STUDY OF CRYPTOCOCCAL MENINGITIS IN HIV POSITIVE PATIENTS AT VIMS, BELLARY.

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ABSTRACT: - BACKGROUND AND OBJECTIVES: Cryptococcal meningitis is a common opportunistic infection and AIDS defining illness in patients with late stage HIV infection, particularly in Southeast Asia and Southern and East Africa. With the increase in the incidence of HIV infection, there is an increase in incidence of cryptococcal meningitis.

Since Vijayanagar institute of medical sciences hospital is a referral center for HIV seropositive patients, the present study was taken up to study the various manifestations of cryptococcal meningitis with respect to its clinical manifestations, diagnostic features, and response to therapy. **MATERIAL AND METHODS:** All patients who presented with features suggestive of meningitis and a low CD4 count were initially screened for cryptococcal meningitis. 30 HIV seropositive patients who were diagnosed to have Cryptococcal meningitis based on CSF culture of *Cryptococcus neoformans* were included in the study. **RESULTS:** Prevalence of cryptococcal meningitis was more in males and most of the patients (75.66%) were in the age group of 26-40 years. Headache (90%), vomiting (80%), and fever (70%) were the most common presenting symptoms. Mean CD4 Count was 72.33 ± 5.32 (range 10-200). Presenting symptoms in cryptococcal meningitis and mixed infection were similar. Frank meningeal signs, raised CSF proteins, and CSF pleocytosis were significantly more in patients with mixed infection. Altered sensorium, raised CSF cell count, CD4 count of ≤ 50 , papilledema, and presence of meningeal signs was associated with poor prognosis. **CONCLUSION:** In half of the patients, cryptococcal meningitis was the AIDS defining illness. Majority of the patients had an acute to subacute presentation and India ink negativity does not rule out cryptococcal meningitis. Response to therapy with Amphotericin B and Fluconazole is well tolerated with a good recovery.

KEY WORDS: Cryptococcal meningitis; Opportunistic infection; AIDS; HIV; *Cryptococcus neoformans*; Neuro infection; Amphotericin B.

INTRODUCTION: Cryptococcosis is an infection caused by the yeastlike fungus, *Cryptococcus neoformans*. Fever and headache in a patient with AIDS or with risk factors for HIV infection suggest the possibility of cryptococcosis.¹

Cryptococcal meningitis is a common opportunistic infection and AIDS defining illness in patients with late stage HIV infection, particularly in Southeast Asia and Southern and East Africa.^{1,2,3} With the increase in the incidence of HIV infection, there is an increase in incidence of cryptococcal meningitis.⁴ Cryptococcal *neoformans* is the leading cause of meningitis in patients with AIDS.⁵

Cryptococcal meningitis also occurs in patients with other forms of immunosuppression and in apparently immunocompetent individuals. Cryptococcal meningitis has been reported as the most common opportunistic infection of the CNS of Indian patients with HIV.^{6,7,8} Cryptococcal meningitis is now the leading cause of community acquired meningitis, accounting for 20-45% of laboratory confirmed cases of meningitis in Southern Africa.⁹

It is the initial AIDS defining illness in ~2% of patients and generally occurs in patients with CD4 counts <100/ μ l.^{10,11} Most of the patients have an acute to subacute presentation. Cryptococcosis in AIDS patients is notable for the relative paucity of symptoms and signs, even in severe disease. Headache is present in ~90% of cases and fever in ~75%. Prostate gland may serve as a reservoir for smoldering cryptococcal infection¹. Lumbar puncture is the single most useful diagnostic test

Mortality from HIV associated cryptococcal meningitis remains high (10–30%), even in developed countries, because of the inadequacy of current antifungal drugs and combinations, and the complication of raised intracranial pressure.¹²

In the developing world, patients tend to present later, flucytosine is often not available and there may not be the resources or facilities for inpatient intravenous therapy with amphotericin B. The result of this combination of factors is that in cohorts of HIV-infected patients from sub-Saharan Africa, cryptococcosis has accounted for 13–44% of all deaths.¹³

However the expansion of antiretroviral programmes now raises the prospect of transforming the long term prognosis of these patients, provided that they survive the acute phase of the illness.¹⁰

Since Vijayanagar institute of medical sciences hospital is a referral center for HIV seropositive patients after the establishment of antiretroviral therapy center. The number of patients being referred with HIV/AIDS has increased considerably and more and more cases of cryptococcal meningitis are diagnosed.

Hence the present study is taken up to study the various manifestations of cryptococcal meningitis with respect to its clinical manifestations, diagnostic features, and response to therapy.

AIMS AND OBJECTIVES: The present study is undertaken to study Cryptococcal meningitis in HIV seropositive subjects with respect to-

1. Clinical course
2. Laboratory findings like-
 - CSF profile
 - Radiological profile
 - CD4 count
3. Response to treatment.

4. Prognostic factors.

MATERIAL AND METHODS: - SOURCE OF DATA: 30 patients of Cryptococcal meningitis who were HIV seropositive were studied prospectively at Vijayanagar institute of medical sciences. The study was done over a period of 1 year.

METHOD OF COLLECTION OF DATA: 30 patients of Cryptococcal meningitis who were HIV seropositive were included in the study. A detailed clinical evaluation (history and examination) and relevant laboratory investigations were done for all subjects as per the proforma.

Inclusion criteria: All patients who presented with features suggestive of meningitis and a low CD4 count were initially screened for cryptococcal meningitis. All the HIV seropositive patients who were diagnosed to have Cryptococcal meningitis based on CSF culture of *Cryptococcus neoformans* were included in the study.

EXCLUSION CRITERIA:

1. Cryptococcal meningitis in patients who are not HIV seropositive.
2. Children < 12 years of age.

INVESTIGATIONS DONE:

1. ELISA test for HIV
2. CD4 count was determined by BD FACS Count system.
3. CSF analysis for
 - a. Proteins and sugar
 - b. Cell count and cell type
 - c. India ink smear
 - d. Cryptococcal antigen
 - e. Cryptococcal culture
4. CT Brain
5. Renal function tests
6. Blood culture (optional)
7. Chest X-ray

STATISTICAL METHODS: Chi-square and Fisher exact test have been used to test the significance of study parameters between Group A and Group B. Odds Ratio has been used to find the strength of relationship between study parameters and the groups. Student t test (independent samples) has been used to find the significance of investigations between the two groups.

1. Chi-Square Test

$$\chi^2 = \frac{\sum (O_i - E_i)^2}{E_i}, \text{ Where } O_i \text{ is observed frequency and } E_i \text{ is Expected frequency}$$

2. Fisher Exact Test

	Class1	Class2	Total
Sample1	A	B	a+b

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Sample2	C	D	c+d
Total	a+c	b+d	N

$$\text{Fisher Exact Test statistic} = \sum p = \frac{(a+b)(c+d)(a+c)(b+d)}{n} \frac{1}{\sum abcd}$$

3. Odds Ratio OR=ad/bc

4. Student t test (Independent)

Objective: To investigate the significance between the means of two populations

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{s^2 (1/n_1 + 1/n_2)}}$$

$$\text{Where } s^2 = \frac{(n_1 - 1) \sum_{i=1}^{n_1} (x_1 - \bar{x}_1)^2 + (n_2 - 1) \sum_{i=1}^{n_2} (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

STATISTICAL SOFTWARE: The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS AND OBSERVATIONS: Results are as shown in the tables

DISCUSSION: - AGE: In the present study the mean age of patients was 33.55 years, Most of the patients (76.66%) were in the age group between 26 and 40 years.. This is in accordance with the study done by Imwidthaya P et al,²⁰ where the mean age of patients was 32.1 years.

Sex: In the present study, the number of males was more as compared to the number of females (25 Vs 5). In the study done by Imwidthaya P et al,²⁰ 80.46% of the patients were males.

Duration of HIV seropositive status: Cryptococcal meningitis was the first manifestation of AIDS in 46.66% of the patients. This finding is similar to the study done by Chuck SL et al,¹⁴ wherein cryptococcal meningitis was AIDS defining illness in 45.28% of the patients..

Presenting symptoms: In the present study headache, vomiting and fever were the most common presenting symptoms. In the study done by Woldemanuel Y et al ,²² fever and headache were the most common presenting symptoms.

TABLE 15: COMPARISON OF PRESENTING SYMPTOMS WITH OTHER STUDIES

Presenting symptoms	Chuck SL et al ¹⁴ (n=106)	Martinez Fernandez EM et al ²¹ (n=25)	Darras-Joly C et al ²³ (n=65)	Kalra SP et al ²⁴ (n=15)	Present study (n=30)
Headache	73%	88%	67%	80%	90%
Vomiting	42%	-	-	-	80%
Fever	45%	68%	86%	86.6%	70%
Altered sensorium	28%	-	29%	26.6%	30%
Seizures	-	20%	-	-	10%

MENINGEAL SIGNS: In the present study 83.33% of the patients had meningeal signs. Frank meningeal signs were present only in 33.33% of the patients. This is in accordance with the study done by Mwaba P et al,¹⁶ where 85% of the patients had meningeal signs. Maher D et al,²⁵ noted that 74% of the patients with cryptococcal meningitis had meningeal signs.

CSF INDIA INK, CRYPTOCCAL ANTIGEN, And CRYPTOCCAL CULTURE POSITIVITY: CSF India ink was positive in 90% of the patients. In all the 30 patients, CSF cryptococcal antigen and CSF cryptococcal culture was positive.

TABLE 16: COMPARISION OF CSF INDIA INK, CRYPTOCCAL ANTIGEN, AND CRYPTOCCAL CULTURE POSITIVITY WITH OTHER STUDIES

	Imwidthaya P et al ²⁰ (n=87)	Darras-Joly C et al ²³ (n=65)	Khanna N et al ²⁵ (n=87)	Present study (n=30)
CSF India Ink positivity	91%	87%	87.36%	90%
CSF Cryptococcal antigen positivity	100%	92%	98.81%	100%
CSF Cryptococcal culture positivity	100%	100%	100%	100%

India ink preparation is a simple procedure and it is used as a screening procedure in cases of cryptococcal meningitis. The positivity in the present study with respect to India ink preparation is similar to studies done by Imwidthaya P et al,²⁰Darras-Joly C et al,²³ and Khanna N et al.²⁶

The CSF culture for *Cryptococcus neoformans* is diagnostic test, hence all the studies cited above have 100% positivity. There is a high correlation between the culture positivity and the presence of cryptococcal antigen in the CSF. Present study correlates with study done by Imwidthaya P et al,²⁰ whereas in the study done by Darras-Joly C et al²¹ the cryptococcal antigen positivity was less compared to the present study.

CSF GLUCOSE LEVELS: In the present study the mean CSF glucose level was 45.63mg/dl and 53.32% of the patients had a CSF glucose level of <40mg/dl. In the study done by Hakim et al,⁹52.7% of the patients had a CSF glucose level of <40mg/dl. Because of minimal cellular inflammation of meninges, CSF glucose levels are not grossly decreased. This correlates with other studies.

CSF PROTEIN LEVELS: In the present study the mean CSF protein level was 106.33mg/dl and 93.34% of the patients had a CSF protein level of >40mg/dl. This is in accordance with the study

done by Hakim et al,⁹ where 80.6% of the patients had a CSF protein level of >40mg/dl. Mildly raised CSF proteins is a common finding in patients with cryptococcal meningitis

CD4 COUNT: In the present study mean CD4 Count was 72.33. In the study done by Imwidthaya P et al,²⁰ mean CD4 Count was 45. In another study done by Darras-Joly C et al,²³ mean CD4 Count was 46. In the present study, all the 30 patients (100%) had a CD4 count of <200. Martinez Fernandez EM et al²¹ noted that 84% of the patients had a CD4 count of <200. 56.66% of the patients in the present study had a CD4 count of <50. In the study done by Bava AJ et al,²⁰ 83.63% of the patients had a CD4 count of <50. This is in accordance with the observation that cryptococcal meningitis presents in late stage of HIV infection; and lower the CD4 count, higher the occurrence of cryptococcal meningitis.

MORTALITY: In the present study mortality was 33.33%. In the study done by Khanna N et al,²⁶ mortality was 32.18%. Mortality was 36.7% in the study done by Metta HA et al.²⁷ Mortality in cryptococcal meningitis is high and it is mainly due to the immunodeficiency seen in the patients. In the present study the mortality was slightly less compared to the study done by Khanna N et al²⁶ and Metta HA et al.⁶¹ As all the patients were put on Amphotericin B and fluconazole, there is a better prognosis seen in present study compared to the above two studies.

POOR PROGNOSTIC FACTORS: In the present study the poor prognostic factors included altered sensorium, raised CSF cell count, CD4 count of ≤ 50 , papilledema, and presence of meningeal signs. Altered sensorium was a significant predictor of bad prognosis (death). Altered sensorium was 14.28 times more likely to predict death. This is concordance with various other studies which have shown that altered sensorium is associated with poor prognosis.^{15,17,18} Raised CSF cell count (>5 cells) was 3.58 times more likely to predict death. A CD4 count of ≤ 50 was 3.40 times more likely to predict death. This is in concordance with study done by Robinson PA et al.¹⁹

SUMMARY: The present study was undertaken to study the Clinical course, Laboratory findings, outcome, and prognostic factors in HIV seropositive patients with Cryptococcal meningitis. For this, 30 HIV seropositive patients with cryptococcal meningitis were randomly selected.

Review of literature regarding cryptococcal meningitis in HIV positive patients was done. Present study was viewed in comparison with other national and international studies.

The salient features of this study are-

1. Prevalence of cryptococcal meningitis was more in males.
2. Most of the patients were in the age group of 26-40 years.
3. Majority of patients had an acute to subacute presentation.
4. In half of the patients, cryptococcal meningitis was the first manifestation of AIDS.
5. Headache, vomiting, and fever were the most common presenting symptoms.
6. CSF India Ink was positive in 90% of the patients only.
7. Most of the patients had a CD4 count of <100/ μ l.
8. Disseminated Cryptococemia was not common.
9. Presenting symptoms in cryptococcal meningitis and mixed infection were similar.
10. Frank meningeal signs were significantly more in patients with mixed infection.
11. Altered sensorium, raised CSF cell count, CD4 count of ≤ 50 , papilledema, and

12. Presence of meningeal signs was associated with poor prognosis.
13. Reinfection was seen in 4.29% of the patients.
14. Cryptococcal meningitis due to Immune reconstitution syndrome was seen in 5.71% of the patients.
15. Response to therapy with Amphotericin B and Fluconazole was good and recovery was seen in 71.4% of the patients.
16. All survivors were subsequently started on HAART.

Statistical analysis was done as per research guidelines. Vancouver format was used in writing bibliography. Master chart reveals all patient data.

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RESULTS AND OBSERVATIONS: TABLE 1: AGE DISTRIBUTION

Age in years	Number of patients
20-25	4(13.33%)
26-30	6(20%)
31-35	10(33.33%)
36-40	7(23.33%)
41-45	2(6.66%)
46-50	1(3.33%)
Total	30
Mean $\pm$ SD	33.55 $\pm$ 7.002

TABLE 2: GENDER DISTRIBUTION

Gender	Number of patients (n=30)
Male	25(83.0%)
Female	5(17%)

TABLE 3: DURATION OF HIV POSITIVE STATUS

HIV positive status since	Number of patients (n=30)
Freshly detected	14(46.66%)
<6 months	7(23.33%)
6-12 months	4(13.33%)
> 12 months	5(16.66%)

TABLE 4: PRESENTING SYMPTOMS

Symptoms	Number of patients (n=30)
Headache	27(90%)
Vomiting	24(80%)
Fever	21(70%)
Altered sensorium	9(30%)
Seizures	3(10%)

TABLE 5: DURATION OF NEUROLOGICAL SYMPTOMS

Duration of symptoms in days	Number of patients (n=30)
Up to 15	14(46.66%)
16-30	7(23.33%)
30-60	6(20%)
>60	3(10%)

TABLE 6: LEVEL OF CONSCIOUSNESS

Consciousness	Number of patients (n=30)
Conscious	20(66.66%)
Drowsy	6(20%)
Comatose	4(13.33%)

TABLE 7: MENINGEAL SIGNS

Meningeal signs	Number of patients (n=30)
Absent	5(16.66%)
Terminal neck stiffness	15(50%)
Frank meningeal signs	10(33.33%)

TABLE 8: PERCENTAGE OF CSF INDIA INK, CSF CRYPTOCOCCAL ANTIGEN AND CSF CRYPTOCOCCAL CULTURE POSITIVITY

CSF findings	Number of patients (n=30)
CSF India Ink positivity	27 (90.0%)
CSF Cryptococcal antigen positivity	30 (100.0%)
CSF Cryptococcal culture positivity	30 (100.0%)

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TABLE 9: CSF GLUCOSE LEVELS

Glucose levels in mg/dl	Number of patients (n=30)
0-20	2(6.66%)
21-40	14(46.66%)
41-60	10(33.33%)
>60	4(13.33%)
Mean	45.63

TABLE 10: CRYPTOCOCCAL BLOOD CULTURE AND BLOOD CRYPTOCOCCAL ANTIGEN POSITIVITY

Blood test	Number of patients (n=30)
Blood Cryptococcal antigen positivity	18 (60.0%)
Cryptococcal Blood Culture positivity	10 (33.33%)

TABLE 10: CD4 COUNT

CD4 count	Number of patients (n=30)
0-50	17(56.66%)
51-100	8(26.66%)
101-200	5(16.66%)
Mean	72.33

TABLE 11: CT BRAIN

CT brain	Number of patients (n=30)
Normal	10(33.33%)
Diffuse cerebral atrophy	13(43.33%)
Diffuse cerebral atrophy with ventricular dilation	7(23.33%)

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TABLE 12: OUTCOME

Outcome	Number of patients (n=30)
Dead	10(33.33%)
Alive	20(66.66%)

TABLE 13: RISK FACTORS ASSOCIATED WITH DEATH

Risk factors	Improved (n=20)		Dead (n=10)	
	Number	%	Number	%
Duration of neurological symptoms (<7 days)	4	20	3	30
Altered sensorium	2	10	7	70.0
Presence of meningeal signs	16	80	9	90
Presence of Papilledema	5	25	3	30
CSF protein (>40mg/dl)	19	95	9	90
CSF cell count (>5)	13	65	7	70
CD4 count $\leq$ 50	10	50.0	7	70
Abnormal CT brain	13	65	7	70.0

TABLE 14: ANALYSIS OF MULTIVARIATE LOGISTIC REGRESSION TO FIND THE EFFECT OF POOR PROGNOSTIC FACTORS FOR PREDICTING THE DEATH (N=30)

Prognostic factors	Logistic efficient	co-Standard Error	Wald	P value	Adj. OR
Duration of neurological symptoms (<7 days)	-0.01	0.98	0.00	0.989	0.99
Altered sensorium	2.66	0.76	12.13	<0.001	14.28
Presence of meningeal signs	0.23	1.22	0.03	0.853	1.25
Presence of Papilledema	0.29	0.92	0.10	0.752	1.34
CSF protein (>40mg/dl)	-0.60	0.94	0.40	0.528	0.55
CSF cell count (>5)	1.28	0.89	2.05	0.152	3.58
CD4 count $\leq$ 50	1.22	0.71	2.97	0.085	3.40
Abnormal CT brain	-1.04	0.80	1.67	0.196	0.35