

CRYPTOCOCCAL MENINGITIS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-POSITIVE AND HIV-NEGATIVE PATIENTS

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Abstract. This study compared clinical manifestations, blood biochemistry and cerebrospinal fluid (CSF) results of HIV-positive and HIV-negative patients with cryptococcal meningitis. We collected 57 cases of cryptococcal meningitis from cytological specimens submitted to the Department of Tropical Pathology, Faculty of Tropical Medicine. Pertinent clinical data were analyzed retrospectively in 47 cases for clinical manifestations, laboratory features and outcomes of 38 HIV-positive and 9 HIV-negative patients. Headache was the most common symptom seen in all cases, of which 70.2% occurred with fever. CSF examination of both groups revealed elevated opening pressure. Increased CSF protein and depressed CSF glucose levels were seen in HIV-negative cases, which differed from HIV-positive cases, where a slight change was noted. CSF pleocytosis in HIV-positive patients was variable. Forty-eight percent of HIV-positive patients had CSF leukocyte counts below 20 cells/mm³. None was found in the HIV-negative patients. Specific treatments with amphotericin B and fluconazole were given. Five fatal cases of cryptococcal meningitis were noted, all of which were HIV-positive. There were statistically significant differences in blood neutrophils, blood eosinophils, CSF leukocyte counts, CSF neutrophils, CSF lymphocytes, CSF glucose, and CSF total protein, in HIV-positive and HIV-negative patients ($p = 0.050$, $p = 0.022$, $p = 0.002$, $p = 0.016$, $p = 0.047$, $p = 0.031$, $p = 0.009$, respectively).

INTRODUCTION

The incidence of cryptococcosis, particularly cryptococcal meningitis (CM), has increased as a consequence of acquired immunodeficiency syndrome (AIDS). Cryptococcosis, a disease caused by a fungus, *Cryptococcus neoformans* has become a serious worldwide opportunistic infection, representing the most common opportunistic fungal infection in patients with AIDS, contributing to increased morbidity and mortality (Mitchell and Perfect, 1995). CM, often seen in immunocompromised hosts, is also a disease of the immunocompetent individual. Meningitis is the usual presenting manifestation. Fever and headache were the most common manifestations of CM in Thailand (Tansuphasawadikul *et al*, 1999). Usually, cerebrospinal fluid (CSF) findings were nonspecific with low cell count, abnormal glucose level, and abnormal protein. The specific CSF findings were demonstration of encapsulated yeast and isolation of the organism. The immediate mortality rate was 60% (Tansuphasawadikul *et al*, 1999). CM in AIDS has

been well documented all over the world (Kalra *et al*, 1999; Hakim *et al*, 2000; Mwaba *et al*, 2001). However, few publications are related to clinical manifestations and laboratory features, especially CSF findings of CM among HIV-positive and HIV-negative patients (Khan *et al*, 1996; Khanna *et al*, 1996; Moosa and Coovadia, 1997; Tunlayadechanont *et al*, 1997).

The clinical findings of CM are usually headache, fever, and malaise, which are not characteristic of any other opportunistic disease in AIDS. Altered mental status commonly occurs and represents a poor outcome. Cranial nerve abnormalities, nausea, and vomiting are common findings (Tansuphasawadikul *et al*, 1999). Standard laboratory assessment for CM includes cell count, CSF glucose, CSF total protein, *C. neoformans* culture and cryptococcal polysaccharide antigen. CSF usually shows an elevated opening pressure during spinal or occipital tap. Indian ink preparation gives a rapid diagnosis, allowing direct visualization of cryptococcus. Cryptococcosis may also be diagnosed by determination of cryptococcal antigen in the CSF, however, a positive CSF culture is still the gold standard diagnostic test for *C. neoformans*.

This study retrospectively reviewed the clinical manifestations, blood and CSF findings, treatment and outcome of CM in HIV-positive and HIV-negative patients in Thailand. Primary CM was enrolled into the study. All collectable cases were admitted to the Hospital for Tropical Diseases, Faculty of Tropical

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Medicine, Mahidol University, Bangkok, Thailand. This study was designed to assess laboratory features and clinical manifestations of CM in HIV-positive and HIV-negative patients.

MATERIALS AND METHODS

A retrospective descriptive study (1987-2001) was conducted by reviewing the pathological records of the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. All cytologically diagnosed CM specimens were collected. Diagnostics were based on a positive Indian ink stain of CSF and/or a positive culture for *C. neoformans*. Indian ink preparation was examined using low power (10×) and high power (40×) light microscopy. Characteristics of *C. neoformans* were based on round thick capsular with budding yeasts. The results of clinical profiles were collected from the clinical charts. Clinical histories were revised to collect clinical therapeutic data. Laboratory blood and CSF findings, biochemical analysis, culture and antigen testing were assayed for *C. neoformans*. Clinical manifestations, laboratory features, treatments and outcomes of each individual were collected to find significant differences between HIV-positive and HIV-negative patients.

Data analysis

The data gathered were subjected to descriptive statistics. The results of blood anti-HIV testing were compared with clinical profiles and laboratory features by χ^2 test, Fisher's exact test, and Mann-Whitney *U* test. Data were recorded by Borland Visual dBASE version 5.5. All statistical analyses were performed by Epi Info version 6.03 and SPSS 9.0 for Windows.

RESULTS

Of the 215 CSF specimens submitted to the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Thailand, 57 (26.5%) were cytologically diagnosed as cryptococcal meningitis (CM). There were 50 clinically suspected CM but 42 (84%) cases were cytologically diagnosed as CM and 8 cases (16%) were due to other causes. They were pathologically diagnosed as fungal infection (1), eosinophilic meningoencephalitis (1), tuberculous (TB) meningitis (3), chronic inflammation (1), CSF with a few inflammatory cells (1), and inadequate specimen (1). Fifteen cases not clinically suspicious of CM showed cryptococcal organisms in the CSF. They were clinically diagnosed as TB meningitis (3), meningitis (3), headache of unknown cause (2), and

fever of unknown origin (7). Of these 57 cases of CM confirmed by cytological diagnosis, 47 were analysed in this study. Data gathered showed the prevalence of CM to be 26.5%. Taking pathological diagnosis as the screening method, the sensitivity and specificity of clinical diagnosis were 73.7 and 94.9%, respectively. The predictive value for true positive and negative diagnosis of CM was 84 and 90.9%, respectively. The efficiency for clinical diagnosis of CM was 89.3%.

Age, sex, and habitat

There were 48 male (84.2%) and 9 female (15.8%) patients. Of these 57 cases, 38 cases (80.9%) were HIV-positive (male/female=35/3) and 9 (19.1%) were HIV-negative (male/female=8/1). The age range was 16-58 years, and the average age 34 years. CM was often seen in the age range 27-42 years (73.7%).

Regarding patients' residences, CM is prevalent and widely distributed in Bangkok (29.8%), central parts (23.4%), north (14.9%), and northeastern (12.8%) Thailand. The prevalences of those from the south, west, and east were 2.1, 4.3%, and 6.4%, respectively. Most patients worked as employees (38.3%) and in government service (19.1%). The rest were merchants (4.3%), housekeepers (2.1%), monks (2.1%), and students (2.1%). Fifteen cases (31.9%) were lost to record.

Signs and symptoms

Most of the patients (93.6%) were admitted with a chief complaint of persistent headache, of which 70.2% occurred with fever, and 23.4% for headache only. Nine HIV-negative cases were admitted with a chief complaint of persistent headache, of which 8 (88.9%) occurred with fever and one case (2.1%) presented with headache only. HIV-positive patients were admitted with the common chief complaint of headache with fever (67.6%) followed by headache only (2.7%) and one case was admitted with the chief complaint of fever alone (2.7%). One of HIV-positive case was unconscious on admission (2.1%). The duration of headache in this series varied from 1-120 days, with an average of 20 days. There was no previous history of pigeon contact.

Several associated symptoms were noted in both groups. Most of the patients had fever (78.7%); nausea and vomiting were found in 44.7 and 51.1%, respectively. Other associated symptoms included general weakness (55.3%), convulsion (2.1%), and blurred vision (6.4%). For physical examination, fever presented in 17.0%. Stiffness of the neck was detected in 48.9%. Papilledema was noted in 2 cases (4.3%) and one patient was unconscious (2.1%).

The past medical histories of patients were generally unremarkable. There was no previous history of CM.

Treatment and outcome

The drugs of choice for treating cryptococcosis in this series were amphotericin B and fluconazole. The mainstay of treatment was amphotericin B (53.2%). Of these, 23.4% were treated with amphotericin B alone and 29.8% were prescribed fluconazole with previous amphotericin B usage. Treatment by fluconazole alone was found in 21.3%. Lumbar punctures (LP) were performed to reduce the intracranial pressure in cases with severe headache. Generally, patients needed only one lumbar puncture (61.1%). Two cases (5.6%) required 9 lumbar taps (one HIV-positive and one HIV-negative patient). They generally were relieved (44.7%), stable (8.5%), and one case was referred to another hospital and lost to follow-up. Five cases (10.6%) died.

Length of admission

All patients needed hospitalization: 28.1% left the hospital within the first week, 25% within 2 weeks, 40.7% within 1 month and 6.2% within 1½ months.

Laboratory finding

Anti-HIV blood testing was positive in 38 cases (80.9%) and negative in 9 (19.1%). The average values for complete blood count and biochemical findings are presented in Table 1. CSF findings and related biochemical data are shown in Table 2. Antigen testing was performed in 2 cases (4.3%), with positive results

for *C. neoformans*. Culture collection of *C. neoformans* was possible in 65.9%, of these, 57.4% were positive and 8.5% negative. Tables 3 and 4 show p-values of analytical data by χ^2 test (recommend Fisher's exact test) and Mann-Whitney *U* test. There were no significant differences between length of admission and duration of headache ($p = 0.784$) and these CSF parameters: opening pressure of lumbar puncture, leukocyte count, neutrophils, lymphocytes, eosinophils, glucose, total protein ($p = 0.983$, $p = 0.397$, $p = 0.287$, $p = 0.536$, $p = 0.623$, $p = 0.949$, $p = 0.321$, respectively). Moreover there were no significant differences between the three patterns of chief complaint (headache, fever, headache with fever) and duration of headache (1-16 weeks), treatment (amphotericin B, fluconazole, amphotericin B+fluconazole) and outcome (relieve, stable, dead) ($p = 0.862$, $p = 0.102$, $p = 1.000$). Treatment either with amphotericin B alone, fluconazole alone, or amphotericin B+fluconazole, were not statistically significantly related to outcome ($p = 0.513$).

Cerebrospinal fluid

In this CM series, open pressure (OP) of the first spinal tap was >20 cmH₂O in 25 cases (92.6%). Two cases (7.4%) were within normal limits. All HIV-negative patients had OP >20 cmH₂O (100%). Most of HIV-positive cases had OP >20 cmH₂O (90%). Only 2 cases (10%) of HIV-positive had normal limit CSF. Open pressure >60 cmH₂O was seen in 4 HIV-positive cases (20%). The general appearance of CSF was clear (44.7%) and colorless (72.3%).

Table 1
Complete blood count and biochemical findings of HIV-positive and HIV-negative patients with cryptococcal meningitis.

Blood and biochemical findings	Median		Minimum-maximum value (min-max)		Normal range (min-max)
	HIV-positive (n=38)	HIV-negative (n=9)	HIV-positive (n=38)	HIV-negative (n=9)	
Leukocyte count (cells/l)	6×10 ⁹	8.9×10 ⁹	2-13.9×10 ⁹	3.8-31.5×10 ⁹	5-10×10 ⁹
Neutrophils (%)	74	64	58-89	36-80	60-70
Lymphocytes (%)	16.5	24	5-34	19-48	25-33
Monocytes (%)	3	5	0-8	0-11	1-4
Eosinophils (%)	1	4	0-14	1-7	2-6
Basophils (%)	0	0	0-1	0-1	0-1
Blood glucose (g/l)	108	125.5	79-160	84-164	70-120
Blood total protein (g/l)	76	75	63-92	72-80	60-80

Table 2
CSF findings of collectable cryptococcal meningitis cases.

CSF findings		Median		Minimum-maximum value (min-max)		Normal range (min-max)
		HIV-positive (n=38)	HIV-negative (n=9)	HIV-positive (n=38)	HIV-negative (n=9)	
Leukocyte count (cells/mm ³)	(27/47)	20	152	1-210	80-224	0-10
Neutrophils (%)	(27/47)	0	15	0-87	0-29	0-1
Lymphocytes (%)	(27/47)	100	85	10-100	71-100	1-7
Eosinophils (%)	(27/47)	0	0	0-5	0-0	0
CSF volume (cc)	(12/47)	13.7	24	3-20	3-20	-
CSF color	(47/47)	Colorless	Colorless	Colorless	Colorless	Colorless
CSF turbidity	(47/47)	Clear	Clear	Clear to turbid	Clear to turbid	Clear
Open pressure of lumbar puncture (LP)	(27/47)	37	36	14.5 to >60	21-52	10-20 (cm H ₂ O)
CSF glucose g/l	(31/47)	42	24	10-75	18-33	40-70
CSF total protein g/l	(31/47)	42	165	18-275	71-264	20-40

Table 3
P-value of χ^2 test^a.

Analysis with anti-HIV blood testing	HIV-positive		HIV-negative		p-value
	n	(%)	n	(%)	
Sex (n=47)					0.5861
Male	35	(74.5)	8	(17.0)	
Female	3	(6.4)	1	(2.1)	
Associated symptom -Nausea (n=36)					0.6625
Positive	18	(50.0)	3	(8.3)	
Negative	13	(36.1)	2	(5.6)	
Associated symptom -Vomiting (n=38)					0.4832
Positive	19	(50.0)	5	(13.1)	
Negative	12	(31.6)	2	(5.3)	
Associated symptom -Fever (n=45)					0.4889
Positive	29	(64.4)	8	(17.8)	
Negative	7	(15.6)	1	(2.2)	
Physical examination -Fever (n=36)					0.4029
Positive	6	(16.7)	2	(5.5)	
Negative	24	(66.7)	4	(11.1)	
Physical examination -Neck stiffness (n=40)					0.3264
Positive	20	(50.0)	3	(7.5)	
Negative	13	(32.5)	4	(10.0)	
Physical examination -Papilledema (n=33)					0.2841
Positive	1	(3.0)	1	(3.0)	
Negative	27	(81.8)	4	(12.2)	
CSF culture for <i>C. neoformans</i> (n=31)					0.4422
Positive	24	(77.4)	3	(9.7)	
Negative	3	(9.7)	1	(3.2)	

^aFisher's exact test

Table 4
P-value of Mann-Whitney *U* test.

Analysis with anti-HIV blood testing	HIV-positive (n=38)		HIV-negative (n=9)		p-value
	Median	(min-max)	Median	(min-max)	
Length of admission (days)	14	(3-32)	13	(3-45)	0.977
Duration of headache (days)	12	(1-60)	22.5	(4-120)	0.166
Blood leukocyte count (cells/l)	6×10^9	($2-13.9 \times 10^9$)	8.9×10^9	($3.8-13.5 \times 10^9$)	0.200
Blood neutrophils (%)	74	(58-89)	64	(36-80)	0.050 ^a
Blood lymphocytes (%)	16.5	(5-34)	24	(19-48)	0.063
Blood monocytes (%)	3	(0-8)	5	(0-11)	0.667
Blood eosinophils (%)	1	(0-14)	4	(1-7)	0.022 ^a
Blood basophils (%)	0	(0-1)	0	(0-1)	0.176
Blood glucose (g/l)	108	(79-160)	125.5	(84-164)	0.458
Blood total protein (g/l)	76	(63-92)	75	(72-80)	0.790
CSF leukocyte count (cells/mm ³)	20	(1-210)	152	(80-224)	0.002 ^a
CSF neutrophils (%)	0	(0-87)	15	(0-29)	0.016 ^a
CSF lymphocytes (%)	100	(10-100)	85	(71-100)	0.047 ^a
CSF eosinophils (%)	0	(0-5)	0	(0-0)	0.492
CSF glucose (g/l)	42	(10-75)	24	(18-33)	0.031 ^a
CSF total protein (g/l)	42	(18-275)	165	(71-264)	0.009 ^a
Number of LP	1	(1-9)	2	(1-9)	0.245
Open pressure of LP (cm H ₂ O)	37	(14.5-60)	36	(21-52)	0.618

^asignificant difference < 0.05

DISCUSSION

C. neoformans is an important pathogen of immunocompromized hosts and most patients (90%) develop meningitis. Of the 47 patients diagnosed with CM in this study, 38 (80.9%) were HIV-infected, higher than previously recorded in South Africa (68%) (Moosa and Coovadia, 1996) and Thailand (68%) (Tunlayadechanont *et al*, 1997). These results reflect an increase in the HIV epidemic in Thailand. Comparative studies of CM in immunocompromized and immunocompetent patients were very rare, the clinical study of CM in Thailand was not updated since 1997. CM was predominant in males (84.2%) in the present study, and 35 cases (81.4%) were HIV-infected. More male admitted than female cases (11.7:1 HIV-positive; 8:1 HIV-negative), which was higher than the previous study in Thailand (Tunlayadechanont *et al*, 1997) and contrasted with the study in South Africa (Moosa and Coovadia, 1996), in which CM among HIV-positive cases occurred more in female cases. The lowest and highest ages were 16 and 58 years old (they were HIV-negative), which was similar to the previous study in Thailand (12/57 years old; HIV-negative) (Tunlayadechanont *et al*, 1997), and slightly differed

from the previous study in South Africa (9/72 years old; HIV-negative) (Moosa and Coovadia, 1996).

The diagnosis of CM was established on the basis of clinical criteria, laboratory features, especially CSF parameters. Most of the patients (HIV-positive and HIV-negative) were admitted with the chief complaint of headache with fever, more than headache alone. In comparing the clinical presentations of HIV-positive and HIV-negative groups, headache and neck stiffness were more common among HIV-positive patients, which may due to the larger proportion of HIV-positive cases in the present series. This finding is different from other researchers', who have reported that HIV-positive patients with CM have fewer neurological signs, probably as a result of a relatively poor CSF inflammatory response (Dismukes, 1988; Ennis and Saag, 1993). However, this finding is similar to previous studies in South Africa (Moosa and Coovadia, 1996) and Thailand (Tunlayadechanont *et al*, 1997). Elevation of intracranial pressure was found in both groups (> 20 cmH₂O). The very high opening pressure of >60 cmH₂O was found only in HIV-positive patients (4 cases). This finding is similar to the previous study in Thailand (Tunlayadechanont *et al*, 1997). The high

detection rate in this series may be due to the progressive of CM in HIV infection. The relative presence of CSF pleocytosis (>20 cells/mm³) in HIV-positive patients was similar to the finding in the HIV-negative group. This finding is slightly different from the studies in South Africa (Moosa and Coovadia, 1996) and Thailand (Tunlayadechanont *et al*, 1997).

Reaching a definitive diagnosis for patients with CM is important, as the clinical presentation and routine hematologic, biochemical, and CSF results may overlap with tuberculosis. CM is often misdiagnosed as tuberculous meningitis because of the limitations of routine CSF examination. Specific tests should be relied upon in confirming the suspected diagnosis, including India ink smear, antigen detection, and culture of *C. neoformans*. In this study, three cases were initially misdiagnosed as tuberculous meningitis but later confirmed to be CM. This finding is similar to the previous study in South Africa (Moosa and Coovadia, 1996).

In conclusion, differential diagnosis of cryptococcal meningitis should be pursued when investigating febrile immunocompromized patients with signs and symptoms involving the central nervous system.

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