Cryptococcal Meningitis : A Clinicopathological Account of Seven Cases Encountered in a Military Setting

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Abstract

The clinicopathological profile of 7 cases of *Cryptococcal* meningitis encountered over one year period in a military hospital has been described. Severe persistent headache was the main form of presentation followed by features of progressive ill-health. Age range of cases was 34-55 years (mean 40.8 years). All were males and 6 were serving soldiers. Duration of symptoms ranged from 10 days to 6 weeks. Six patients were positive for HIV-1 antibodies. This was the initial presentation of AIDS in 6 patients accounting for 10.34% among all cases of HIV infection during that year. Both clinical and laboratory findings were subtle. Nuchal rigidity was rare, cerebro spinal fluid (CSF) cell count and protein level was normal in 3 out of 7 cases. Although culture for *Cryptococcus* was positive in all, it took 10 days to grow in 2 cases. India-ink stain showed scanty number of organisms in 3 cases. Low cell counts in CSF and presence of associated hyponatremia appear to be bad prognostic indicators in *Cryptococcal* infections of central nervous system. Latex agglutination test is a worthwhile screening test.

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Key Words : *Cryptococcal* meningitis; *Cryptococcus neoformans*; Opportunistic infection

Introduction

Cryptococcal meningitis is the commonest life threatening opportunistic fungal infection in patients afflicted by the human immunodeficiency virus [1-8]. About 6% - 10% of patients with AIDS have *cryptococcal* meningitis as the first AIDS defining illness [1,2,7,9]. However, the condition is known to occur in other immunocompromised conditions [10,11,12] as also in immunocompetent individuals [13]

Cryptococcus neoformans, the organism causing the condition is present ubiquitously and affects those prone to it. It is a yeast like fungus, round or oval, usually 4-6 µm in diameter with double wall and distinct capsule. Reproduction is by budding [13-17]. It exists as a saprobe in nature. Entry into the human host is through the respiratory route with propensity to localise predominantly in the basal ganglia and cortical grey matter of the central nervous system causing meningoencephalitis. Selective nutritional factors such as simple sources of nitrogen (asparagine and creatinine) present in the spinal fluid may stimulate growth [9,13]. Large focal lesions may occur causing pressure effects [4,13]. Even when widespread in the central nervous system, many may be asymptomatic. There need not be any occupational predisposition or history of exposure to pigeons, dust or flowering eucalyptus trees [9].

The organism is classified into two serovars : *Cryptococcus neoformans var neoformans* and

Cryptococcus neoformans var gatti. The former is exclusively associated with infections in patients with AIDS [12-17]. We present the clinical and laboratory findings in 7 cases of *cryptococcal* meningitis presenting over a period of one year in a 650 bedded military hospital. Some vital aspects of laboratory diagnosis have been highlighted.

Material and Methods

The 7 cases discussed in this study presented to this hospital during the period January 2000 to January 2001. Detailed history was taken in every case and complete neurological evaluation was carried out by the clinician. The initial work-up of every case included a complete hemogram, urine analysis (routine examination for albumin, sugar as well as microscopic examination for pus cells, red blood cells and yeast cells), blood biochemical parameters (sugar, urea, creatinine, liver function tests), VDRL test and test for Hepatitis B surface antigen (HBsAg). Blood culture was taken in every case. Antibodies to HIV 1 and 2 were looked for by ELISA in all cases. All positive results for HIV antibody were confirmed on two more fresh blood samples using three different ELISA systems. All positive samples were further confirmed from National AIDS Control Organization (NACO), New Delhi. An X-ray of chest was done in every case and three days' sputum was checked for acid and alcohol fast bacilli, yeast cells *Cryptococcus* and *Pneumocystis carinii*. The CSF submitted to laboratory was subjected to following tests :

(a) Staining for microbiological examination : Gram stain, Ziehl-Neelsen (ZN) stain and India ink/10% Nigrosin

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stain of deposit from 3-4 ml of CSF.

(b) Cytology for predominant cells on Geimsa and Leishman stained smears from deposit.

(c) Biochemical tests : protein, sugar and globulin estimation (a simultaneous sample for blood sugar was also drawn).

(d) Cultures on blood agar, chocolate agar (in presence of 5-10% CO₂) and sabouraud agar (without antibiotics). Culture on blood agar and chocolate agar were incubated at 37°C and sabouraud agar was incubated at room temperature. All cultures in suspected cases were observed for a period of six weeks. Care was taken to maintain adequate humidity within the incubator during the period of incubation by keeping a petri dish with water within.

(e) Culture for acid fast bacillus (AFB) was put up in every case.

Follow up of CSF cultures

Culture plates inoculated with CSF were incubated at 37°C for upto six weeks in all suspected cases of *Cryptococcal* meningitis. Plates were checked daily for growth. Sabouraud's agar was incubated at room temperature for six weeks and checked daily for growth. Any growth obtained in any of the cultures was subjected to Gram stain. If conforming to yeast cells, capsule stain was done using serum eosin / India-ink/10% Nigrosin stain. Test for hydrolysis of urea using Christensen's urea agar and test for nitrate reduction were undertaken (broth method). Cultures were handled in a Class II bio-safety cabinet with strict enforcement of masks and gloves. All AFB cultures on Lowenstein-Jensen (LJ) medium were observed for 8 weeks before being labelled negative.

Other cultures

Blood cultures (in brain heart infusion broth) were subcultured every 48 hours on blood agar, chocolate agar (with 5% carbon dioxide) and sabouraud's agar. All blood cultures were observed for two weeks for any growth. All sputum cultures were observed for yeast colonies conforming to *Cryptococci*.

Results

The 7 patients of *Cryptococcal* meningitis in this study presented in a variety of ways as given in Table 1. The table also gives the figures in three other studies [3,6,7]. The age range at presentation was 34-55 years with mean age at 40.8 years. All were males. Six out of the 7 were serving soldiers in this sector while one was an ex-serviceman who had retired five years back. All were heterosexual individuals and except for one patient, all gave history of sexual promiscuity. The time of first exposure varied between three years to seven years prior to illness.

The commonest mode of presentation was headache of 10 days to 4 weeks duration (average 20 days) followed by general ill health and unexplained "confusion state". Symptomatic period ranged from 10 days to 6 weeks with average of 3 weeks duration. Three patients had history of nausea, vomiting and fever. Non specific reports of fever

Table 1

Modes of presentation and salient laboratory findings in *Cryptococcal* meningitis - a comparative analysis

	This study % (n=7)	Chuck et al % (n=106)	Satishchandra et al % (n=37)	Kalra et al % (n=15)
I. Clinical presentation				
Headache	6 (85.7)	73	97.3	12 (80)
General ill health	5 (71.4)	76	-	-
Fever	3 (42.8)	45	86.5	13 (86.6)
Personality change	3 (42.8)	17	-	-
Nausea and vomiting	3 (42.8)	42	51.3	-
Confusional state	2 (28.5)	28	75.7	4 (26.6)
Stiff neck	1 (14.2)	22	-	-
Visual complaints	1 (14.2)	8.1	8.1	2 (13.3)
Sudden blindness	1 (14.2)	15	16.2	-
II. Signs				
Raised ICT	4 (57.1)	66	-	-
Fever > 38.4° C	3 (42.8)	56	-	-
Nuchal rigidity	2 (28.5)	27	-	-
Papilloedema	1 (14.2)	-	18.9	4 (26.6)
Focal CNS signs	1 (14.2)	15	16.2	-
Cranial nerve palsy	1 (14.2)	-	29.7	2 (13.3)
III. Salient laboratory findings				
Hyponatremia	2 (28.5)	22	-	-
Abnormal LFT	1 (14.2)	27	-	-
In CSF :				
Glucose < 40 mg/dl	1 (14.2)	24	-	-
Protein > 45 mg/dl	3 (42.8)	55	-	-
WBC > 20/cumm	2 (28.5)	21	-	-
India ink +ve	7 (100)	73.5	-	15 (100)
(Very scanty in three)				

and malaise were often the only complaints on admission. Visual complaints were present in 3 patients (blurring of vision in 2, sudden onset blindness in one). Clinical signs were subtle and at least in one case presenting with features of cerebrovascular accident the diagnosis came as a surprise. This patient had hemiplegia, aphasia and sudden onset of blindness. Nuchal rigidity, the cornerstone of diagnosing meningitis, was absent in the majority of cases. Focal neurological deficit was noted in one case. Two of the patients died within 96 hours of admission. The other 4 were transferred ahead soon after diagnosis was made.

Laboratory findings were minimal and are summarised in Table 2. Routine investigations of blood and urine were essentially normal in all cases except for anaemia in 2 patients (haemoglobin 9 g% and 10.5 g%). There was no leucocytosis and peripheral smears did not show toxic changes. Biochemical tests gave normal results in all except the 2 fatal cases in whom blood urea and serum creatinine were raised (urea 65 mg% and 84 mg%, creatinine 2.0 and 2.5 mg%), liver function tests (LFT) were deranged (raised AST and ALT values) and serum electrolytes showed hyponatremia.

CSF was visibly clear in all patients. Normal cell counts were recorded in 3 out of 7 cases. The predominant cells in CSF were lymphocytes in 5 out of 7 cases (other 2 had a

Table 2

Laboratory findings in seven cases of *Cryptococcal* meningitis

	Patient number						
	1	2	3	4	5	6	7
Age/Sex	50/M	35/M	36/M	40/M	55/M	36/M	34/M
At presentation	Headache Nausea Blurred vision	Headache	Headache	Headache	Headache Sudden loss of vision and hemiplegia	Confusion state	Headache
HBsAg status	Negative	Negative	Negative	Negative	Negative	Negative	Negative
HIV status	Positive	Positive	Positive	Positive	Positive	Positive	Negative
VDRL status	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Final outcome	Fatal-48h	Transferred	Transferred	Transferred	Fatal-96h	Transferred	Transferred
CSF findings							
Protein mg/dl	120	60	30	40	60	150	250
Sugar mg/dl	54	45	63	50	52	10	56
Globulins	Raised	Raised	Raised	Raised	Raised	Raised	Raised
Total cells/cu mm	03	18	04	06	06	240	320
Predominant cell	Lympho	Lympho	Lympho	Lympho	Lympho	Pleocytosis	Pleocytosis
Organisms in India ink prep	Numerous	Very few	Very few	Few	Many	Numerous	Very few
Gram stain for yeast cells	Positive	Scanty	Scanty	Scanty	Positive	Positive	Scanty
Ziehl-Neelsen stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Culture for <i>Cryptococcus</i>	+ve (48h)	+ve (10 days)	+ve (7 days)	+ve (96h)	+ve (7 days)	+ve (48h)	+ve(72h)
Culture for AFB	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Blood culture for <i>Cryptococcus</i>	+ve (72h)	-ve	-ve	-ve	+ve(96h)	-ve	-ve

mixture of lymphocyte and polymorph). There was slight increase in CSF proteins in 5 patients (range 60-250 mg/dl of CSF). CSF glucose level was normal in most cases. One patient (Case No. 6) was suspected clinically to have associated tubercular meningitis. He had a higher number of cells in CSF (240 cells/cu mm) and raised value of CSF proteins (150 mg/dl CSF). Case No.7 who was negative for HIV antibodies also had a higher cell count in CSF (320 cells/cu mm) and a higher protein level (250 mg/dl CSF). The level of globulins was, however increased in all.

In 3 patients out of 7, India-ink staining showed numerous budding capsulated yeast cells in CSF. In other cases one had to diligently search all fields of smear from deposit before being rewarded. Gram stain of deposit of CSF showed the organisms clearly when numerous but in others could have been dismissed as artifacts by the unsuspecting observer. Although culture of CSF was positive for *Cryptococcus* in all, most cultures required more than 48 hours of incubation to show the first evidence of growth. Two of the cultures took ten days to show growth. None of the cultures of CSF grew AFB. Two blood cultures from fatal cases were positive for *C. neoformans*.

The sputum from all patients was negative for yeast cells (Gram stain), AFB (ZN stain) as well as for *Pneumocystis carinii* (methenamine silver). None of the sputum cultures was positive for *Cryptococci* or for AFB.

Six out of 7 patients tested positive for HIV-1 antibodies. One sample was negative for HIV antibodies despite repeat test by three different ELISA systems. In the 6 patients testing positive for HIV-1 antibodies, it was the initial AIDS defining illness. This worked out to 10.34% individuals

presenting with *Cryptococcal* meningitis as the initial AIDS defining illness during the period of study.

The patient testing negative for HIV antibodies denied history of exposure to commercial sex workers, drug abuse or blood transfusion. There was history of general ill health of two months duration associated with persistent intractable headache for past two weeks. Clinically there was no nuchal rigidity and fundoscopy was normal. No obvious precipitating factor (diabetes mellitus, immunosuppressive therapy, history of organ transplant, steroid therapy, previous diagnosis of sarcoidosis, previous history of cryptococcosis, occupational predilection) was identified during his short stay prior to transfer ahead. The cell count in his CSF was higher than in others, so was the protein content in CSF. There was pleocytosis in CSF and organisms were difficult to come by in initial stain of deposit.

Discussion

Central nervous system (CNS) *Cryptococcosis* is known to have a variety of presentation from insidious symptoms such as mild headaches, memory lapses or personality changes to overt signs of meningitis, sometimes with localising signs [1,2-4,6,13]. Depending on the location of the lesion, visual aberrations, at times with acute onset blindness may occur [4,13]. The 7 patients encountered in the span of a year presented with a variety of symptoms. What needs to be stressed is the fact that even with widespread *Cryptococcosis*, no abnormality is usually seen in routine laboratory tests. There may be little if any change in the findings of cytology and biochemistry of the CSF. Positive results by negative staining of CSF may be obtained

in the presence of apparently normal findings by cytology and biochemical analysis. It is therefore advisable to stain sediment from at least 3 ml of CSF in all laboratory CSF analysis with negative stain such as India ink/10% Nigrosin so as to pick up even a lone unsuspected case. All fields need to be searched carefully as number of organisms may at times be scanty. In this series, two samples had very few organisms on negative staining and if only few fields had been checked, would have resulted in a false negative report. In the 2 fatal cases, an otherwise bland CSF analysis revealed numerous *Cryptococci* on India-ink staining of deposit signifying increased fungal load with minimal host response. Although Gram stain shows the organisms, this is often difficult especially in those with low fungal load.

Growth on culture can take considerable time in some cases [15,17]. In this series, growth has been obtained as late as 10 days after start of incubation in 2 cases. It is also known that culture results may be rewarding in the absence of findings on staining CSF [13,15]. In this series however, all samples of CSF in clinically suspected cases were positive by both negative staining and by culture.

As results of staining are not always rewarding, biochemical results are often non contributory, and culture results unavailable for some time, some cases may be diagnosed with an immunological test such as a latex agglutination test for detection of *Cryptococcal* antigen in serum/CSF [1-4,12,13]. False positive results are known to occur especially in the presence of rheumatoid factor, systemic lupus erythematosus and sarcoidosis. Such false positive reactions may be neutralised by pre-treatment of the sample with pronase or with 2- β -mercaptoethanol [12,13-15]. Immunological tests, however were not carried out in this study.

The level of protein has shown a wide variation in this series (30-250 mg/dl). *Cryptococcal* meningitis is part of a syndrome complex of AIDS. It is possible that other co-infections may have resulted in a variation of the protein content of CSF. Case No. 6 whose CSF showed protein concentration of 150 mg/dl was clinically suspected to harbour associated tubercular meningitis. However, ZN stain did not reveal AFB nor was the culture for AFB rewarding.

The wide variation in cell count in CSF is also noteworthy. Most cases had cell count of <20 cells/cumm CSF. Both the moribund cases who died within 96 hours of admission had low cell count in CSF (3 cells/cumm in one, 6 cells/cumm in another). Case No. 6 could have had associated tubercular meningitis and thus had higher cell counts in CSF. Raised cell count was also found in the HIV negative patient presenting with *Cryptococcal* meningitis. In this case, the high cell count could be due to a better immune status. Reports of *Cryptococcal* meningitis in association with sarcoidosis, idiopathic CD4 lymphopenia and neoplastic disease is known [10, 11-13]. *Cryptococcal* meningitis can occur in immunocompetent individuals also [14]. The HIV negative patient in this series could not be fully evaluated in this centre as he was transferred ahead soon after diagnosis.

Although the series is small a few important points were noticed and are being highlighted below :

(a) *Cryptococcal* meningitis can present with very subtle clinical and laboratory findings. Often cell counts and biochemical results in CSF study give bland results.

(b) Negative staining should be done for all CSF to pick up even an unsuspected case.

(c) In cases with high degree of suspicion, cultures should be observed for prolonged periods (at least 4 weeks).

(d) A diligent search is required as organisms are often scanty.

(e) It is worth while to have latex agglutination test for *Cryptococcal* antigen available to screen samples. Serum offers better correlation than CSF [2,12].

(f) Other associated co-infections should also be looked for as more than one infection may be present in cases of full-blown AIDS.

Are there any prognostic indicators? Some authors [2,3,13] suggest that persistent hyponatremia, low initial CSF cell counts, isolation of *Cryptococci* from other body fluids, low CSF glucose and a high titer of *Cryptococcal* antigen [2] spell a bad prognosis. Both fatal cases in this series had associated hyponatremia. Both also had low cell counts in CSF and deranged biochemical parameters in blood.

Although, prognosis is guarded in *Cryptococcal* meningitis, especially in those with HIV infection, early diagnosis and therapy undoubtedly go a long way in offering some relief to those afflicted and every effort should be made to achieve this goal.

References

1. Powderly WG. *Cryptococcal* meningitis and AIDS. Clin Infect Dis 1993;17:837-42.
2. Dismukes WE. *Cryptococcal* meningitis in patients with AIDS. J Infect Dis 1999;52:928-30.
3. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the Acquired Immunodeficiency Syndrome. N Engl J Med 1989;321:794-9.
4. Khanna N, Chandramukhi A, Desai A, Ravi V. *Cryptococcal* infections of the central nervous system : an analysis of predisposing factors, laboratory findings and outcome in patients from South India with special reference to HIV infection. J Med Microbiol 1996;45:376-9.
5. Aquina SR, Tarey SD, Ravindran GD, Nagmani D, Ross C. *Cryptococcal* meningitis in AIDS-need for early diagnosis. JAPI 1996;44:178-80.
6. Satishchandra A, Gouri Devi NM, Khanna N et al. Profile of neurologic disorders associated with HIV/AIDS from Bangalore, South India (1989-96). Indian J Med Res 2000;111:14-23.
7. Kalra SP, Chadha DS, Singh AP, Sanchette PC, Mohapatra AK. *Cryptococcal* meningitis in Acquired Immunodeficiency Syndrome. JAPI 1999;47:958-61.
8. Kaur A, Babu PG, Jacob M. Clinical and laboratory profile of AIDS in India. J Acquir Immune Defic Syndr 1992;5:883-9.

9. Diamond RD. *Cryptococcus neoformans*. In: Mandell, Douglas and Bennett, editors. Principles and Practice of Infectious Diseases. 4th ed. McGraw Hill, Philadelphia 1995;2332-41.
10. Botha RJP, Wessels E. *Cryptococcal* meningitis in an HIV negative patient with systemic sarcoidosis. J Clin Pathol 1999;52:928-30.
11. White M, Cirincione C, Blevius A, Armstrong D. *Cryptococcal* meningitis-outcome in patients with AIDS and patients with neoplastic diseases. Clin Infect Dis 1992;165:960-3.
12. Chakrabarty A, Sharma A, Sood A et al. Changing scenario of *cryptococcosis* in a tertiary care hospital in North India. Indian J Med Res 2000;12:56-60.
13. Madan M, Ranjitham M, Chandrasekharan S, Sudhakar. *Cryptococcal* meningitis in immunocompetent individuals. JAPI 1999;47:933-4.
14. Mitchell DH, Sorrel TC, Allworth AM. *Cryptococcal* disease of the CNS in immunocompetent hosts : influence of *cryptococcal* variety on clinical manifestations and outcome. Clin Infect Dis 1995;20:611-6.
15. Mycology. In: Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. Colour Atlas and Textbook of Diagnostic Microbiology, 5th ed. Philadelphia : Lippincott, 1997.
16. *Cryptococcosis*. In: John Willard Rippon, editor. Medical Microbiology, the pathogenic fungi and pathogenic actinomycetes, 3rd ed. 1988;603.
17. Koneman EW, Roberts GD, editors. Practical Laboratory Mycology 3rd ed. Baltimore: Williams and Wilkins, 1985.

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