Comparison of Clinical Features and Survival between Cryptococcosis in Human Immunodeficiency Virus (HIV)-Positive and HIV-Negative Patients

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SUMMARY: Cryptococcosis is a life-threatening fungal infection among human immunodeficiency virus (HIV)-positive patients and also occurs frequently in HIV-negative patients. A retrospective cohort study was conducted among patients with cryptococcosis. Clinical manifestations, laboratory findings, treatment, and outcomes for 149 HIV-positive and 29 HIV-negative patients were compared. Neurological involvement occurred more frequently in HIV-positive patients (91.9 versus 20.7%, \( P < 0.001 \)), whereas pulmonary involvement was more frequently observed in HIV-negative patients (34.5 versus 2.7%, \( P < 0.001 \)). Ninety percent of HIV-positive patients and 74% of HIV-negative patients had positive serum cryptococcal antigen (\( P = 0.119 \)). HIV-positive patients were more likely to have a cerebrospinal fluid (CSF) preparation that is positive for India ink staining (81 versus 50%, \( P < 0.001 \)) and a CSF cryptococcal antigen titer of \( \geq 1:1,024 \) (61.1 versus 16.7%, \( P = 0.038 \)). Most of the patients in both groups received amphotericin B as the primary therapy. Cryptococcosis-related mortality was high and did not differ between the two groups (22.2 versus 34.5%, \( P = 0.162 \)). Kaplan-Meier analysis revealed that HIV-positive patients had a higher relapse rate (\( P = 0.011 \)), especially among those lacking antiretroviral therapy. In conclusion, the clinical presentation of cryptococcosis among HIV-negative patients varies and differs from that of HIV-positive patients. Awareness and prompt management are crucial for establishing a diagnosis and initiating proper treatment.

INTRODUCTION

Cryptococcosis is a systemic infection caused by \textit{Cryptococcus neoformans}, a yeast that can be isolated from the environment. \textit{C. neoformans} causes human diseases ranging from asymptomatic pulmonary colonization to meningitis and disseminated infection. Cryptococcosis is seen in apparently normal hosts as well as in immunocompromised hosts such as patients with hematologic malignancy, organ transplant, rheumatic disease, and those receiving immunosuppressive therapy (1,2). Cryptococcosis is also one of the most common opportunistic fungal infections in patients with acquired immunodeficiency syndrome (AIDS), and in this subgroup, infection is associated with high morbidity and mortality (3).

The natural history of cryptococcal infection in patients with AIDS in the pre-highly active antiretroviral therapy (HAART) era has been well documented in many studies (4–8). In addition, the clinical characteristics and natural history of cryptococcosis in human immunodeficiency virus (HIV)-negative patients have been described in the literature (9–15). Some reports differ with regard to cryptococcosis infection in HIV-negative and HIV-positive patients (13,16). To date, few studies have reported comparative data regarding the clinical manifestations, laboratory findings, and survival of HIV-positive and HIV-negative patients, especially those affected during the HAART era. Information about similarities and differences between the two populations with respect to clinical features and survival rates may be useful for the recognition, diagnosis, and management of cryptococcosis in clinical practice. Here, we aimed to compare the clinical features and survival associated with cryptococcal infections in these two groups of patients.

PATIENTS AND METHODS

A retrospective cohort study was conducted by reviewing the medical records of adult patients (\( \geq 15 \) years old) who were diagnosed with cryptococcal infection at Ramathibodi Hospital (an 800-bed medical school hospital in Bangkok, Thailand) from January 1996 through December 2005. Patients were categorized into two groups according to the anti-HIV antibody seropositivity. The reviewed data included clinical manifestations, underlying conditions, laboratory findings, treatment, survival, and treatment outcomes. In addition, information about the following were retrieved for the HIV-positive group: treatment with an antiretroviral therapy (ART) regimen, CD4 cell count, and HIV RNA levels. The diagnosis of cryptococcosis was based on one or more of the following: a positive culture, a positive histopathological study, a positive India ink preparation of the cerebrospinal fluid (CSF), or a positive serum cryptococcal antigen titer. Disseminated infection was defined by the presence of a positive culture for \textit{C. neoformans} from more than one site, or a positive culture for \textit{C. neoformans} from one site (other than from a blood sample) plus a positive test for serum cryptococcal antigen.

All available patient records were reviewed from the time of cryptococcal diagnosis until the patients died or were lost to follow up, or until the end of the study on 31 December 2005. The clinical features, laboratory findings, treatment, and outcomes (including survival) for the two groups of patients were compared. Factors that would predict a poor outcome were also evaluated. The duration of follow-up was measured from the time of cryptococcal diagnosis to the date of the last visit or the date on which a primary end point was reached. The primary end point of the study was death from...
cryptococcosis. Secondary end points were a confirmed diagnosis of relapse of cryptococcal disease. Relapse was defined as a culture-positive condition with or without clinical disease or clinical evidence of relapse with histopathological confirmation. The study protocol was approved by the Institutional Review Board.

**Statistical analysis:** Categorical data are presented as the median and range. Continuous data are expressed either as the mean and standard deviation (SD) or median and range based on the distribution. Categorical variables were compared by using the \( \chi^2 \) test or Fisher’s exact test (two-tailed), depending on which was appropriate. Numerical variables were compared using Student’s \( t \) test or the Mann-Whitney rank-sum test based on the distribution. Patients who had at least one follow-up evaluation after diagnosis were included in the survival analysis. Kaplan-Meier survival analysis with the log-rank test was used to compare the two groups in terms of time-to-relapse and time-to-death due to cryptococcosis. Cox’s proportional hazard regression analysis was carried out to identify the factors associated with relapse and death. All analyses were performed by using SPSS statistical software version 13.0. A \( P \)-value of <0.05 was considered statistically significant.

**RESULTS**

During the 10-year period between 1996 and 2005, there were 178 patients diagnosed with cryptococcal infection. Of these, 149 (83.7%) patients were HIV-positive. A comparison of the clinical features of HIV-positive and HIV-negative patients is shown in Table 1. HIV-positive patients had cryptococcal infection at a younger age than HIV-negative patients (32 versus 44 years, \( P < 0.001 \)). According to the site of infection, HIV-positive patients were more likely to have central nervous system (CNS) involvement (91.7 versus 21.7%, \( P < 0.001 \)), while HIV-negative patients were more likely to have pulmonary cryptococcosis (34.5 versus 2.7%, \( P < 0.001 \)). Disseminated infection was found in approximately half of the subjects in both groups. There was a trend toward a higher frequency of positive serum cryptococcal antigen test in the HIV-positive patients, but this difference was not significantly different (90.3 versus 73.7%, \( P = 0.119 \)).

For HIV-positive patients, the median CD4 cell count was 22 (interquartile range, 5 - 71) cells/mm\(^3\). Cryptococcosis was an initial AIDS-defining illness in 77 (51.7%) patients. Of the HIV-negative patients, 20 (69%) had associated illnesses, 15 (51.7%) received immunosuppressive therapy, 7 (24.1%) had systemic lupus erythematosus, 6 (20.7%) had malignancies, 3 (10.3%) had diabetes mellitus, 2 (6.9%) had chronic inflammatory demyelinating disease, 2 (6.9%) had cirrhosis, and 6 (20.7%) patients had other conditions.

CSF findings for the 137 HIV-positive patients and 6 HIV-negative patients with meningitis are shown in Table 2. There was a higher median CSF leukocyte count among HIV-negative patients than HIV-positive patients, but the difference was not statistically significant. HIV-positive patients

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**Table 1. Clinical and laboratory findings among HIV-positive and HIV-negative patients with cryptococcosis**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of HIV-positive (%) (( n = 149 ))</th>
<th>No. of HIV-negative (%) (( n = 29 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>85 (57)</td>
<td>9 (31)</td>
<td>0.014</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>32 (20 - 64)</td>
<td>44 (16 - 83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type of infection</td>
<td></td>
<td></td>
<td>0.413</td>
</tr>
<tr>
<td>Localized infection</td>
<td>87 (58.4)</td>
<td>14 (48.3)</td>
<td></td>
</tr>
<tr>
<td>Disseminated infection</td>
<td>62 (41.6)</td>
<td>15 (51.7)</td>
<td></td>
</tr>
<tr>
<td>Site of localized infection</td>
<td>87</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Meningitis</td>
<td>84 (96.6)</td>
<td>3 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>1 (1.1)</td>
<td>1 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>2 (2.3)</td>
<td>5 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Bone and joint</td>
<td>0</td>
<td>2 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>3 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>4 (2.7)</td>
<td>10 (34.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>137 (91.9)</td>
<td>6 (20.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive serum cryptococcal antigen</td>
<td>65/72 (90.3)</td>
<td>14/19 (73.7)</td>
<td>0.119</td>
</tr>
<tr>
<td>Positive hemoculture for <em>C. neoformans</em></td>
<td>58 (38.9)</td>
<td>11 (37.9)</td>
<td>0.869</td>
</tr>
</tbody>
</table>

1): Gastrointestinal tract, peritoneum and scalp.
CNS, central nervous system.

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**Table 2. CSF findings of patients with cryptococcal meningitis**

<table>
<thead>
<tr>
<th>CSF findings</th>
<th>No. of HIV-positive (%) (( n = 137 ))</th>
<th>No. of HIV-negative (%) (( n = 6 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median open pressure, cmH(_2)O (range)</td>
<td>25 (5 - 60)</td>
<td>25.5 (8 - 58)</td>
<td>0.799</td>
</tr>
<tr>
<td>Median leukocyte, cells/mm(^3) (range)</td>
<td>10 (0 - 900)</td>
<td>89 (0 - 165)</td>
<td>0.052</td>
</tr>
<tr>
<td>Median protein, g/l (range)</td>
<td>60.5 (10 - 495)</td>
<td>104 (28 - 136)</td>
<td>0.264</td>
</tr>
<tr>
<td>Median glucose, g/l (range)</td>
<td>45 (11 - 160)</td>
<td>34.5 (25 - 138)</td>
<td>0.620</td>
</tr>
<tr>
<td>Positive CSF culture</td>
<td>115 (83.9)</td>
<td>4 (33.3)</td>
<td>0.314</td>
</tr>
<tr>
<td>Positive India ink preparation</td>
<td>111 (81)</td>
<td>3 (50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive cryptococcal antigen</td>
<td>86/99 (86.9)</td>
<td>5 (83.3)</td>
<td>0.558</td>
</tr>
<tr>
<td>Cryptococcal antigen titer ≥ 1:1024</td>
<td>44/72 (61.1)</td>
<td>1 (16.7)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid.
were more likely to have positive results on India ink preparation of the CSF (81.0 versus 50.0%, \( P < 0.001 \)). Although the CSF cryptococcal antigen was positive in >80% of patients in both groups (\( P = 0.558 \)), HIV-positive patients were more likely to have a CSF cryptococcal antigen titer of \( \geq 1:1,024 \) (61.1 versus 16.7%, \( P = 0.038 \)). There was no statistically significant difference between the two groups in terms of open pressure, protein level, glucose level, or positive CSF culture.

The mainstay of treatment in both groups was amphotericin B. Of the HIV-positive patients, 120 (80.5%) were initially treated with an amphotericin B-based regimen, and 29 (19.5%) received a non-amphotericin B-based regimen including fluconazole (17 patients), liposomal nystatin (9 patients), and itraconazole (3 patients). Of the HIV-negative patients, 21 (72.4%) were initially treated with an amphotericin B-based regimen, and 4 (13.8%) were treated with fluconazole. The median cumulative dose of amphotericin B in these HIV-negative patients was 800 mg (range 40 - 4,000 mg) and the median duration of oral fluconazole treatment was 90 days (range 15 - 1,080 days). Four (13.8%) patients did not receive any antifungal drugs because they were initially misdiagnosed.

Overall cryptococcal-related mortality was 24.2%. The cryptococcal-related mortality did not differ significantly between groups (22.2 versus 34.5%, \( P = 0.162 \)). The Kaplan-Meier survival curve demonstrating survival for cryptococcal-related mortality is shown in Figure 1. The median survival duration of HIV-positive patients and HIV-negative patients was >59.9 and >67.9 months, respectively (\( P = 0.428 \)). Seventy-five-percent survival durations for the corresponding groups were 15.2 and 2.1 months, respectively.

The overall relapse rate was 19.1%. The relapse rates in HIV-positive patients with ART, HIV-positive patients without ART, and HIV-negative patients were 13.5% (7 of 52 patients), 24.7% (24 of 97 patients), and 10.3% (3 of 29 patients), respectively. The Kaplan-Meier survival curve demonstrating relapse-free survival for HIV-positive and HIV-negative patients is shown in Figure 2. The median survival (free) from relapse duration was 49.1 months in the HIV-positive group and >67.9 months in the HIV-negative group (\( P = 0.011 \)). Moreover, there was a statistically significant difference in relapse-free survival between HIV-positive patients who did not receive ART and that of the other two groups (\( P < 0.001 \)) (Figure 2). Cox’s regression demonstrated that the only significant risk factor for a relapse of cryptococcal infection was HIV infection (hazard ratio \([HR]\) 5.83; 95% confidence interval \([CI]\) 1.14 - 29.79, \( P = 0.034 \)). Male gender \((HR \ 0.51; 95\% \ CI \ 0.24 - 1.06; P = 0.071)\), age \((HR \ 0.96; 95\% \ CI \ 0.91 - 1.03; P = 0.064)\), CNS involvement \((HR \ 0.65; 95\% \ CI \ 0.23 - 1.88; P = 0.427)\), and pulmonary involvement \((HR \ 2.31; 95\% \ CI \ 0.37 - 14.69; P = 0.327)\) were not risk factors.

**DISCUSSION**

In the course of the AIDS epidemic, cryptococcosis has been one of the major AIDS-defining illnesses in HIV-infected patients (17). The decreasing incidence of cryptococcosis among HIV-infected patients has been documented after the introduction of ART. In contrast, a slight increase in cryptococcosis in HIV-negative patients has been observed due to the increased use of immunosuppressive therapy. An underlying immunosuppressed condition was identified in the majority of our patients. However, approximately one-third of our patients had no known underlying medical condition. These data are similar to findings from a previous study (14).

To the best of our knowledge, the present study is among the larger-scale cohort studies of cryptococcosis, especially with respect to comparisons between these two particular groups of patients.

Our main observations were that cryptococcal meningitis occurred more commonly in HIV-positive patients, but pulmonary involvement was found more frequently in HIV-negative patients. According to the largest reported study of cryptococcosis in HIV-negative patients, pulmonary involvement and CNS involvement was found in 36 and 51% of patients, respectively (14). The organism is trophic to the CNS, and the majority of cases have been found to be meningitis.
As the site of entry, the respiratory tract is the most frequently involved among the organ systems when cryptococcal infection develops (18, 19). In animal models, the role of immune status and an organism’s particular ability to induce a pulmonary inflammatory response may establish the course of clinical features (20). For example, *C. neoformans* may remain primary in the lung or may undergo hematogenous spread to other systems (16).

Meningitis was found in a fewer number of HIV-negative patients in the present study. This difference may be due to the lower proportion of patients (58.6%) who underwent lumbar puncture comparing with that of the HIV-positive group (96.6%). Asymptomatic meningitis can develop in patients with T-cell suppression and it may also be under-recognized (10). Although it was recommended in the year 2000 that all patients with cryptococcal disease undergo lumbar puncture (21), this procedure had not been routinely performed in earlier years.

According to the CSF findings in patients with meningitis, the mean open intracranial pressure was high (>20 mmH2O) in both groups. This finding is similar to those of previous studies of Thai patients (13,22). In the present study, a higher proportion of HIV-positive patients tested positive on India ink preparation and had high titers of cryptococcal antigen in the CSF. Although, there was a trend toward higher CSF pleocytosis among HIV-negative patients, the difference was not statistically significant. No other between-group differences in the CSF findings were observed. The diagnosis of cryptococcosis in HIV-negative patients is thus rather difficult and warrants an aggressive procedure to establish diagnosis.

The choice of therapy for cryptococcosis depends on both the site(s) of involvement and the host’s immune status. Amphotericin B plus flucytosine followed by fluconazole is preferred for the treatment of CNS infection and for patients with severe symptoms (21). Fluconazole is recommended for patients with pulmonary disease (21). The regimens for treatment in the present study were heterogeneous secondary to a long study period. Flucytosine has not been available in Thailand for at least 10 years, and some patients in the study received liposomal nystatin, an investigational drug. However, most of the patients in both groups received amphotericin B as the initial treatment.

The mortality rate was high in both groups, and no statistically significant difference (22.2 and 34.5%) was observed between them in this regard. This high rate is concordant with the year 2000 (data not shown). Unfortunately, due to the small sample, this analysis was not feasible in the group of HIV-negative patients. Second, because this was a retrospective study, there were missing data regarding the CSF findings. Third, a variety of *C. neoformans* was not identified, which might have had an impact on outcomes due to differences in the distribution of the gatti and neoformans varieties in non-immunosuppressed and immunosuppressed populations (26). Lastly, a higher number of HIV-positive patients was included in the study due to the high prevalence of HIV infection in Thailand, and cryptococcosis is the third-most common AIDS-defining illness among Thai patients (27). An inappropriate proportion of HIV-positive versus HIV-negative patients for the analysis may have yielded a lack of statistical significant difference for some variables.

In summary, due to a high mortality rate, cryptococcosis is a threatening infectious disease in both HIV-positive and HIV-negative patients. Cryptococcal-related mortality was found to be comparable between HIV-positive and HIV-negative patients. The results of the present study provided substantial infomation or differentiating between HIV-positive and HIV-negative patients based on clinical features, laboratory findings, and cryptococcosis outcomes. ART was found to be an important factor associated with a lower relapse rate in HIV-infected patients and it should be administered to HIV-infected patients with cryptococcosis.

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REFERENCES


