cancers.

Globally , it is estimated that approximately 957,900 caSes of cryptococcal meningoencephalitis occur each year, resulting in more than 600,000 deaths.(2,3) The region with the highest number of estimated cases in 2006 was subsaharn Africa(720,000 cases; range 144,000 to 1.3 million), followed by south and southeast Asia(120,000 cases; range, 24,000 to 216,000) .(2)

Cryptococcal meningitis (c. meningitis) is the most common clinical manifestation of cryptococcosis and the most common cause of fatality. It occurs in AIDS patients with D 4 count of <200/ul. Chronic meningitis is a main manifestation of this infection. Early cases with no symptoms referable to the central nervous system(CNS) may only have a positive culture of cerebrospinal fluid(CSF) with no other abnormaity of the fluid.(4,5,6) Including smears for Indian ink preparation and cryptococcal antigen(c. antigen). C. mengitis has been reported as one of the most common OI of the CNS of India patients with HIV accounted for 2-4.7 percent of all OI in two large HIV positive cohorts in Mumbai and Chennai. (7,8).Reports of it, from various states of India have increased more in the AIDS era.(9)

In patients with AIDS, Amphotericin B(AmB) (0.7mg/kg IV) given for 2 weeks followed by fluconazole(FCZ) 400mg PO for a further 8 weeks is associated with the best outcome to date in prospective trials.(10) The combination of AmB deoxycholate and flucytosine(5FC) is generally considered as the gold standard of initial therapy of cryptococcosis.(11)

BACKGROUND

HIV infection is a global pandemic with cases reported from virtually every country. In India the first case of HIV infection was detected in 1986 and since then it is rising at an alarming rate. In july 1987 the first transfusion related HIV infection was diagnosed. The donor reported that his last contact with a commercial sex worker had been in 1984 suggesting that HIV was present in India at least by that time.

HIV belongs to the family of human retroviruses(retroviridae) and the subfamily of lentiviruses. The HIV 1 and HIV 2 are cytopathic viruses. Both HIV 1 and HIV 2 are zoonotic infections. HIV 2 is more phylogenetically related to the Trogiodytes species of chimpanzees, in which the virus had co-evolved over centurieThere are thre subgroups of HIV 1; group M(major) which is responsible for most of the infections in the world and is divided into 9 subtypes, designated A,B,C,D,F,G,H,I,J,K and crfS(circulating recombinant forms. Subtype is prevelant in India. Other subgroups are group O(outlier) and group N.

CRYTOCOCCAL MENINGITIS

C. Meningitis was first identified in 1905 by Hansemann, even though Curtis had made the observation of meningeal complication in his patient from 1895. The predisposing factor to Cryptococcus worldwide is currently AIDS.CD4 cell counts are usually below 200/Ul in AIDS patients who develop cryptococcal infection. In patients with AIDS, C. mengitis is a well known cause of neuro-ophthalmic disorders, such as papiloedema and ocular motor nerve palsies. However visual loss in the absence of direct ocular involvement is an uncommon complication. Cryptococcoma is a rare entity, characterized by localized, solid, tumor like masses, usually found in the cerebral hemispheres or cerebellum, or more rarely in the spinal cord. Infection with C. neoformans can involve sites other than the meninges.

Treatment :

Amphotericin B(AmB) binds with ergesterol, a component of fungal cell membranes, forming a transmembrane channel that

leads to monovalent ion(k, Na, H and Cl) leakage which is the primary effect leading to fungal cell death. AmB can form pores in the host membrane as well as the fungal membrane. This impairment in barrier function can have lethal effects.(12,13,14) Recommended dose is 1.7 -1mg/kg/day. Liposomal AmB can be given at a rate of 4mg/kg/day .

Fluconazole (FCZ) is a triazole antifungal drug that inhibits fungal ergesterol synthesis, has excellent absorption and csf penetration and is safe. FCZ inhibits fungal cytochrome450enzyme 14alpha demethylase.

OBJECTIVE OF THE STUDY

Preliminary investigation into the effect of amphotericin B with Fluconazole in Cryptococcal meningitis patient with amphotericin B as control.

MATERIAL AND METHODS

MATERIALS:

This is a randomized prospective unbiased control study where all hiv positive patientsof more than 12 yr age with prior informed consent from pt or relatives who fulfill the exclusion criteria with symptoms of meningitis were admitted in a tertiary care hospital in a period of one year. Then patients were separated according to the etiology of meningitis. Out of them only cryptococcal meningitis patients diagnosed by csf study were selected for study population which comprises of 84 cases . sample size chosen according to estimation method (confidence interval approach) by ready made table method where relative precision e is 0.28 and confidence level is 99%. Then the study population were devided into two groups randomly by simple randomization method, random number table method.

METHODOLOGY:

Inclusion criteria

1. Age > 12 years
2. Patient infected with HIV with cryptococcal meningitis.
3. Written informed consent for each patient either from the patient or from the patient legal guardian.

Exclusion criteria

1. Pregnancy
2. Breast-feeding
3. Moderate to severe liver disease
4. Moderate to severe renal disease
5. Evidence of acute or chronic meningitis based upon any etiology other than cryptococcosis like Tubercular meningitis, Toxoplasmosis, Histoplasmosis, Bacterial meningitis.
6. Diabetes, hypertension, electrolyte imbalance, Isochemic heart disease, cerebro vascular accident, malignancy, pulmonary disease.
7. Relapse after prior therapy
8. H/o allergy or intolerance to imidazole, azoles or amphotericin
9. H/o prior systemic antifungal therapy for Cryptococcu

Detail demographic, clinical, biochemical data of the patients were collected in a exel sheet.

1. Name, age ,sex, occupation,
2. Fever, headache, neck stiffness, altered sensorium, focal neurological-deficit.
3. Onset, progress and duration of disease.
4 CD4 count,hiv 1& 2
5 On ART- yes/no with duration of treatment.
6. Complete blood count
7. Renal function test
8. Liver function test
9. CT brain was done where possible
10. CSF study done for r/m. ada, sugar, protein, viral markers, gene expert for MTB.
11. DIAGONOSIS OF CRYPTOCOCCAL MENINIGITIS

CSF for Indian ink preparation and csf culture for CryptococcusOut of 84 study population divided into two groups by simple randomization using a random number table one treated with both inj. Amphotericin B and inj Fluconazole and other group only with inj. Amphotericin.B.

1. GROUP A- Inj. AmB (0.7-1mg/kg/day) alone for 2 weeks.
2. GROUP B-Inj. AmB (0.7-1mg/kg/day) plus fluconazole 400mg for 2 weeks.

After two weeks of treatment both groups were compaired according to there cinical profile like persistence of fever, headache ,neck stiffness, altered sensorium and focal neurological deficit along with biochemical parameters for efficacy of treatment.

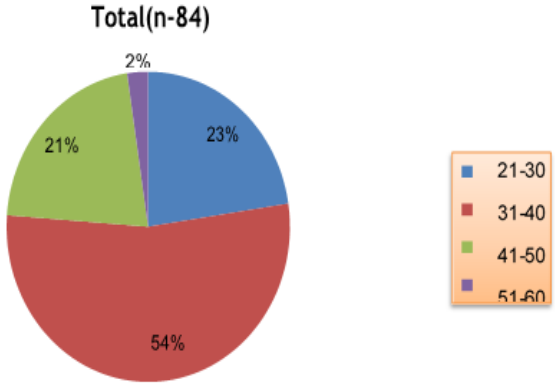
During treatment monitoring of CBC, Renal function test, liver function test done for toxicity determination.. If toxicity occurs during treatment, discontinuation of treatment was done. After 2 weeks, evaluation of improvement in clinical profile and CSF culture negativity for Cryptococcus done to compare the efficacy of above treatment modalities in terms of both clinical and pathological parameter.

RESULTS AND STATISTICAL ANALYSIS

All the results were analysed with chi-square test with a p value of less than 0.05 considered to be significant A total of 84 HIV-Positive patients having C. meningitis were enrolled into the study conducted at a tertiary health care centre. Patients were randomised in two groups as

1. GROUP A (n-42):Inj. AmB(0.7-1mg/kg/day)IV alone
2. GROUP B (n-42):Inj. AmB(0.7-1mg/kg/day)IV plus FCZ Above therapy given for 14 days.

FIGURE 1
1: Age distribution in the study



Descriptive statistics of CFS findings:
Maximum number of patients in the study was in the age group of 31-40 years.

Table 1: Distribution of CSF protein in both groups:

CSF protein (mg/dl)	Minimum	Maximum	Mean	SD
Group A	20	100	62.238	25.132
Group B	20	104	58.095	24.817

Above table shows mean of CSF protein in group A-62.238 mg/dl and group B-58.095 mg/dl.

Table 2: Distribution of CSF sugar in both groups:

CSF protein (mg/dl)	Minimum	Maximum	Mean	SD
Group A	22	71	43.42	12.034
Group B	22	72	42.11	12.215

Above table shows mean of CSF sugar group A-43.42 mg/dl and group B-42.11 mg/dl.

FEVER:
TABLE 3: FEVER; Group A Vs. Group B(On admission)

Fever	Group A	Group B	Total (%)
	No.(%)	No.(%)	

Yes	36(90.47)	38(90.5)	76(90.47)
No	6(9.53)	4(9.50)	8(9.53)
Total	42(100)	42(100)	84(100)
Chi-square value-0.4541; p value-0.5004; Non-significant			

FEVER:

TABLE 4: FEVER; Group A Vs. Group B (After 14 days of therapy).

Fever	Group A	Group B	Total(%)
	No (%)	No (%)	
Yes	12 (28.6)	10 (23.81)	22 (28.5)
No	30 (71.4)	32 (76.19)	62 (71.4)
Total	42 (100)	42 (100)	84 (100)
Chi-square value- 0.2463; p value- 0.6197; non-significant			

There was no statistical significant difference among the two groups A and B in terms of fever as a clinical outcome after 14 days of treatment (p value-0.6197).

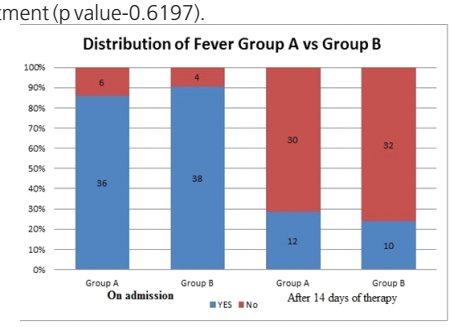


FIGURE 2: Distribution of FEVER Group A Vs. Group B

HEADACHE:

TABLE 5: HEADACHE; Group A Vs. Group B (0n admission)

Headache	Group A	Group B	Total(%)
	No.(%)	No.(%)	
Yes	36(85.71)	34(80.95)	70(83.33)
No	6(14.29)	8(19.05)	14(16.67)
Total	42(100)	42(100)	84(100)
Chi-square value-0.3429; p value-0.5582;Non-significant			

HEADACHE:

TABLE 6:HEADACHE; Group A Vs.Group B(After 15 days of therapy)

Headache	Group A	Group B	Total (%)
	No.(%)	No.(%)	
Yes	18(42.9)	14(33.3)	32(38.1)
No	24(54.5)	28(66.7)	52(61.9)
Total	42(100)	42(100)	84(100)
Chi-square value-1.061; p value-0.303; Non-significant			

There was no statistical significant difference among the two groups A and B in terms of altered sensorium as a clinical outcome after 14 days of treatment (p value-0.303; Non-significant).

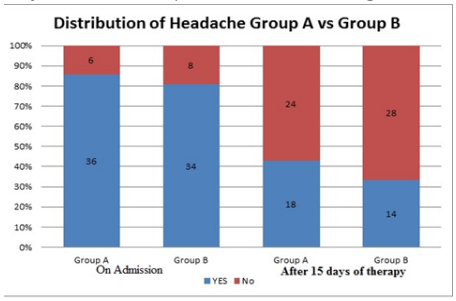


FIGURE 3: Distribution of HEADACHE; Group A Vs.GroupB

Neck stiffness :

Table 7: Neck stiffness; Group A Vs. Group B (After 14 days of therapy)

Neck stiffness	Group A	Group B	Total(%)
	No.(%)	No.(%)	
Yes	10(23.8)	10(23.8)	20(23.8)
No	32(76.2)	32(76.2)	64(76.2)
Total	42(100)	42(100)	84(100)
Chi-square value-0.00; p value-1;Non significant			

There was no statistical significant difference among the two groups A and B in terms of Neck stiffness as a clinical outcome after 14 days of treatment(p value-1; Non-significant)

NECK STIFFNESS:

TABLE 8: NECK STIFFNESS: Group A Vs. Group B (On admission)

Neck stiffness	Group A	Group B	Total(%)
	No. (%)	No. (%)	
Yes	24(67.1)	26(61.90)	50(59.52)
No	18(42.9)	16(38.10)	34(40.48)
Total	42(100)	42(100)	84(100)
Chi-square value-0.1967; p value-0.6666;Non-significant			

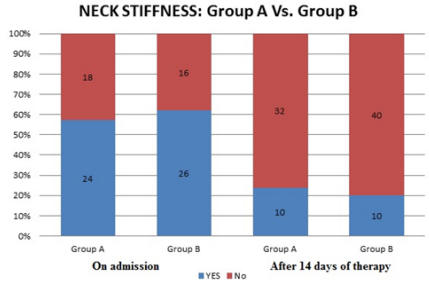


FIGURE 4: Distribution of NECK STIFFNESS: Group A Vs. Group B

ALTERED SENSORIUM

TABLE 9: ALTERED SENSORIUM; Group A Vs. Group B (after 14 days of therapy)

Altered sensorium	Group A	Group B	Total(%)
	No.(%)	No.(%)	
Yes	6(14.29)	1(2.38)	7(0.33)
No	36(85.71)	41(97.62)	77(99.67)
Total	42(100)	42(100)	84(100)
Chi-square value-3.8961; p value-0.0484; Significant			

There was statistical significant difference among the two groups A and B in terms of altered sensorium as a clinical outcome after 14 days of treatment (p value-0.0484; Significant).

ALTERED SENSORIUM

TABLE 10: ALTERED SENSORIUM: Group A Vs. Group B (On admission).

Altered sensorium	Group A	Group B	Total (%)
	No.(%)	No.(%)	
Yes	18(42.9)	16(38.1)	34(40.5)
No	24(57.1)	26(61.9)	50(59.5)
Total	42(100)	42(100)	84(100)
Chi-square value-0.198; p value-0.657; Non-significant			

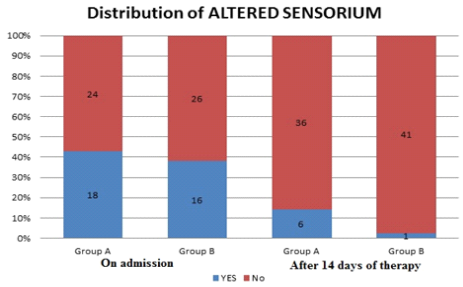


FIGURE 5: Distribution of Altered Sensorium: Group A Vs. Group B

FOCAL NEUROLOGICAL DEFICIT

Table 11: FOCAL NEUROLOGICAL DEFICIT; Group A Vs. Group B (on admission).

Focal neurological Deficit	Group A No.(%)	Group B No.(%)	Total
Yes	8(19)	6(14.3)	16(16.7)
No	34(81)	36(85.7)	70(83.3)
Total	42(100)	42(100)	84(100)
Chi-square value-0.343; p value-0.558; Non-significant			

Table 12: FOCAL NEUROLOGICAL DEFICIT; Group A Vs. Group B (after 14 days of therapy).

Focal neurological deficit	Group A No.(%)	Group B No.(%)	Total
Yes	8 (19)	2 (4.8)	10(11.9)
No	34 (81)	40 (95.2)	74(88.1)
Total	42 (100)	42 (100)	84(100)
Chi-square value-0.343; p value-0.558; Significant			

There was statistical significant among the two groups A and B in terms of Focal neurological deficit as a clinical outcome after 14 days of treatment (p value-0.0484; Significant).

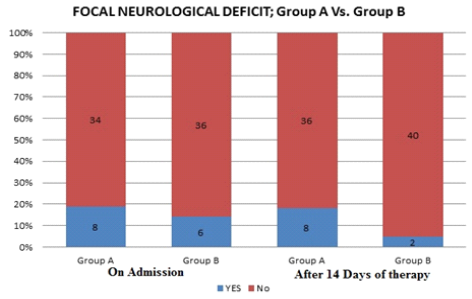


FIGURE 6: Distribution of FOCAL NEUROLOGICAL DEFICIT; Group A Vs. Group B

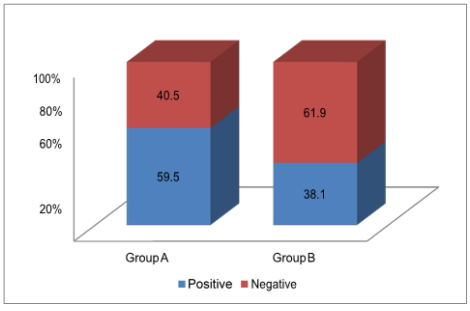
CULTURE:

TABLE 13: CULTURE Group of A and Group B (After 14 days of therapy).

Culture	Group A No.(%)	Group B No.(%)	Total(%)
Positive	25(59.5)	16(38.1)	41(47.6)
Negative	17(40.5)	26(61.9)	43(52.4)
Total	42(100)	42(100)	84(100)
Chi-square value-3.859; p value-0.049; Significant			

There was no statistical significant difference among the two groups A and B in terms of CSF culture study after 14 days of treatment (p value-0.049; Significant).

FIGURE 7: CULTURE Group of A and Group B (After 14 days of therapy).



DISCUSSION

Duration of ART:

In group A duration of ART was 3.26±1.84 years In group B duration of ART was 2.83±1.68 years.

CSF finding:

In study values of CSF protein in group A and B was 62.23±25.13 and 58.09±24.81 mg/dl respectively CSF sugar in group A and B was 43.42±12.03 and 42.11±12.21 mg/dl respectively.

CSF lymphocyte in group A and B was 42.19±37.69 and 46.19±37.37 cells/µl respectively.

Clinical profile:

- **Fever:**

On admission.In group A 36 and group B 38 patients had fever.

After 14 days of treatment. Patients with fever in group A 12 and group B 10. Improvement in fever was similar in both group A and B after 14 days of treatment

There was no statistical significant difference among the two groups A and B in terms of fever as a clinical outcome after 14 days of treatment.

- **Headache:**

On admission. In group A 36 and group B 32 patients had headache. After 14 days of treatment. Patients with headache in group A 18 and group B 14.

Improvement in headache was similar in both groups A and B after 14 days of treatment. There was no statistical significant difference among the two groups A and B in terms of headache as clinical outcome after 14 days of treatment.

- **Neck stiffness:**

On admission. In group A 24 and group B 26 patients had neck stiffness.

After 14 days of treatment. Patients with neck stiffness in group A 10 and group B 10. Improvement in neck stiffness was similar in both groups A and B after 14 days of treatment.

There was no statistical significant difference among the two groups A and B in terms of neck stiffness as clinical outcome after 14 days of treatment.

- **Altered sensorium:**

On admission. In group A 18 and group B 16 patients had altered sensorium After 14 days of treatment. Patients with altered sensorium in group A 6 and group B 1. There was statistical significant difference among the two groups A and B in terms of altered sensorium as a clinical outcome after 14 days of treatment. After 14 days of treatment, in group B statistical significant improvement in altered sensorium as compared to group A.

- **Focal neurological deficit**

On admission. In group A 8 and group B 6 patients had focal neurological deficit.

After 14 days of treatment. Patients with focal neurological deficite in group A 8 and group B 2.

There was statistical significant difference among the two groups A and B in terms of focal neurological deficit as a clinical outcome after 14 days of treatment.

After 14 days of treatment, in group B statistical significant improvement in focal neurological deficit as compared to group A.

There was no baseline data available to compare improvement in clinical profile after treatment. But our study shows improvement in altered sensorium and focal neurological deficit for limited duration of 14 days. To draw other significant difference between two treatment categories, large sample size and long term follow up and may be desirable.

CSF CULTURE AFTER 14 DAYS of TREATMENT:

Patients enrolled in study were HIV positive with cryptococcal meningitis diagnosed with India ink test and CSF culture.

After 14 days of treatment, Patients with CSF culture negativity in group A 17 and group B 26.

There was statistical significant among the two groups A and B in terms of CSf culture study after 14 days of treatment.

After 14 days of treatment, in group B statistical significant CSf fungal (cryptococcal neoformans) culture negativity as compare to group A.

SUMMARY

1. Total n-84 patients HIV positive with cryptococcal meningitis diagnosed by India ink study and confirmed by CSF culture for Cryptococcal neoformans were studied.
2. Prospective study, for comparison total patients divided in 2 groups. Group A(n-42): On Amphotericine alone
   Group B(n-42): On Amphotericine combined with fluconazole
   Both groups were studied for 14 days hospital stay [84-87]
3. Maximum number of patients with cryptococcal meningitis in an age group of 31- 40 years. Male predominance was seen i.e. 83.33% male and 16.27% females.
4. Duration of hospital stay was more in group A(21.57 days) compared to group B(17.69 days).
5. All 84 patients with CD4 count <200 cell/µl. Group A-43.54±31.98 cell/µl. and Group B-51.21±21.81 cell/µl.
6. CSF finding- CSF protein was 50mg/dl in both groups. (Group A-62.23±25.13 and Group B-58.09±24.81mg/dl) CSF sugar was 45mg/dl in both groups (Group A-43.42±12.03 and Group B-42.11±12.21mg/dl) CSF lymphocytes were 5cell/µl in both groups.
7. Clinical profile- After 14 days of treatment in group A and group B, there was no difference in terms of fever, headache, neck stiffness, as a clinical outcome Which was **Statistical not significant.**

But in group B there was improvement in altered sensorium forcal and neurological deficit as compare to group A which was **statistically significant.**

8. CSF culture- After 14 days of treatment, CSF culture negativity was more in group B as compared to group A which is statistically significant.

CONCLUSION

1. The following conclusions were drawn from the study
2. Study involved 84 patients equally matched for most of the confounding factor like age, CD4 count and duration ART.
3. Maximum number of patients with cryptococcal meningitis with HIV positive in an age group of 31-40 years.
4. Duration of hospital stay was reduced with combination therapy. Amphotericine B+fluconazole compared to amphotericine B alone.
5. In study improvement in altered sensorium and focal neurological deficit with combination therapy amophotericine B+ fluconazole better than amphotericine B alone.
6. Study shows that amphotericine B + fluconazole was the most rapidly fungicidal combination in CSF, compared with amphotericine B alone.

Above study signifies combination therapy improves morbidity in short term evaluation and thereby can reduce cost and complications of prolonged bed ridden state and extended immobilisation without any additional side effects associated with combination therapy.

REFERENCES

1. Global statistics/HIV:gov
2. Park Bj, Wannemuehler KA, Marston BJ, et al. Estimation of the current burden of cryptococcal meningitis among persons living with HIV/AIDS 2009; 23:525
3. Desalermos A, Kourkoumpetis Tk, mylonakis E. Update on the epidemiology and management of cryptococcal meningitis. Expert opinion pharmacother 2012;13:783
4. Kwon KJ- Drung: bennet John E mycology Lea and febiser 1992,398-439
5. kauffmanL, BlumerS, cryptococcosis: Awakening of giant in the black and white yeats. Proceedings of the 4th international conference and science Publication 1978: p 176-184
6. Drouhet L, overview of fungal antigens. In: Drouhet E, Cole GT, d Rependigny L, Latg JP, Doupon B ,eds. Fungalantigen: isolation, purification and detection, new yrk: plenum press. 1988;1-36
7. Wadia RS pujari SN, Kothari S, Udhar M, kulkarni s, Bhagat S, nanivadeka a. Neurological manifestations of HIV disease: J assoc physicians India 2001;49: 343-8
8. Kumarasamy N, Solomon S, Flanigan Tp, HemlathaR, Thyagarajan SP, Mayer Kh. Natural history of HIV disease in south india: lin inf Dis 2003: 36:79-85
9. Vajpayee M, kanswal S, seth P, wig N. Spectrum of opportunistic infections and profile of D4 cell count among AIDS patients in northern India.
10. Horst CM, saag NS, cloud GA, et al. Treatmnent of cryptococcal meningitis associated with the aids. National institute of allergy and infectious diseases mycoses study group and AIDS clinical trials Group. N Engl. J med.1997; 337: 15-21
11. Saag Ms, gaybbill RJ, LarsenR A etal. Practise guidelines for the management of cryptococcal disease. Infectious disease society of America. Clin infect dis 2000:-0-710-8
12. Baginski, M; Czub, J(2009). Amphoterecin B and its new derivatives- mode of action.
13. Laniado- Laborin R. And abrales- Vargas MN. Amphotericin B: side effects and toxicity. Revista Iberoamericana de micologia 2009(223-7)