

CRYPTOCOCCOSIS IN HIV-INFECTED CHILDREN

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Abstract. Between January 1994 and December 2001, 21 HIV-infected children were diagnosed as having cryptococcosis. The 8-year point prevalence of cryptococcosis among hospitalized HIV-infected patients was 2.97%. Medical records of 19 patients were available for review. Sixteen patients had cryptococcal meningitis. Of these patients, cryptococcal antigen in the cerebrospinal fluid and sera were positive in all tested samples. India ink preparations were positive in 94% of cases. However, the routine CSF examination was normal in 50% of cases. All patients but one received antifungal treatment. Six patients died during treatment, the others (13 patients) were successfully treated. Relapse occurred in 2 patients despite secondary prophylaxis. Two patients died later from other causes and nine were lost to follow-up. We conclude that cryptococcal meningitis was the most common clinical presentation of cryptococcosis among HIV-infected children. HIV-infected children who present with fever, with or without central nervous system signs, should have a lumbar puncture and CSF sent for cryptococcal antigen and culture.

INTRODUCTION

Cryptococcosis is a systemic infection caused by *Cryptococcus neoformans*, a ubiquitous encapsulated fungus. It is an important opportunistic infection affecting between 5-10% of adults with AIDS in the USA (Currie and Casadevall, 1994), but this proportion may be as high as 20% in Thailand (Chariyalertsak *et al*, 2001). It is the leading cause of meningoencephalitis in HIV-infected adults (Hakim *et al*, 2000). Cryptococcosis is relatively rare in children with a prevalence of 0.85-1.4% (Gonzalez *et al*, 1996; Abadi *et al*, 1999). Only 11 cases had been reported in Thailand (Pancharoen *et al*, 2001; Lolekha *et al*, 2002). Since there is little data on the clinical characteristics and laboratory findings of this infection in HIV-infected children, we conducted a retrospective study of cryptococcosis cases in this group of patients at Chiang Mai University Hospital in northern Thailand.

MATERIALS AND METHODS

Medical records of HIV-infected children (aged 15 years or younger), who were admitted to Chiang Mai University Hospital between January

1994 and December 2001, were searched. Those with cryptococcosis were reviewed. The presence of HIV antibodies was determined by two tests, enzyme-linked immunosorbent assay (ELISA) (Enzymun test, Anti-HIV 1+2, Boehringer Mannheim GmbH Diagnostics, Germany or Enzygnost Anti-HIV 1/2 Plus, Dade Behring Marburg GmbH, German) and the particle agglutination test (Serodia-HIV, Fujirebio Inc, Tokyo). Only children who were older than 18 months and seropositive on both HIV antibody tests were considered infected with HIV. They were classified into clinical categories according to the US Center for Disease Control and Prevention (CDC) classification (CDC, 1994).

Demographic data, clinical manifestations, physical examination, laboratory findings and patients' outcomes were extracted from medical records using a standardized form.

A normal white blood cell count in the cerebrospinal fluid (CSF) was defined as 7 cells/ml³ or less and a normal CSF glucose level as 40 mg/dl or greater. A normal CSF protein level was defined as 40 mg/dl or less and a normal opening pressure as 20 cmH₂O or less (in a lateral recumbent position) (McMillan *et al*, 1999).

The diagnosis of cryptococcosis was made by positive culture and/or histopathology.

The isolation of *C. neoformans* from clinical specimens was performed by incubating the

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specimens at 25°C on Sabouraud dextrose agar (BBL®, Becton Dickinson, Cockeysville, MD), direct examination with India ink, germ tube test (in-house preparation) and urease test (BBL). Further tests for confirmation of *C. neoformans* were performed by the carbohydrate assimilation test (in-house preparation) and caffeic acid agar (Caffeic®, Sigma Chemical, MO).

Detection of cryptococcal capsular polysaccharide antigen titers in sera and CSF was performed using the latex agglutination test (CALAS®, Meridian Bioscience, Cincinnati, OH, USA).

Response to treatment is defined as improvement of clinical symptoms and/or negative cultures for *C. neoformans*.

Statistical analysis was performed with SPSS (version 10) software, and a $p < 0.05$ was considered significant. Variables were compared using the *t*-test, chi-square test, or Mann-Whitney test as appropriate.

RESULTS

Seven hundred and eight HIV-infected children were admitted to the hospital between January 1994 and December 2001. Of these, 21 were diagnosed as having cryptococcosis. Medical records from 19 of these 21 patients were available for review. The diagnosis of cryptococcal infection was made by positive culture in 14 cases, culture plus histopathology in 4, and histopathology alone in one. None of the 21 cases received antiretroviral agents. The characteristics of these patients are shown in Table 1. There were 11 males and 8 females with a median age of 7 years (range 4.8 -10.8 years). Seventeen (90%) acquired HIV infection from perinatal transmission, and 2 (10%) from blood products (one from coagulation-factor replacement and the other from a blood transfusion). Seventeen patients were classified in CDC clinical category C (11 with wasting syndrome alone, 6 with wasting syndrome and a history of opportunistic infection). Previous opportunistic infections included tuberculosis (2 patients), disseminated herpes zoster (2), nocardiosis (1) and penicilliosis (1). The CDC clinical categories were unknown in 2 patients, since they died within 48 hours of admission and their previous clinical histories were not available.

Of the seventeen patients who had lumbar punctures, 16 showed evidence of central nervous system (CNS) involvement. One patient, without CNS involvement, presented with fever and cervical lymphadenopathy. Biopsy of his lymph node revealed histologic evidence of cryptococcal in-

Table 1
Characteristics of HIV-infected children with cryptococcosis.

| | |
|---------------------------------------------------------------|--------------|
| No. of patients | 19 |
| Median age (yrs), (range) | 7 (4.8-10.8) |
| Sex (M/F) | 11/8 |
| Route of HIV acquisition | |
| Perinatal transmission | 17 |
| Coagulation-factor replacement | 1 |
| Blood transfusion | 1 |
| US Clinical Categories | |
| Category C | 17 |
| Unknown | 2 |
| Prior opportunistic infection ^a | 6 |
| Disease presentation | |
| Meningitis | 16 |
| Sepsis | 2 |
| Pericarditis | 1 |
| Median duration of illness prior to admission (days), (range) | 7 (2-120) |

^aTuberculosis (2), disseminated herpes zoster (2), nocardiosis (1), penicilliosis (1)

Table 2
Clinical manifestations of cryptococcal meningitis in HIV-infected children.

| Clinical manifestations | No. of children/ findings (%) |
|-------------------------|----------------------------------|
| Symptoms | |
| Fever | 13/16 (81%) |
| Headache | 13/16 (81%) |
| Nausea/vomiting | 12/16 (75%) |
| Seizures | 3/16 |
| Abdominal pain | 2/16 |
| Altered mental status | 2/16 |
| Dyspnea | 1/16 |
| Signs | |
| Meningeal irritation | 10/16 (63%) |
| Papilledema | 7/16 (44%) |
| Respiratory distress | 1/16 |
| Papular skin lesions | 3/16 |

Table 3
Laboratory findings of cryptococcal meningitis in HIV-infected children.

| Laboratory results | No. with finding/ No. tested (%) | Range |
|--------------------------------------------------|-------------------------------------|----------------------------------------|
| Cerebrospinal fluid | | |
| Culture positive | 15/16 (94) | |
| Cryptococcal-antigen titer | | |
| Positive | 15/15 (100) | |
| Titer not done | 1/15 | |
| 1:100 | 2/15 | |
| 1:1,000 | 8/15 | |
| ≥1:10,000 | 4/15 | |
| Indian ink test positive | 15/16 (94) | |
| Opening pressure > 20 cmH₂O | 10/12 (83) | 22 to >50 cmH ₂ O |
| Biochemical and microscopic | | |
| Normal | 8/16 (50) | |
| White cell count > 7 cell/ml ³ | 6/16 (38) | 0-130 cell/mm ³ (mean 17.6) |
| Glucose < 40 mg% | 3/16 (19) | 22-112 mg% (mean 53.9) |
| Protein > 40 mg% | 3/16 (19) | 8-115 mg% (mean 37.6) |
| Blood | | |
| Culture positive | 6/16 (44) | |
| Cryptococcal antigen titer | | |
| Positive | 8/8 (100) | |
| 1:100 | 1/8 | |
| 1:1,000 | 1/8 | |
| >1:10,000 | 6/8 | |

fection. Lumbar puncture was not performed in 2 patients. One patient presented with fever and positive blood cultures for *C. neoformans*. He died before the diagnosis of cryptococcosis was made. The other, a thalassemic child, presented with congestive heart failure and positive pericardial fluid cultures.

The clinical manifestations of the 16 patients who had evidence of meningitis are shown in Table 2. The most common presenting symptoms were fever (81%), headache (81%), and nausea or vomiting (75%). The median duration of illness prior to admission was 4 days (range 2-15 days). On physical examination, signs of meningeal irritation and papilledema were found in 63% and 44% of patients, respectively. There were no focal neurologic signs. Two patients, who had abdominal pain as one of the presenting symptoms had intraabdominal lymph node enlargement detected by abdominal ultrasound. One patient underwent fine needle aspiration of an intraabdominal lymph

node and cryptococcus was found histologically.

The laboratory findings are shown in Table 3. One patient with a negative CSF culture had a positive blood culture and a positive CSF cryptococcal antigen (1:100). Cryptococcal antigen was detected in all tested CSF (15) and blood samples (8). India ink preparation was positive in 15 patients (94%). Eight patients (50%) had normal biochemical and microscopic CSF findings (other than India ink preparation).

The antifungal therapies and outcomes of all the patients are summarized in Table 4. All the patients but one received antifungal treatment. This consisted of either amphotericin B alone for 6-8 weeks (total dose of 35 mg/kg body weight) or amphotericin B, as the initial antifungal drug for at least 2 weeks, followed by an oral triazole for a 10-week course of therapy. Increased intracranial pressure was treated by repeat lumbar puncture. Six patients (32%) died within 4 weeks after treatment (range 2-28 days, median 5 days). The causes of death were severe sepsis in 3 patients, sepsis with respiratory failure in 2 and uncontrolled increased intracranial pressure in 1. The other 13 patients were successfully treated.

The mean duration of illness prior to admission, in patients who survived was significantly longer than those who died within 4 weeks after treatment (mean 22.8 and 3.8 days respectively, $p=0.009$).

All the patients who survived were given secondary antifungal prophylaxis. Six received oral itraconazole. The other seven received oral fluconazole. One patient from each group relapsed and died within 5 months after initial diagnosis. Two more patients died from other causes within 7 months of the diagnosis.

Table 4
Antifungal therapy and outcome of
cryptococcosis in HIV-infected children.

| Therapy/outcome | No. |
|--------------------------------|-----|
| Therapy | |
| None | 1 |
| Amphotericin B | 16 |
| Amphotericin B+ Itraconazole | 1 |
| Amphotericin B + Fluconazole | 1 |
| Outcome | |
| Initial outcome | |
| Failure (death within 4 weeks) | 6 |
| Response to treatment | 13 |
| Long term outcome | |
| Relapse and died | 2 |
| Died from other causes | 2 |
| Lost to follow-up | 9 |

DISCUSSION

Cryptococcosis was found in 21 HIV-infected patients in our hospital over a period of 8 years. The 8-year point prevalence of this infection among hospitalized HIV-infected children was 2.97%. Since only cases with cryptococcosis proven by culture and/or histopathology were included in this study, the actual number of cases may be higher than that reported here. The majority of patients presented with cryptococcal meningitis and/or disseminated cryptococcosis. The presenting symptoms were similar to those in reports from the USA and Africa (Leggiadro *et al*, 1991; Abadi *et al*, 1999; Gumbo *et al*, 2002). However, unusual symptoms were found in our study, such as abdominal pain from intraabdominal lymphadenopathy and congestive heart failure secondary to pericardial effusion.

Similar to the American and African studies (Abadi *et al*, 1999; Gumbo *et al*, 2002), most children in our study were between 6-10 years of age. Cryptococcosis usually occurs late in the course of HIV infection in patients with severe immunosuppression, as reflected by their low CD4 cell counts (Gonzalez *et al*, 1996; Abadi *et al*, 1999). Although lymphocyte subset determination had not been performed in our study because of its high cost, almost all of our patients were in CDC clinical category C, the most advanced stage of HIV infection. Our patients had a rather acute onset of

the disease, which was similar to the African patients (Gumbo *et al*, 2002). This was in contrast to the subtle symptoms and signs reported from patients in the US study (Gonzalez *et al*, 1996; Abadi *et al*, 1999).

Examination of the CSF in AIDS patients with cryptococcal meningitis usually shows prominent signs of fungal infection, *eg* the presence of a large number of yeasts by direct examination and positive fungal culture. Signs of meningeal inflammatory response, such as pleocytosis and increased CSF protein level, are less apparent (Livramento *et al*, 1992). In about 50% of our patients, biochemical and microscopic examination (other than India ink preparation) of the CSF revealed normal findings. In contrast, yeast cells were seen on India ink preparation in 94% of our patients. These findings are similar to those in a study from Africa (Gumbo *et al*, 2002).

Detection of the cryptococcal antigen was very helpful in the diagnosis of cryptococcal infection (Chuck and Sande, 1989). The sensitivity and specificity of cryptococcal antigen determination in both serum and CSF of these patients were high (Tanner *et al*, 1994). All patients with meningitis in our study had positive capsular cryptococcal polysaccharide in tested blood and CSF samples. In addition, in one child who presented with meningitis and a negative CSF culture, the cryptococcal antigen test in the CSF was positive. A high CSF titer at baseline has been shown to be associated with a poor outcome (Zuger *et al*, 1986). Our limited data did not show this predictive value. Serial monitoring of serum and CSF cryptococcal antigens has been shown to be of a limited value in the management of patients with cryptococcal meningitis. However, early in the course of treatment patients who responded favorably tended to have a decreasing CSF titer (Powderly *et al*, 1994). In our study, 7 out of 8 patients who survived had a decreasing titer during treatment (data not shown).

Therefore, we suggest that fungal cultures, India ink preparations of the CSF and detection of CSF and blood for cryptococcal polysaccharide capsular antigens be performed in every AIDS child who presents with prolonged fever, with or without signs of meningeal irritation, even with normal CSF findings.

Current treatment for AIDS-related cryptococcosis includes amphotericin B plus flucytocine for 2 weeks followed by fluconazole for 10 weeks in total (Saag *et al*, 2000). Increased intracranial pressure was found in 83% of the patients in our study. This is similar to study involving adult patients (Graybill *et al*, 2000). The pathogenesis of increased intracranial pressure is thought to be from mechanical obstruction of the CSF pathway and lymph channels (Graybill *et al*, 2000). Treatment includes large-volume CSF drainage (Graybill *et al*, 2000) or a ventriculoperitoneal shunt (Saag *et al*, 2000).

Despite early treatment with antifungal drugs, the immediate mortality rate in our study was slightly higher than that of the American study (Abadi *et al*, 1999). Most of our patients died from fulminant septicemia, respiratory failure or uncontrolled increased intracranial pressure. Two patients relapsed and died despite maintenance therapy with fluconazole or itraconazole. The overall outcome of patients who had cryptococcal infection was not good, with at least 53% of the patients dying within 7 months of the initial diagnosis. With the prospect of increased access to highly active antiretroviral therapy for patients in developing countries, the incidence of cryptococcosis is expect to decrease. Until then, pediatricians need to know how to diagnose and manage this life-threatening infection.

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