HISTOPLASMOSIS AND PENICILLIOSIS AMONG HIV-INFECTED THAI PATIENTS: A RETROSPECTIVE REVIEW

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Abstract. Histoplasmosis and penicilliosis are fungal infections with similar clinical presentation and laboratory findings that were reported mainly in the era prior to highly active antiretroviral therapy. We conducted a retrospective review at two hospitals in Central Thailand of the medical records of HIV-positive patients with microbiologic evidence of histoplasmosis or penicilliosis between January 2003 to September 2007 when antiretrovirals became widely available in Thailand. Fifty patients met inclusion criteria; 36 had histoplasmosis, and 14 had penicilliosis. Symptoms and laboratory findings on presentation were similar between the two infections except for a greater incidence of tachypnea and neutropenia among patients with histoplasmosis (both $p < 0.05$). For histoplasmosis, blood culture had a significantly lower yield for detecting infection compared to tissue microscopic examination highlighting the importance of obtaining tissue for diagnosis ($p < 0.05$).

Keywords: histoplasmosis, penicilliosis, HIV infected patients, review

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INTRODUCTION

Fungal infections are a potentially devastating complications of HIV infection, and their incidence is inversely related to CD4 lymphocyte counts (Ruhnke, 2004). Severe, life-threatening fungal infections are typically found in HIV patients with CD4 lymphocyte counts <200 cells/mm^3.

Histoplasmosis and penicilliosis are infections caused by dimorphic fungi, *Histoplasma capsulatum* and *Penicillium marneffei,* respectively. Penicilliosis marneffei is an endemic mycosis that has been reported throughout Southeast Asia (Sirisanthana and Supparatpinyo, 1998). The number of reported cases of penicilliosis has increased in the AIDS era (Sirisanthana and Supparatpinyo, 1998). *P. marneffei* infection was found in 3% of all Thai HIV-infected patients, with a higher prevalence of 6.8% among HIV-positive patients from northern Thailand (Chariyalertsak *et al*, 2001). *H. capsulatum*, a mycosis endemic to the Midwestern United States, has a prevalence there of 2-25%, and an estimated prevalence in Thailand of 0.3-1.0% (Chariyalertsak *et al*, 2001; Anekthananon *et al*, 2004; Ruhnke, 2004).

This case series examine patients with HIV and *H. capsulatum* or *P. marneffei* co-infection at two hospitals in Thailand, in order to describe and compare the clinical, laboratory and microbiological findings of these fungal infections.

MATERIALS AND METHODS

A retrospective review was conducted of HIV-infected patients with clinical evidence of invasive penicilliosis or histoplasmosis hospitalized at Chulalongkorn University, Bangkok, Thailand and Chon Buri Hospital, Chon Buri, Thailand from January 2003 to September 2007. The inpatient admissions with these infections were discovered by searching the ICD-9 codes. Inclusion criteria were: 1) HIV-positive patients aged >15 years, 2) having a diagnosis of invasive penicilliosis or histoplasmosis by clinical features and positive fungal cultures or positive fungal stains from clinical specimens. Patients with other concurrent opportunistic infections were excluded.

Comparisons between the groups was assessed with a Fisher's exact test for categorical variables. A two tailed *p*-value ≤0.05 was considered significant. All analyses were performed using Microsoft Excel (Seattle, Washington, USA) and GraphPad online statistical calculators (www.graphpad.com). The study was approved by the Chulalongkorn University, Chon Buri Hospital, and Duke University institutional review boards.

RESULTS

During the five-year study period, 50 patients with 65 inpatient admissions, met our inclusion criteria. Thirty-six patients (72%) had histoplasmosis, and 14 patients (28%) had penicilliosis. Of the 36 patients with histoplasmosis, 28 were male and 8 were female patients, with a mean age of 34.5 ± 8.11 years. Of the 14 patients with penicilliosis, 13 were male and 1 was female, with a mean age of 37.4 ± 3.4 years. The mean baseline CD4 count at presentation among histoplasmosis patients was 31 cells/mm^3 (SD±47) (22 of 36 patients with available results). The baseline plasma HIV RNA level was 1,078,000 (35,000-3,660,000) copies/ml among the 4 out of 36 patients in whom a result was available. The mean baseline CD4 count among penicilliosis patients at presentation was 30 cells/mm^3 (SD±36) for the 9 of 14 patients in whom a result was available and the mean baseline plasma HIV RNA level was 880,700 (260,000-1,840,000) copies/ml in the 3 of 14 patients in whom a result was available. Four of 36 patients with histoplasmosis (11%) were on highly active antiretroviral thereapy (HAART) on presentation; 1 of the 14 patients with penicilliosis (7.1%) was on HAART on presentation.

Table 1
Presenting signs and symptoms of HIV-positive patients infected with histoplasmosis or penicilliosis.

	Histoplasmosis (%) $n = 36$	Penicilliosis (%) $n = 14$
Fever	30 (83)	14 (100)
Rash[a]	25 (69)	13 (93)
Tachypnea[b]	21 (58)	3 (21)
Lymphadenopathy	18 (50)	10 (71)
Weight loss	15 (42)	10 (71)
Cough	15 (42)	5 (36)
Anorexia	14 (39)	6 (43)
Hepatomegaly	12 (33)	6 (43)
Tachycardia	11 (31)	6 (43)
Diarrhea	9 (25)	6 (43)
Abdominal pain	8 (22)	5 (36)
Vomiting	8 (22)	4 (29)
Dyspnea	7 (19)	2 (14)
Ulcers[c]	6 (17)	1 (7.1)
Altered mental status	6 (17)	1 (7.1)
Jaundice	6 (17)	1 (7.1)
Splenomegaly	6 (17)	3 (21)
Hypotension	5 (14)	1 (7.1)
Pleural effusion	4 (11)	1 (7.1)
Bleeding	4 (11)	1 (7.1)
Chest pain	2 (5.6)	1 (7.1)
Headache	1 (2.8)	0 (0)

[a] Plaques, papules or umbilicated papules; [b] $p<0.05$; [c] Penile, perianal, oral or conjunctival ulcers

The two groups of patients presented with similar clinical findings (Table 1). The most common presenting symptoms in both groups were fever (88%), rash (plaques, papules or umbilicated papules, 76%) and weight loss (50%). Cough (40%) and gastrointestinal symptoms (30%) were also common. The most common signs were lymphadenopathy (56%), tachypnea (48%) and hepatomegaly (36%). Tachypnea was significantly more common among histoplasmosis patients than penicilliosis patients (58% *vs* 21%, $p < 0.05$). Laboratory results were available for all patients except one. The most common abnormalities were anemia (85%), hyponatremia (73%) and neutropenia (63%). Neutropenia was significantly more common among patients with histoplasmosis (77%) than patients with penicilliosis (29%, $p < 0.05$). The other laboratory abnormalities did not differ significantly between the two groups of patients ($p > 0.05$).

Microbiological data are shown in Table 2. The diagnostic yield for fungal blood culture was lower for histoplasmosis (3 of 13) compared to penicilliosis (7 of

Table 2
Results of fungal cultures and microscopic examination of HIV-positive patients with histoplasmosis or penicilliosis.

	Histoplasmosis (%)	Penicilliosis (%)
Positive cultures	6/16 (38)	7/12 (58)
Blood	3/13 (23)	7/12 (58)
Skin	1/1 (100)	0/0
Lymph node	1/1 (100)	0/0
Bone marrow	1/1 (100)	0/0
Positive microscopic exam	39/44 (89)	12/16 (75)
Skin	14/14 (100)	7/9 (78)
Lymph node	9/9 (100)	4/5 (80)
Bone marrow	13/17 (76)	1/2 (50)
Small intestine	2/2 (100)	0/0
Hypopharyngeal mass	1/1 (100)	0/0
Sputum	0/1 (0)	0/0

12), but the difference was not significant ($p = 0.45$). Microscopic examination of biopsy specimens had a higher diagnostic yield than blood culture for histoplasmosis (89% *vs* 23% positive specimens, $p < 0.05$) but not for penicilliosis (75% *vs* 58% positive specimens, $p = 0.43$).

Immune reconstitution inflammatory syndrome (IRIS) was diagnosed in 2 of 36 patients with histoplasmosis (5.6%) and 1 of 14 patients with penicilliosis (7.1%). The mean baseline CD4 counts in the four IRIS cases was 13.67 (SD ± 5.51) cells/mm^3. The mean time from initiation of HAART to presentation with IRIS symptoms was 31 (SD ± 24) days.

DISCUSSION

In this study, the majority of the patients with HIV and fungal co-infection presented with late, disseminated infection and very low CD4 counts, similar to other reports (Anekthananon *et al*, 2004; Kiertiburanakul *et al*, 2008). The symptoms and laboratory abnormalities on presentation were similar between the histoplasmosis and penicilliosis patients except for a greater incidence of tachypnea and neutropenia in the histoplasmosis patients. In a study from northeastern Thailand among 32 histoplasmosis and 36 penicilliosis patients, no differences were seen between the 2 groups in symptoms, signs and laboratories findings except for a greater frequency of hyperbilirubinemia among penicilliosis patients (Mootsikapun and Srikulbutr, 2006). A higher diagnostic yield from microscopic examination on biopsy specimens compared to fungal blood cultures was seen for histoplasmosis, but not for penicilliosis. Two patients with histoplasmosis and one patient with pencilliosis subsequently developed IRIS. Approximately 15-20% of HIV-infected Thai patients develop IRIS following HAART (Bonnet *et al*, 2006; Puthanakit *et al*, 2006). Despite being treated at HIV referral centers, some of our patients did not have their fungal infections detected

and treated before HAART initiation. This illustrates the need to be more vigilant in diagnosing opportunistic infections.

A primary limitation of this study was the majority of histoplasmosis and penicilliosis infections were diagnosed solely by morphological examination of biopsy specimens. Typically, *H. capsulatum* is a small, round or oval intracellular budding yeast enclosed by an achromatic refractile capsule, *P. marneffei* is a larger, more elongated intracellular yeast with a central transverse septum that is indicative of their characteristic division by fission. In our case series, 58% of *P. marneffei* infections were confirmed by blood cultures while only 23% of *H. capsulatum* infections had positive blood cultures. In contrast, 75% and 89% of *H. capsulatum* and *P. marneffei* infections, respectively, were confirmed by microscopic exam, signifying the importance of obtaining tissue samples for microscopic examination, particularly for *H. capsulatum* diagnosis, as reported by others (Mootsikapun and Srikulbutr, 2006; Nacher *et al*, 2006). The lower yield of blood cultures compared to microscopic examination to diagnose histoplasmosis could be related to the longer time to positivity for *H. capsulatum* blood cultures (average 14 days) (Guerra-Romero, 1987). Samples that show no growth by day 7 are discarded by our laboratories. *P. marneffei* grows more rapidly, and takes an average of 4 days to be detected in a blood culture (Wong and Wong, 2011).

Inherent limitations of this study stems from its retrospective design. The chart review resulted in a non-standardized history, thus symptom onset and duration was often difficult to determine. A history of HAART use and compliance was often incompletely documented in the charts. Most critically, CD4 counts and HIV RNA levels were frequently not available. It was also difficult to determine whether other opportunistic infections or adverse drug events were excluded.

In summary, we found that histoplasmosis and penicilliosis infections manifested similarly among HIV-infected patients with a slightly more severe clinical presentation among histoplasmosis patients. When diagnosing these infections, it is important to obtain tissue for microscopic examination due to the low diagnostic yield of blood cultures, particularly among histoplasmosis-infected patients.

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