CRYPTOCOCCAL MENINGITIS IN HIV-INFECTED PATIENTS AT CHIANG MAI UNIVERSITY HOSPITAL: A RETROSPECTIVE STUDY

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Abstract. Cryptococcal meningitis (CM) is a common central nervous system infection in HIV-infected patients. This study aimed to determine treatment outcomes among HIV-infected patients who had cryptococcal meningitis and to determine predictors of death. We conducted a retrospective cohort study among HIV-infected patients receiving care at Chiang Mai University Hospital from January 1, 2005 to December 31, 2010. We studied 79 patients; 45 (57.0%) were male and the mean age was 35.1±7.2 years. Eleven patients (13.9%) had previous opportunistic infection. The most common presenting symptoms were headache (63 patients, 79.8%), fever (49 patients, 62.0%), and altered consciousness (21 patients, 26.6%). The median CD4+ cell count was 20 cells/mm^3 [Interquartile range (IQR) 10, 53]. The in-hospital, 90-day, and 1-year mortality rates were 24.1%, 32.4%, and 52.2%, respectively. The CM attributable in-hospital, 90-day and 1-year mortality rates were 13.9%, 20.3%, and 23.2%, respectively. Predictors associated with a 1-year mortality were a high cerebrospinal (CSF) cryptococcal antigen titer (>1:10,000) [Odds Ratio (OR) =7.08, 95% confidence interval (CI): 1.62-31.00, p=0.009], and altered consciousness at presentation (OR=5.27; 95% CI: 1.16-24.05; p=0.032). Cryptococcal meningitis is an important cause of death in HIV-infected patients. HIV-infected patients with a low CD4+ cell count, a headache, fever and altered consciousness should be investigated for CM and those with a high CSF cryptococcal antigen titer are at high risk for mortality.

Keywords: cryptococcal meningitis, HIV, mortality

INTRODUCTION

Globally, an estimated 957,900 cases of cryptococcal meningitis (CM) occur each year among patients with acquired immune deficiency syndrome (AIDS), resulting in 624,700 deaths by 3 months of infection (Park *et al*, 2009). The majority of cases occur in Sub-Saharan Africa. In South and Southeast Asia, an estimated 120,000 cases occur each year with a 90-day mortality rate of 55% (Park *et al*, 2009). In Thailand cryptococcal meningitis is the third most common opportunistic infections (OIs), followed tuberculosis and *Pneumocystis jiroveci* pneumonia (PCP) (Bureau of Epidemiology, 2011). It was the most common central nervous system (CNS) infection among a study of 148 HIV-infected patients (37.8%), followed

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by tuberculosis (35.8%), and cerebral toxoplasmosis (12.8%) (Kongsiriwattanakul and Suankratay, 2011). Between 1984 and 2011, 50,021 cases of cryptococcal meningitis were reported to the Ministry of Public Health for Thailand (Bureau of Epidemiology, 2011). The mortality rate varies depends on the period of mortality defined. For example, a study among 87 HIV-infected patients from January 1997 to December 1998 found an inpatient mortality rate of 42.5% (Imwidthaya and Poungvarin, 2000). Another study reported 14-day, 70-day, and 168-day mortality rates among 140 HIV-infected patients from May 2005 to August 2007 of 5.7%, 15%, 19.3%, respectively (Anekthananon *et al*, 2011). Amphotericin B with flucytosine is the recommended treatment during the intensive phase (van der Horst *et al*, 1997; Brouwer *et al*, 2004; Perfect *et al*, 2010) since it cleared cryptococci from the cerebrospinal fluid (CSF) faster than amphotericin B with fluconazole or a triple-drug combination (Brouwer *et al*, 2004). However, amphotericin B with fluconazole may be an alternative regimen when flucytosine is not available (Perfect *et al*, 2010). In the era of combination antiretroviral therapy (cART), early initiation of cART may decrease the risk of death depending on the treatment regimen for cryptococcal meningitis (Bisson *et al*, 2008; Manosuthi *et al*, 2008; Zolopa *et al*, 2009; Makadzange *et al*, 2010). This study aimed to determine treatment outcomes among HIV-infected patients who had cryptococcal meningitis and to determine the risk factors related to death.

MATERIALS AND METHODS

Study design and population

A retrospective cohort study was conducted at Chiang Mai University Hospital, a 1,800-bed, tertiary-care hospital in northern Thailand. All patients with HIV infection treated at the HIV Clinic between January 1, 2005 and December 31, 2010 who met the following criteria were included: 1) a documented HIV infection; 2) age ≥15 years; and 3) a new diagnosis of cryptococcal meningitis defined by a positive culture for *Cryptococcus neoformans* or finding cyptococcal antigen in the CSF. All patients were followed up until December 31, 2011. Data extracted from the medical records included demographic information (sex and age), clinical information, laboratory results (CD4+ cell count within 6 months of the diagnosis of cryptococcal meningitis and CSF examination results, a computed tomography of the brain), type of antifungal therapy, type of cART regimen and treatment outcome of the cryptococcal meningitis. The cause of death was defined as the last event responsible for the patient's death. Patients who were lost to follow-up were contacted to obtain their vital status.

Immune reconstitution inflammatory syndrome (IRIS) was defined as a clinical deterioration of cryptococcal meningitis within 12 months of cART initiation in a patient who had an initial clinical response to antifungal therapy with partial or complete resolution of signs and symptoms, or lesions, or a reduction in a CSF cryptococcal antigen titer or quantitative culture, whose signs and symptoms could not be explained by non-adherence or suboptimal antifungal therapy or an alternate infection (Haddow *et al*, 2010).

This study was approved by the ethics committee of Faculty of Medicine, Chiang Mai University.

Statistical analysis

The overall mortality rate was expressed as a percentage. Demographic

Table 1
Characteristics of HIV-infected patients who survived and who died related to cryptococcal meningitis (CM) during hospitalization.

Variables	Patients who survived (*n*=68)	Patients who died (*n*=11)	*p*-value
Male	39 (57.4)	6 (54.5)	0.861
Age at diagnosis, years (mean ± SD)	35.5 ± 7.9	34.8 ± 6.2	0.796
Previous opportunistic infections	10 (14.7)	1 (9.1)	0.618
Symptoms and signs			
Fever	44 (64.7)	5 (45.5)	0.222
Headache	56 (82.4)	7 (63.6)	0.152
Altered consciousness	10 (14.7)	5 (45.4)	0.016
Nausea/vomiting	11 (16.2)	2 (18.2)	0.868
Seizures	6 (8.8)	4 (36.4)	0.011
Duration of illness prior to admission	5 (3, 14)	4 (2, 7)	0.411
Computed tomography of the brain	*n* = 55	*n* = 11	
Hypodense lesion	13 (23.6)	3 (27.3)	0.797
Leptomeningeal enhancement	9 (16.4)	3 (27.3)	0.392
Communicating hydrocephalus	9 (16.4)	2 (18.2)	0.883
Cerebrospinal fluid (CSF) studies			
Opening pressure (cmH_20)[a]	31 (20, 45)	32 (30, >60)	0.389
White blood cell count ($cells/mm^3$)[b]	25 (5, 75)	10 (0, 60)	0.157
Positive India ink preparation	44/56 (78.6)	9/10 (90)	0.403
Glucose (mg/dl)	39 (27, 51)	47 (18, 50)	0.815
Protein (mg/dl)	87.5 (48, 173)	69 (50, 100)	0.512
CSF/serum glucose ratio	0.36 (0.18, 0.46)	0.32 (0.17, 0.48)	0.912
Positive cryptococcal antigen	68 (100)	11 (100)	-
Cryptococcal antigen titer	*n* = 50	*n* = 11	0.100
1: 10	1 (2)	1 (9.1)	
1: 100	10 (10)	0 (0)	
1: 1,000	18 (36)	2 (18.2)	
1: 10,000	21 (42)	8 (72.7)	
Positive culture for *C. neoformans*	51/55 (92.7)	8/9 (88.9)	0.691
Fungemia	25/48 (52.1)	6/8 (75.0)	0.227
Positive serum cryptococcal antigen	42/42	6/6	-
Serum cryptococcal antigen titer	*n* = 27	*n* = 5	0.222
1:100	8 (29.6)	0 (0)	
1:1,000	8 (29.6)	1 (20.0)	
1:10,000	11 (40.7)	4 (80.0)	
CD4+ cell count at CM diagnosis ($cells/mm^3$)[c]	21 (10, 50)	33 (11, 79)	0.784
Year of diagnosis			0.075
2005	15 (22.1)	6 (54.6)	
2006	13 (19.1)	0 (0)	
2007	6 (8.8)	0 (0)	
2008	9 (13.2)	3 (27.3)	
2009	13 (19.1)	2 (18.2)	
2010	12 (17.6)	0 (0)	

Data are presented as number (%) or median (IQR), unless otherwise specified.
[a]*n*=57 for those who survived and *n*=9 for those who died.
[b]*n*=61 for those who survived and *n*=11 for those who died.
[c]*n*=56 for those who survived and *n*=6 for those who died.

information, clinical and laboratory data, treatment, and treatment outcome were expressed in percentages, means ± standard deviations (SD), medians and interquartile ranges (IQR) where appropriate. Comparisons of clinical characteristics between the 2 groups were performed using the chi-square test or Fisher's exact test for categorical data and the Student's *t*-test or Mann-Whitney *U* test for continuous data. Variables predictive of death in a univariable model with a *p*-value <0.10 were included in a multivariable model using forward stepwise procedures.

All statistical analyses were performed using Stata statistical software, version 10.0 (Stata Corporation, College Station, TX). A two-sided test was used to indicate statistical significance at a *p*-value of <0.05.

RESULTS

Of a total of 1,493 adult HIV-infected patients attending the HIV Clinic, Chiang Mai University Hospital, between January 1, 2005 and December 31, 2010, 79 newly diagnosed cryptococcal meningitis patients met inclusion criteria. Forty-five patients (57.0%) were male with a mean age of 35.1±7.2 years (range 20-53). Eleven patients (13.9%) had previous opportunistic infections: 5 had tuberculosis, 4 had *Pneumocystis jiroveci* pneumonia (PCP) and 1 each had candida esophagitis, and cerebral toxoplasmosis. Seventy patients (88.6%) were HIV-treatment naïve and had never received primary prophylaxis for fungal infections. Nine patients had initiated cART prior to the diagnosis of CM. Four patients with CD4+ cell counts of 13, 20, 138, and 133 cells/mm^3 developed CM 5 days, 2 weeks, 9 weeks, and 9 weeks after cART initiation, respectively. None of these patients received fluconazole prophylaxis. Five patients had received cART prior to CM being diagnosed. One patient received cART for 9 months prior to the diagnosis of CM. This patient had an undetectable HIV-1 RNA level and a CD4+ cell count of 133 cells/mm^3 when CM was diagnosed. This patient did not receive fluconazole prophylaxis. The other 4 patients had received cART but were either lost to follow-up or developed drug resistance and had CD4+ cell counts < 100 cells/mm^3 prior to presenting with CM. These 4 patients had received fluconazole prophylaxis but were poorly compliant with the medicine. Clinical manifestations of CM included: headache (63 patients, 79.8%), fever (49 patients, 62.0%), altered consciousness (21 patients, 26.6%), intractable vomiting (13 patients, 16.5%), seizures (10 patients, 12.7%), diplopia due to sixth cranial nerve palsy (3 patients, 3.8%), blurred vision (2 patients, 2.5%), ataxia (2 patients, 2.5%), aphasia (1 patient, 1.3%) and left hemiparesis (1 patient, 1.3%). The median duration of symptoms prior to seeking medical care was 5 days (IQR 3, 10).

A lumbar puncture was performed in all patients. The CSF opening and closing pressures were measured in 66 and 63 patients, respectively. The CSF findings were as follows: the median opening and closing pressures were 315 mm H_2O (IQR 200, 450) and 200 mm H_2O (IQR 150, 250), respectively and the CSF white blood cell count was 20 cells/mm^3 (IQR 5, 75) with mononuclear cells comprising 99% (25, 100). The CSF glucose was 40 mg/dl (IQR 26, 51) and the protein level was 82 mg/dl (IQR 50, 168). India ink staining was found in 53 of 66 patients performed (80.3%). All patients had positive cryptococcal antigen in the CSF. Of the 61 patients in whom CSF cryptococcal antigen titers were measured, 2 (3.3%) had a titer of 1:10,

10 (16.4%) had a titer of 1:100, 20 (32.8%) had a titer of 1:1,000, and 29 (47.5%) had a titer of 1:10,000. Forty-eight patients (60.8%) also had positive serum cryptococcal antigen test. Of the 32 patients in whom a serum cryptococcal antigen titer was performed, 8 (25.0%) had a titer of 1:100, 9 (28.1%) had a titer of 1:1,000, and 15 (46.9%) had a titer of 1:10,000. *Cryptococcus neoformans* grew out of CSF and blood cultures in 59/64 patients (92.2%) and 31/56 patients (55.4%), respectively.

Computed tomography of the brain was performed in 66 patients (83.5%). Abnormal findings were found in 53 patients (67.1%): cerebral atrophy more advanced than appropriate for age (18 patients, 27.3%), hypodensity lesions (16 patients, 24.2%), leptomeningeal enhancement (12 patients, 18.2%), communicating hydrocephalus (11 patients, 16.7%), diffuse brain edema (1 patient, 1.5%), ring enhancing lesions (1 patient, 1.5%), and cerebritis (1 patient, 1.5%).

CD4+ cell counts upon diagnosis of CM were available in 62 patients (78.5%); the median CD4+ cell count was 20 cells/mm^3 (IQR 10, 53). Fifty-four patients (87.1%) had a CD4+ cell count < 200 cells/mm^3.

Treatment and treatment outcomes

All but 1 patient initially received amphotericin B for at least 2 weeks followed by oral fluconazole at a dose ranging from 400-800 mg/day for 8-10 weeks. The other patient initially received oral fluconazole 1,000 mg/day for 8 weeks.

The in-hospital mortality rate was 24.1% (19/79). Eleven patients died from CM and 8 patients died from other causes not directly related to CM. Three patients died from extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* septicemia, 2 patients died from PCP, 1 patient each died from ESBL producing *E. coli* urinary tract infection (UTI), *Acinetobacter baumannii* UTI, and antibiotic associated enterocolitis. The median time from admission to death among patients who died from CM was 4 days (IQR 3, 6).

At 90 days, the vital status in 5 patients could not be obtained. The mortality rate at 90 days was 32.4% (24/74). An additional 5 cases died, 4 of whom were attributable to CM and the other case died of viral encephalitis. At 1 year, vital status could not be obtained for an additional 5 patients; the mortality rate was 52.2% (36/69). An additional 12 cases died: 1 was related to cryptococcal meningitis. This patient had a relapse of CM 13 weeks after completing the consolidation phase. This patient was lost to follow-up and took neither fluconazole prophylaxis nor cART. The patient eventually died during a second episode of cryptococcal meningitis. Six cases died from other causes not related to CM, 1 case each died from cerebral toxoplasmosis, lower gastrointestinal tract hemorrhage, PCP, and *E. coli* septicemia, 2 patients were well at the last follow-up but transferred to other hospitals and died; the causes of death in these 2 patients are unlikely to be related to CM. The causes of death could not be determined in an additional 5 cases transferred to local hospitals.

Of note, there was one patient who had a relapse of CM 16 months after the first episode despite secondary fluconazole prophylaxis when her CD4+ cell count was 129 cells/mm^3 and the HIV-1 RNA level was undetectable. Re-treatment was prescribed and the patient survived.

Table 1 shows the characteristics of patients who survived and who died due to CM while hospitalized. Factors associated with in-hospital CM related

Table 2
Characterisitcs of HIV-infected patients who had cryptococcal meningitis and developed IRIS.

Case	Age in years	At the time of cryptococcal meningitis diagnosis				At the time of IRIS diagnosis			
		CD4+ cell count in cells/mm^3	HIV-1 RNA level in log copies/ml	CSF cryptococcal antigen titer	Days after starting cART	CD4+ cell count in cells/mm^3	HIV-1 RNA level in log copies/ml	CSF cryptococcal antigen titer	Treatment
1	20	16	6.2	1:1,000	26	34	ND	1 : 100	Corticosteroids
2	42	16	4.9	1:10,000	220	85	ND	<1 : 10	Corticosteroids
3	37	13	5.0	1:100	85	88	<1.7	1 : 10	Supportive
4	38	13	ND	1:100	85	138	ND	1 : 10	Corticosteroids

IRIS, immune reconstitution inflammatory syndrome; CSF, cerebrospinal fluid; ND, not done; cART, combination antiretroviral therapy.

Table 3
Factors associated with mortality in HIV-infected patients who had cryptococcal meningitis.

Factors	In-hospital mortality (*n*=79)		90-day mortality (*n*=74)		1-year mortality (*n*=69)	
	Univariate OR(95% CI)	Multivariate OR(95% CI)	Univariate OR(95% CI)	Multivariate OR(95% CI)	Univariate OR(95% CI)	Multivariate OR(95% CI)
Altered consciousness	4.83 (1.24-18.90)		3.43 (1.04-11.33)	10.57 (1.73-64.57)	3.94 (1.09-14.28)	5.27 (1.16-24.05)
Seizures	5.90 (1.33-26.12)	5.90 (1.33-26.12)	6.88 (1.57-30.12)	8.86 (1.25-62.52)	5.57 (1.28-24.18)	
CSF cryptococcal antigen titer ≥1:10,000			7.20 (1.75-29.60)	13.15 (2.12-81.67)	5.14 (1.39-19.05)	7.08 (1.62-31.00)
Serum cryptococcal antigen titer ≥1:10,000			12.0 (1.23-117.41)			

mortality on univariate analysis were: altered consciousness and seizures at presentation. However, having a seizure was the only independent risk factor for death (OR=5.90; 95% CI: 1.33-26.12; p=0.019). At 90 days, factors associated with CM-related mortality among the 74 patients evaluated with univariate analysis were: altered consciousness and seizures at presentation, having a CSF cryptococcal antigen titer ≥1:10,000 and having a serum cryptococcal antigen titer ≥1:10,000. Multivariate analysis found having a high CSF cryptococcal antigen titer (OR=13.15; 95% CI: 2.12-81.67; p=0.019), seizure (OR=8.86; 95% CI: 1.25-62.52; p=0.029), and altered consciousness on presentation (OR=10.57; 95% CI: 1.73-64.57; p=0.011) were independently associated with a 90-day mortality. At 1 year, factors associated with CM-related mortality among the 69 patients examined with univariate analysis were: altered consciousness and seizures at presentation and a CSF cryptococcal antigen titer ≥1:10,000. Multivariate analysis showed a high CSF cryptococcal antigen titer (OR=7.08; 95% CI: 1.62-31.00; p=0.009) and altered consciousness at presentation (OR=5.27; 95% CI: 1.16-24.05; p=0.032) were independently associated with a 1-year mortality (Table 3).

Nineteen patients initiated cART at our hospital after begining antifungal therapy. The median time from starting antifungal therapy to cART initiation was 70 days (IQR 42, 135). Ten patients (52.6%) and 9 (47.4%) patients received nevirapine plus 2 nucleoside reverse transcriptase inhibitor (NRTIs), and efavirenz plus 2 NRTIs, respectively. No patients initiated cART during the intensive phase. Nine (47.4%), and 10 (52.6%) patients initiated cART during the consolidation phase and after completing the consolidation phase, respectively. Fifteen patients were transferred and initiated cART at local hospitals.

Among those initiating cART at our hospital, 4 patients (21.1%) developed IRIS. All patients presented with headache. The time from cART initiation to development of IRIS was 26, 85, 85, and 220 days in these 4 patients (Table 2).

DISCUSSION

Cryptococcal meningitis is one of the most common opportunistic infections in HIV-infected patients in resource-limited countries, including Thailand (Park *et al*, 2009; Bureau of Epidemiology, 2011). HIV was diagnosed at the time of CM diagnosis in 68 patients in our study (86.1%). This finding is similar to a study from Sub-Saharan Africa in which CM was the first AIDS-defining illness in 91% of 230 patients (Mwaba *et al*, 2001). Most patients had a low CD4+ cell count at the time of diagnosis in our study [54 of 62 patients (87.1%) had a CD4 cell count <100 cells/mm^3]. The common findings were headache, fever, and alteration of consciousness. Concurrent fungemia was found in nearly 40% of our patients. These findings are similar to other studies (Moosa and Coovadia, 1997; Imwidthaya and Poungvarin, 2000; Chottanapund *et al*, 2001; Mwaba *et al*, 2001; Jarvis and Harrison, 2007; Espie *et al*, 2010; Anekthananon *et al*, 2011).

Finding *Cryptococcus neoformans* on CSF culture is the cornerstone for CM diagnosis (Chayakulkeeree and Perfect, 2006). Negative CSF cultures in patients with cryptococcal meningitis may be due to low yeast burden (Chayakulkeeree and Perfect, 2006). The sensitivity of CSF cryptococcal antigen and India ink were 100% (59/59) and 88.2% (45/51), respectively in our study. The sensitivity of CSF

cryptococcal antigen for diagnosing CM in HIV-infected patients has been found to be 93% to 100% (Prevost and Newell, 1978; Kauffman *et al*, 1981; Tanner *et al*, 1994). All the patients in our study had a positive CSF cryptococcal antigen test. Of the 50 patients in whom both CSF cultures and a cryptococcal antigen titer were performed, 4 had a negative CSF culture for *C. neoformans* (2 had cryptococcal antigen titers of 1:10, and 1 each had cryptococcal antigen titers of 1:100, and 1:1,000). All patients who had a positive CSF cryptococcal antigen titer of 1:10,000 had a positive CSF culture for *C. neoformans*. The negative CSF cultures were likely due to low yeast burden. There was a correlation between a positive CSF culture and a high CSF cryptococcal antigen titer ($p < 0.001$, data not shown). The sensitivity of CSF India ink for detecting Cryptococcus is around 80-91% in AIDS-related CM (Chayakulkeeree and Perfect, 2006; Bisson *et al*, 2008). Similar to the CSF cryptococcal antigen titer, there is a correlation between a positive CSF culture and a positive CSF Indian ink preparation ($p = 0.009$, data not shown). Therefore, detection of cryptococcal antigen in the CSF is a useful tool for diagnosing of CM due to its ease of performance and rapid turnaround time with a high sensitivity and specificity.

Of the 19 patients who initiated cART after being diagnosed with CM, none started cART during the intensive phase. The optimal timing to initiate cART in patients diagnosed with CM is controversial (Bisson *et al*, 2008; Manosuthi *et al*, 2008; Zolopa *et al*, 2009; Makadzange *et al*, 2010). A retrospective study of 281 HIV-infected Thai patients showed no difference in mortality between early and deferred cART initiation (Manosuthi *et al*, 2008). A prospective cohort study among 92 HIV-infected patients with a first episode of CM in Botswana showed cART initiation at the time of CM diagnosis was associated with a decreased risk of in-hospital death (Bisson *et al*, 2008). A randomized control trial of 282 HIV-infected patients with OIs (12% with CM) who initiated cART within 14 days of starting treatment for the OI had fewer AIDS progression and death than those who initiated cART after acute OI treatment was completed (Zolopa *et al*, 2009). However, a randomized control trial among 54 HIV-infected patients in Zimbabwe with the first episode of CM showed initiation of cART within 72 hours of CM diagnosis increased nearly 3-times the 3-year mortality rate compared to those who initiated cART after 10 weeks of treatment (Makadzange *et al*, 2010). The authors concluded the negative outcome might be explained by suboptimal treatment of CM because the antifungal used in that study was fluconazole 800 mg/day only. In our study, the median time from CM treatment to cART initiation is 70 days. This delay in cART initiation was probably due to concern about IRIS during initial CM treatment (Shelburne *et al*, 2005; Bicanic *et al*, 2009). However, the CM-IRIS rate in our cohort was 21.1%, similar to other studies (Haddow *et al*, 2010). Three out of 4 patients received corticosteroids as part of CM-IRIS treatment.

CM can occur at a CD4+ cell count of ≥ 100 cells/mm^3, in which fluconazole prophylaxis was not routinely provided (Chetchotisakd *et al*, 2004; Jarvis and Harrison, 2007; National Institute of Health, 2008). Three patients who had received cART when CM was diagnosed in our study did not receive primary fluconazole prophylaxis due to their CD4+ cell count > 100 cells/mm^3. The other 2 patients had not yet received primary fluconazole prophylaxis when cART was initiated

to avoid confusion if adverse effects occurred.

The overall in-hospital, 90-day, and 1-year mortality rates in our study were 24.1%, 32.4%, and 52.2%, respectively. The CM-attributable in-hospital, 90-day, and 1-year mortality rates were 13.9%, 20.3%, and 23.2%, respectively. The mortality rates in other cohorts vary widely depending on the study population, study design, and period of mortality defined. Studies from Denmark and the US reported 30-day mortality of rates 13.3-14.0% (Sajadi *et al*, 2009; Mathiesen *et al*, 2012). In Sub-Saharan African and India the mortality rates reported are 41.6-100.0% (Mwaba *et al*, 2001; Lessells *et al*, 2011; Berhe *et al*, 2012). In Thailand, retrospective studies reported overall mortality rates of 16.1-42.5% (Imwidthaya and Poungvarin, 2000; Manosuthi *et al*, 2008; Kongsiriwattanakul and Suankratay, 2011), whereas a prospective study reported 14-day, 70-day, and 168-day mortality rates of 5.7%, 15%, 19.3%, respectively (Anekthananon *et al*, 2011). Another prospective study reported 14-day, 28-day, and 1-year mortality rates of 16%, 24%, and 76%, respectively (Pitisuttithum *et al*, 2001). Factors associated with death included seizures and altered consciousness at presentation, and a high CSF cryptococcal antigen titer. These findings correspond to other studies (Diamond and Bennett, 1974; Anekthananon *et al*, 2011; Lessells *et al*, 2011; Berhe *et al*, 2012). Factors associated with death reported in other studies but not in ours included older age at presentation (Mathiesen *et al*, 2012), focal neurological deficits (Lightowler *et al*, 2010), syncope (Sajadi *et al*, 2009), low body weight (Pitisuttithum *et al*, 2001; Anekthananon *et al*, 2011), low Karnofsky performance status (Anekthananon *et al*, 2011), high CSF opening pressure (Diamond and Bennett, 1974; Majumder *et al*, 2011), low CSF WBC count (< 20 cells/mm^3) (Diamond and Bennett, 1974; Anekthananon *et al*, 2011), low CSF glucose (Diamond and Bennett, 1974), high CSF protein (Diamond and Bennett, 1974), a positive India ink preparation (Diamond and Bennett, 1974), concurrent extracranial infection (Diamond and Bennett, 1974), and high serum cryptococcal antigen titer (Diamond and Bennett, 1974). The result from this study in addition to findings from previous reports emphasize the need for aggressive effective antifungal drugs and dosages, and management of elevated CSF pressure in a timely manner, particularly in patients who have risk factors for mortality.

Our study has several limitations. First, incomplete data collection may have occurred due to the retrospective nature of the study. This results in the inability to perform survival analysis. However, we have tried to divide the period of analysis into in-hospital, 90-day, and 1-year mortality to emphasize the risk factors of death at different time points and make it easier to compare with previous studies. Second, some factors associated with death might not have been captured due to the small sample size.

In conclusion, cryptococcal meningitis is an important cause of death in HIV-infected patients even in the era of cART. When it occurs, the morbidity and mortality rates are high. Early detection and treatment of HIV infection is the mainstay for preventing of CM.

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REFERENCES

Anekthananon T, Manosuthi W, Chetchotisakd P, *et al*. Predictors of poor clinical outcome of cryptococcal meningitis in HIV-infected patients. *Int J STD AIDS* 2011; 22: 665-70.

Berhe T, Melkamu Y, Amare A. The pattern and predictors of mortality of HIV/AIDS patients with neurologic manifestation in Ethiopia: a retrospective study. *AIDS Res Ther* 2012; 9: 11.

Bicanic T, Meintjes G, Rebe K, *et al*. Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. *J Acquir Immune Defic Syndr* 2009; 51: 130-4.

Bisson GP, Nthobatsong R, Thakur R, *et al*. The use of HAART is associated with decreased risk of death during initial treatment of cryptococcal meningitis in adults in Botswana. *J Acquir Immune Defic Syndr* 2008; 49: 227-9.

Brouwer AE, Rajanuwong A, Chierakul W, *et al*. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004; 363: 1764-7.

Bureau of Epidemiology, National Trustworthy and Competent Authority in Epidemiological Surveillance and Investigation. AIDS situation, 2011. Nonthaburi: Ministry of Public Health, 2011. [Cited 2012 Jul 28]. Available from: URL: http://www.boe.moph.go.th/files/report/20111206_93584707.pdf

Chayakulkeeree M, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am* 2006; 20: 507-44, v-vi.

Chetchotisakd P, Sungkanuparph S, Thinkhamrop B, Mootsikapun P, Boonyaprawit P. A multicentre, randomized, double-blind, placebo-controlled trial of primary cryptococcal meningitis prophylaxis in HIV-infected patients with severe immune deficiency. *HIV Med* 2004; 5: 140-3.

Chottanapund S, Singhasivanon P, Kaewkungwal J, Chamroonswasdi K, Manosuthi W. Survival time of HIV-infected patients with cryptococcal meningitis. *J Med Assoc Thai* 2007; 90: 2104-11.

Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis. A study in 111 cases. *Ann Intern Med* 1974; 80: 176-81.

Espie E, Pinoges L, Balkan S, *et al*. Cryptococcal meningitis in HIV-infected patients: a longitudinal study in Cambodia. *Trop Med Int Health* 2010; 15: 1375-81.

Haddow LJ, Colebunders R, Meintjes G, *et al*. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *Lancet Infect Dis* 2010; 10: 791-802.

Imwidthaya P, Poungvarin N. Cryptococcosis in AIDS. *Postgrad Med J* 2000; 76: 85-8.

Jarvis JN, Harrison TS. HIV-associated cryptococcal meningitis. *AIDS* 2007; 21: 2119-29.

Kauffman CA, Bergman AG, Severance PJ, McClatchey KD. Detection of cryptococcal antigen. Comparison of two latex agglutination tests. *Am J Clin Pathol* 1981; 75: 106-9.

Kongsiriwattanakul S, Suankratay C. Central nervous system infections in HIV-infected patients hospitalized at King Chulalongkorn Memorial Hospital. *J Med Assoc Thai* 2011; 94: 551-8.

Lessells RJ, Mutevedzi PC, Heller T, Newell ML. Poor long-term outcomes for cryptococcal meningitis in rural South Africa. *S Afr Med J* 2011; 101: 251-2.

Lightowler JV, Cooke GS, Mutevedzi P, Lessells RJ, Newell ML, Dedicoat M. Treatment of cryptococcal meningitis in KwaZulu-Natal, South Africa. *PLoS One* 2010; 5: e8630.

Majumder S, Mandal SK, Bandyopadhyay D. Prognostic markers in AIDS-related cryptococcal meningitis. *J Assoc Physicians India* 2011; 59: 152-4.

Makadzange AT, Ndhlovu CE, Takarinda K, *et al*. Early versus delayed initiation of antiretroviral therapy for concurrent HIV

infection and cryptococcal meningitis in Sub-Saharan Africa. *Clin Infect Dis* 2010; 50: 1532-8.

Manosuthi W, Chottanapund S, Sungkanuparph S. Mortality rate of early versus deferred initiation of antiretroviral therapy in HIV-1-infected patients with cryptococcal meningitis. *J Acquir Immune Defic Syndr* 2008; 48: 508-9.

Mathiesen IH, Knudsen JD, Gerstoft J, Cowan S, Benfield T. Outcome of HIV-1-associated cryptococcal meningitis, Denmark 1988-2008. *Scand J Infect Dis* 2012; 44: 197-200.

Moosa MY, Coovadia YM. Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Clin Infect Dis* 1997; 24: 131-4.

Mwaba P, Mwansa J, Chintu C, *et al*. 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: 769-73.

National Institute of Health (NIH). Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents. Rockville: AIDSinfo, 2008. [Cited 2011 Feb 11]. Available from: URL: http://www.aidsinfo.nih.gov/guidelines

Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009; 23: 525-30.

Perfect JR, Dismukes WE, Dromer F, *et al*. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis* 2010; 50: 291-322.

Pitisuttithum P, Tansuphasawadikul S, Simpson AJ, Howe PA, White NJ. A prospective study of AIDS-associated cryptococcal meningitis in Thailand treated with high-dose amphotericin B. *J Infect* 2001; 43: 226-33.

Prevost E, Newell R. Commercial cryptococcal latex kit: clinical evaluation in a medical center hospital. *J Clin Microbiol* 1978; 8: 529-33.

Sajadi MM, Roddy KM, Chan-Tack KM, Forrest GN. Risk factors for mortality from primary cryptococcosis in patients with HIV. *Postgrad Med* 2009; 121: 107-13.

Shelburne SA, 3rd, Darcourt J, White AC, Jr, *et al*. The role of immune reconstitution inflammatory syndrome in AIDS-related *Cryptococcus neoformans* disease in the era of highly active antiretroviral therapy. *Clin Infect Dis* 2005; 40: 1049-52.

Tanner DC, Weinstein MP, Fedorciw B, Joho KL, Thorpe JJ, Reller L. Comparison of commercial kits for detection of cryptococcal antigen. *J Clin Microbiol* 1994; 32: 1680-4.

van der Horst CM, Saag MS, Cloud GA, *et al*. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. *N Engl J Med* 1997; 337: 15-21.

Zolopa A, Andersen J, Powderly W, *et al*. Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: a multicenter randomized strategy trial. *PLoS One* 2009; 4: e5575.