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Demographic Profile and Clinical Correlation with CD-4 Count in HIV Positive Cryptococcal Meningitis Patients with Comparative Evaluation of Diagnostic Modalities- A Study Done in a Tertiary Level Hospital of Western Rajasthan

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Abstract: This prospective study was conducted in a tertiary level hospital of western Rajasthan, in study duration 48 hospitalised, seropositive patients having CNS manifestation were diagnosed as having co-infection with Cryptococcal meningitis were included in study. There was preponderance of infection in rural, males majority were Labourers, Drivers or Migrant Workers, headache was their main complain. CSF examination by Indian Ink (43 positive), Latex Agglutination for Cryptococcal antigen detection (by Calas) (48) &culture was done on Niger Seed Agar (48) CD-4 Count was done in ICTC BY Fax –Cali bur (In majority was <50) . In study time there was mortality in 12 patients (25%) patients, out of 9 (75%) died within first week of diagnosis & remaining in second week. So there is significant role of early prompt diagnosis &proper management with antifungal & antiretroviral treatment.
Keywords: ART, CD-4 Count, AIDS,HIV, Cryptococcal meningitis.

INTRODUCTION

Cryptococcosis, one of the AIDS defining infections, once considered as "sleeping disease", became an "awakening giant" [1] within a couple of years, and has now been predicted as the "Mycosis of the future" with a predilection that for every million patients with AIDS, 50,000-100,000 will contract cryptococcosis [2,3]. Chronic meningitis is a main manifestation of this infection. Reports of it, from various states of India have increased more in the AIDS era [4]. However, a review in 2007, on the status of cryptococcosis in India strangely reveals more cases from the Northern part, where the HIV prevalence rate is low compared to high HIV prevalent states in the Southern or Western India [5].

Since then the overall scenario has not changed much [6]. This discrepancy probably is due to under reporting and misdiagnosis of cases. The most serious infections usually develop in patients with defective cell-mediated immunity [7]. Cryptococcosis is a defining opportunistic infection for AIDS. Other conditions which pose an increased risk include certain lymphomas (e.g. Hodgkin's lymphoma), sarcoidosis, and patients on long-term corticosteroid therapy [8].

The host response to cryptococcal infection includes both cellular and humoral components. Animal models demonstrate that natural killer cells participate in the early killing of cryptococci and, possibly, antibody-dependent cell-mediated killing [9]. In vitro monocyte-derived macrophages, natural killer cells, and T lymphocytes can inhibit or kill cryptococci. A successful host response includes an increase in helper T-cell activity, skin test conversion, and a reduction in the number of viable organisms in the tissues. In addition to cellular mechanisms, anticryptococcal antibodies and soluble anticryptococcal factors have been described [10]. Antibodies to cryptococcal antigens play a critical role in enhancing the macrophage- and lymphocyte-mediated immune response to the organism. *C. neoformans* infection is usually characterized by little or no necrosis or organ dysfunction until late in the disease [11]. Organ damage may be accelerated in persons with heavy infections. The lack of identifiable

endotoxins or exotoxins may be partly responsible for the absence of extensive necrosis early in cryptococcal infections. Organ damage is primarily due to tissue distortion secondary to the expanding fungal burden. Extensive inflammation or fibrosis is rare [12]. The characteristic lesion of *C. neoformans* consists of a cystic cluster of yeast with no well-defined inflammatory response. Well-formed granulomas are generally absent. *C.neoformans* can cause an asymptomatic pulmonary infection followed later by the development of meningitis, which is often the first indication of disease [13]. If limited to the lungs, *C.neoformans* infection may cause pneumonia, poorly defined mass lesions, pulmonary nodules and rarely pleural effusion[14]. Although immune defects are common in patients with meningitis or disseminated infection, patients with disease that is confined to the lungs are usually immunocompetent [15].

MATERIALS & METHODS

Present study was done in hospitalised patients in a tertiary level hospital in which all HIV positive patients presenting with various neurological manifestations like headache, altered Sensorium, Neck Stiffness, Focal Neurological deficit were included in study. The cases will be diagnosed as cryptococcal meningitis according to the case histories, the clinical manifestations, the physical signs, and the laboratory evaluations. The laboratory diagnosis criteria were a positive cerebrospinal fluid (CSF) India ink stain or a positive culture for the organism in addition to a positive latex agglutination test for the cryptococcal capsular antigen. The fungal culture was done on selective medium at 37°C for 4 weeks and antimycotic drug sensitivity testing was done by disc diffusion technique using CLSI Guidelines.

DATA EVALUATION

In prospective observational study, the data was analyzed by Epi-info statistical and multivariate analysis method used. Statistical analysis of data was done using Chi-square test and a P -value <0.05 was considered statistically significant.

OBSERVATIONS & RESULTS

In present study 48 patients (39 Male & 9 Female) were having coinfection of HIV & Cryptococcal meningitic, were hospitalised and evaluated There was a high incidence of disease among males. Almost 81% of the sample was male as opposed to 19% females. Prevalence of disease was highest among 31-40 age group in both males and females. In study 62.50% (30 out of 48) were rural and 37.50% (18 out of 48) were urban HIV patient. Rural areas were affected more than urban areas overall. Patients were distributed relatively evenly among various occupational groups. 9 Labourers & Drivers each, 8 House Wives, 7 Migrant Workers, 5 Businessmen, 5 Farmers, 3 Teachers and 2 Students were found to be infected with HIV cryptococcal meningitis. Out of 48 Cryptococcal meningitis patients 41% patients presented with headache, 35% with fever, 26% Nausea, 29% Vomiting, 30% Neck stiffness, 13% Seizure, 13% Loss of consciousness, 13% Altered Sensorium, 6% Cranial Nerve Palsy, 6% skin lesion, 5% hearing loss, 2% memory loss and 1% with photophobia. Headache and fever were the most common symptom in cryptococcal meningitis. Out of 48 patients 23 (47.92%) had less than 50 CD4 cell count, 17 (35.42%) patients had CD4 cell count in between 51-100, 7 (14.58%) patients had CD4 cell count in between 100-200 and 1 (2.08%) patient had greater than 200 (but less than 300) CD4 cell count. Cryptococcal infection was found to be more common among those patients who had less than 50 CD4 cell count. Only 6 out of total 48 Cryptococcal Meningitis patients (12.50%) showed Papilloedema All 48 (100%) CSF samples were found positive for Cryptococcal Antigen Test & CSF Culture for Cryptococcus while 43 samples were (89.50%) positive for India ink. So Cryptococcal Antigen Test and CSF Culture for Cryptococcus showed 100% sensitivity to towards Cryptococcus. One-fourth (12 patients) of the total population had died because of the disease under study. Out of those twelve patients, nine died in first week and the rest passed away in the second week. p value less than 0.05 shows that these symptoms and laboratory tests were highly significant in determining the outcome. Patients presented with Altered Sensorium, loss of consciousness, Cranial Nerve Palsy and CD4 cell count of less than 100 met with fatal outcome. Similarly, Laboratory and Imaging Diagnosis Cryptococcal Antigen test (>3+reactive), Fundus Examination (Papilloedema) and CT/MRI Brain Abnormal Imaging showed poor outcome too.

Table-1: CD4 Cell Count Distribution in Cryptococcal Meningitis Patients

	No. of Patient (n=48)	Percentage
<50	23	47.92
51-100	17	35.42
100-200	7	14.58
200-300	1	2.08
>300	0	0.00
Total	48	

Table-2: Symptoms distribution among study population

Symptoms	No. of Patient n=48	Percentage
Headache	41	85.42
Fever	35	72.92
Neck Stiffness	30	62.50
Vomiting	29	60.42
Nausea	26	54.17
Seizure	13	27.08
Altered Sensorium	13	27.08
Loss of Consciousness	13	27.08
Cranial Nerve Palsy	6	12.50
Skin Lesion	6	12.50
Hearing Loss	5	10.42
Memory Loss	2	4.17
Photophobia	1	2.08

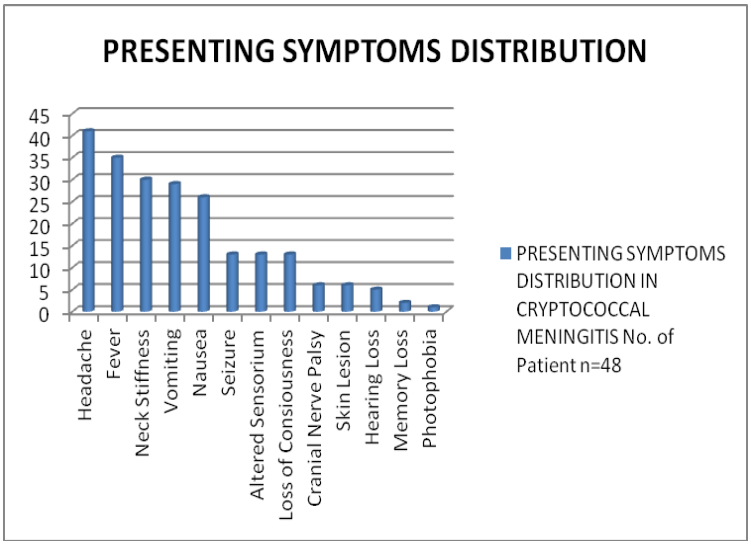

Fig-1: Presenting symptoms distribution

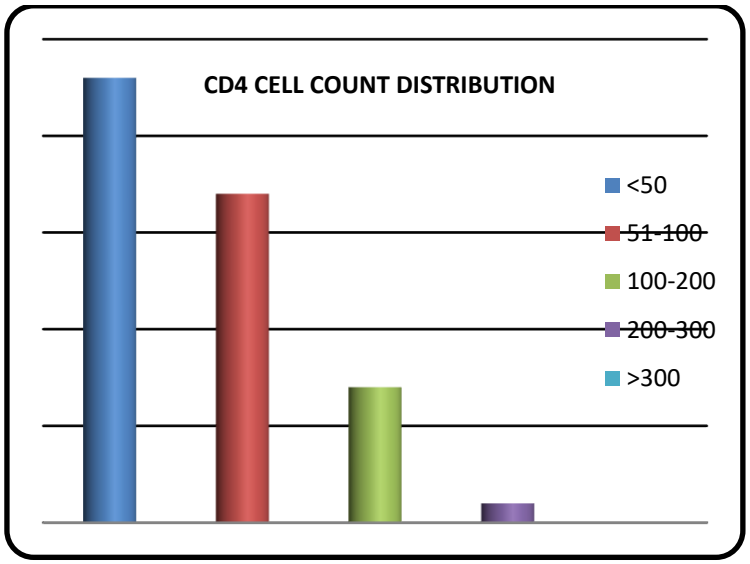

Fig-2: CD-4 Count distribution

Table-3:- CSF examination distribution

CSF EXAM.		No. of Patient (n=48)
India Ink(Yeast cells)		43
ZN Stain (AFB)		2
Cryptococcal Antigen Test (Reactive)	+	20
	++	17
	≥+3	11
CSF Culture for Cryptococcus		48

Table-4: Outcome in cryptococcal meningitis

	Death n=12(%)	Alive n=36 (%)	P = Value
Fever	7 (58.33)	26 (72.22)	0.58
Headache	9 (75.33)	32 (88.89)	0.47
Vomiting	6 (50.00)	23 (63.89)	0.60
Neck Stiffness	7 (58.33)	23 (63.89)	0.73
Seizure	5 (41.67)	8 (22.22)	0.34
Altered Sensorium	10 (83.33)	8 (22.22)	0.0006
Hearing Loss	1 (8.33)	4 (11.11)	0.78
Cranial nerve Palsy	4 (33.33)	2 (5.56)	0.04
Skin Lesion	3 (25.00)	3 (8.33)	0.31
Memory Loss	1 (8.33)	1 (2.78)	0.40
Loss of Consciousness	10 (83.33)	7 (19.44)	0.0003
CD4 Cell Count (<100)	11 (91.67)	20 (55.56)	0.04
India Ink	11 (91.67)	32 (88.89)	0.78
Cryptococcal Antigen Test (>3+ reactive)	12 (100.00)	2 (5.56)	0.0001
Fundus Exam. (Papilledema)	6 (50.00)	1 (2.78)	0.0004
CT &MRI Head Abnormality	10 (83.33)	2 (5.56)	0.0001
TB co-infection	1 (8.33)	1 (2.78)	0.40

DISCUSSION

This is a prospective study of 48 confirmed cryptococcal HIV positive cases admitted in a tertiary level hospital and an attempt was made to ascertain the clinical spectrum of all HIV cryptococcal meningitis cases, their diagnosis and comparison between various diagnostic method and outcome of the treated patients. In our study 81.25% (39 out of 48) patients are male and 18.75% (9 out of 48) are female HIV patients. HIV patients are more common in 31-40 age groups, both among males and females. In this study 62.50% (30 out of 48) are rural and 37.50% (18 out of 48) are urban HIV patient. It shows more HIV patients distribution in rural areas. In this study, out of 48 patients, there were 9 Labourers and Drivers, 8 House wives, 7 Migrant Workers, 5 Agriculture and Businessmen, 3 Teachers and 2 Students. It shows HIV infection more common in migrant worker and illiterate people. In 1993, the US Centres for Disease Control and Prevention reported that patients with AIDS-associated cryptococcal infections now account for 80%-90% of all the patients with Cryptococcosis. The prospective multicenter study CryptoA/D in France (1997–2001) also found that among cryptococcal infection in HIV patients 77% were male while 23% were female. Based on culture results at baseline, Cryptococcosis was more severe in men HIV-positive patients and in patients infected with serotype A. Prevalence of CNS cryptococcosis was observed in 33 men (84.62%) and 6 women (15.38%). The incidence was highest among persons 20 to 40 age category (92.31%) with a mean age of 31 years. In this study, out of 48 Cryptococcal meningitis patients 41% patients presented with headache, 35% with fever, 26% Nausea, 29% Vomiting, 30% Neck stiffness, 13% Seizure, 13% Loss of consciousness, 13% Altered Sensorium, 6% Cranial Nerve Palsy, 6% skin lesion, 5% hearing loss, 2% memory loss and 1% with photophobia. In C. neoformans meningitis headache was the most common symptom.

In Kumar S, Wanchu A *et al.* PGIMER, Chandigarh, India [22] study, Fourty patients were diagnosed with cryptococcal meningitis. Thirty-six (90%) patients presented with headache and eighteen (45%) had altered Sensorium [16].

In our study out of 48 Cryptococcal meningitis patients, 23 (47.92%) have less than 50 CD4 cell count, 17 (35.42%) patients have CD4 cell count in between 51-100, 7 (14.58%) patients' CD4 cell count lies in between 100-200 and 1 (2.08%) patient have 206 CD4 cell count. It shows that cryptococcal infection in HIV positive patient is more common when CD4 cell count is low.

Lakshmi V, Sudha T shows that in CT/MRI showing basilar inflammation and frequent alterations and Cryptococcosis, prevalence increased with declining CD4 $^{+}$ lymphocyte count with risk being greatest at CD4 $^{+}$ cell count below 50 cells per micro litre [17].

Cryptococcal antigen detection is a highly specific and rapid test, and the antigen can remain detectable for several months after infection [18]. It is therefore a suitable choice of laboratory test for screening.CSF examination distribution in our study population shows that all of the 48 (100%) CSF samples are positive for cryptococcal antigen test and culture for Cryptococcus and 43 (90%) of them tested positive with India ink. So, cryptococcal antigen detection and CSF culture are highly specific and rapid tests for early diagnosis.[19] Brouwer, A. E., P. Teparrukkul shows that cerebrospinal fluid India ink test (82%), culture (100%), and cryptococcal antigen (100%) are usually positive [20].

Hakim JG, Gangaidzo IT, Heyderman RS et al argue that all patients suspected to have meningitis had a high HIV sero-positivity irrespective of whether they were later confirmed to have meningitis or not. CM was the most common type of meningitis seen. In-hospital mortality was high irrespective of the cause of meningitis. Compared to other study in our study population, 25% were dead. 9 patients died in first week while remaining 3 patients died in the second week [21].

Patients presenting with altered Sensorium, loss of consciousness, Cranial nerve palsy and CD4 cell count less than 100 shows more fatal outcome. In laboratory and imaging diagnosis Cryptococcal antigen test (>3+reactive), fundus examination (Papilloedema) and CT/MRI Brain abnormal imaging have poor outcome. All of the aforementioned symptoms and tests have a highly significant p value (less than 0.05). This study highlights the significance of these clinical presentation and diagnostic test in management of cryptococcal meningitis in AIDS patients in our region.

SUMMARY AND CONCLUSIONS

Following conclusions were derived from our study

- The incidence of Cryptococcal meningitis has been found to be common in our region.
- Cryptococcal infection should be suspected in all cases of meningitis among HIV infected persons. Early diagnosis and treatment may alter the prognosis for these patients and hence examination of CSF should be considered in all HIV infected persons with symptoms like Fever, Headache, Vomiting, Neck-stiffness, Altered Sensorium, Cranial Nerve Palsy, Hearing Loss etc. Early deaths were more frequent among patients with abnormal neurological signs like Cranial Nerve Palsy 4/12(33.33%) versus 2/36(5.56%) [p=0.04], Loss of Consciousness 10/12(83.33%) versus 7/36(19.44%) [p=0.0003], abnormal brain imaging 10/12(83.33%) versus 2/36(5.56%) [p=0.0001], and Papilloedema 6/12(50%) versus 1/36(2.78%) [p=0.0004].
- For the cryptococcal meningitis, cryptococcal antigen test and CSF culture are the most specific and sensitive tests.

REFERENCES

1. Banerjee U, Dutta K, Majumdar T, Gupta K. “Cryptococcosis in India: Awakening of a giant?” *Med Mycol* (2001); 39: 51-67
2. Antinori S, Galimberti L, Magni C, Casella A, Vago L, Mainini F, Piazza M, Nebuloni N, Fasan M, Bonaccorso C, Vigevani GM, Cargnel A, Moroni M, Ridolfo AL. “Cryptococcus neoformans infection in a cohort of Italian AIDS patients: natural history, early prognostic parameters, and autopsy findings.” *Eur. J. Clin. Microbiol. Infect. Dis.* (2001); 20: 711-717.
3. Banerjee U, Datta K, Casadavell A. “Seroptype distribution of Cryptococcus neoformans in patients in tertiary care center in India.” *Med Mycol* (2004); 42: 181-186.
4. Busari OA, Qdeyemi AO, Agboola SM. “Cryptococcal meningitis in AIDS.” *Int. J. Infect. Dis.* (2009); 7(1).
5. Banerjee U, Dutta K, Diwedi M, Sethi S. “Cryptococcosis due to C neofromans var gattii: a short review and Indian clinical scenario.” *Nat J Infect Dis* (2001); 2: 32-36.
6. Chakrabarti A, Sharma A, Sood A, Grover R, Sakhuja V, Prabhakar S, Verma S. “Changing scenario of cryptococcosis in a tertiary care hospital in north India.” (2000).
7. Chuck SL, Sande MA. “Infections with Cryptococcus neoformans in the acquired immunodeficiency syndrome.” *N. Engl. J. Med.* (1989); 321:794.
8. Mitchell DH, Sorrell TC, Allworth AM, Heath CH, McGregor AR, Papanaoum K, Richards MJ, Gottlieb T. “Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome.” *Clin. Infect. Dis.* (1995); 20: 611-616.
9. Datta K. “Characterization of Indian clinical isolates of Cryptococcus neoformans and assessment of protective efficacy of anti-cryptococcal antibody in murine model of cryptococcosis.” *Ph D. Thesis* (2002). *All India Institute of Medical Sciences New Delhi*.
10. Manfredi R, Maroni A, Mazzoni A, Nanetti A, Donati M. “Isolated detection of cryptococcal polysaccharide antigen in cerebrospinal fluid samples from patients with AIDS.” *Clin Infect Dis* (1996); 23: 849–850.
11. Zuger A, Lourie E, Holtzman R, Sunberkoff MS, Rahal JJ. “Cryptococcal disease in patients with

the acquired immunodeficiency syndrome: diagnostic features and outcome of treatment." *Ann Intern Med* (1986); 104: 234 -240.

12. Hoang LMN, Maguire JA, Doyle P, Fyfe M, Roscoe DL. "Cryptococcal neoformans infections at Vancouver Hospital and Health Sciences Center (1997–2002)." *Epidemiology, Microbiology and Histopathology* (2004).
13. Mwaba P, Mwansa J, Chintu C. "Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions." *Postgrad Med J* (2001); 77: 769–773.
14. Dromer F, Mathoulin-Pélissier S, Launay O, Lortholary O, the French Cryptococcosis Study Group. "Determinants of Disease Presentation and Outcome during Cryptococcosis: The CryptoA/D Study." *PLoS Med* (2007) 4(2): e21.
15. Cunha, BA. "Central nervous system infections in the compromised host: a diagnostic approach." (2001).
16. Kumar S, Wanchu A, Chakrabarti A, Sharma A, Bambery P, Singh S. "Cryptococcal meningitis in HIV infected: Experience from a North Indian tertiary center." *Neurol India* (2008); 56: 444-449.
17. Lakshmi V, Sudha T, Teja VD, Umabala P. "Prevalence of central nervous system cryptococcosis in human immunodeficiency virus reactive hospitalized patients." *Indian J Med Microbiol.* (2007); 25: 146-149.
18. Lu H, Zhou Y, Yin Y, Pan X, Weng X. "Cryptococcal antigen test revisited: significance for cryptococcal meningitis therapy monitoring in a tertiary Chinese hospital." *J Clin Microbiol* (2005); 43: 2989–2990.
19. Powderly WG, Cloud GA, Dismukes WE, Saag MS. "Measurement of cryptococcal antigen in serum and cerebrospinal fluid: value in the management of AIDS-associated cryptococcal meningitis." *Clin. Infect. Dis.* (1994); 18: 789-792.
20. Brouwer AE, Teparrukkul P, Pinpraphapora S, Larsen RA, Chierakul W, Peacock S, Duy N, White NJ, Harrison TS. "Baseline correlation and comparative kinetics of cerebrospinal fluid colony-forming unit counts and antigen titers in cryptococcal meningitis." *J. Infect. Dis.* (2005); 192: 681-684.
21. Hakim JG, Gangaidzo IT, Heyderman RS, Mielke J, Mushangi E, Taziwa A, Robertson VJ, Musvaire P, Mason PR. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. Aids. 2000 Jul 7;14(10):1401-7.