ORIGINAL ARTICLE

A Retrospective Review on Successful Management of *Penicillium Marneffei* Infections in Patients with Advanced HIV in Hospital Sungai Buloh

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SUMMARY

***Penicillium marneffei* is a dimorphic fungus which commonly causes a life threatening systemic fungal infection in an immunocompromised host. It has been recognized as an AIDS defining illness in Malaysia since the beginning of the HIV pandemic. The presence of various non specific clinical presentations, especially the characteristic umbilicated papular rashes with central necrosis which lead to significant ill health in immunocompromised patients should alarm clinicians to the possibility of *Penicillium marneffei* infection and prompt investigations accordingly. Simple investigations like blood culture and fungal staining of the skin scrapping can confirm the diagnosis in the majority of cases. Early treatment with appropriate systemic antifungal for a definite duration will significantly decrease the mortality rate from penicilliosis.**

KEY WORDS:
Penicillium marneffei; Penicilliosis; dimorphic fungus; HIV; AIDS; Amphothericin B; Itraconazole

INTRODUCTION

Penicillium marneffei infection is not an uncommon opportunistic infection in Malaysia. The clinical presentations of penicilliosis are non-specific and this has posed great challenge to clinicians. It is undeniably a fatal disease if left untreated. The purpose of this review is to describe the common clinical presentations, mycology and management of our experience in HIV patients who have been successfully treated for disseminated penicilliosis in order to promote awareness to clinicians that timely diagnosis and early appropriate antifungal therapy is crucial to ensure survival of these patients.

MATERIALS AND METHODS

This one year retrospective review (from January to December 2008) was conducted at the Hospital Sungai Buloh (HSB). For the purpose of this study, only successfully treated HIV patients with laboratory confirmed *Penicillium marneffei* infection during January to December 2008 in Hospital Sungai Buloh, Malaysia were identified from the database of the Microbiology Laboratory and the Pharmacy Department in HSB. In the context of our study, successful treatment was defined as no relapse or mortality from *Penicillium marneffei* infection upon completion of the 12 weeks of scheduled therapy and at 3 months of follow-up. Patients with advanced HIV infection who may have died from *Penicillium marneffei* infections were not included in this review as we are not able to track down their records due to incomplete documentation of such patients such as no confirmatory laboratory results of the diagnosis or no documentation of penicilliosis as the cause of death in these patients.

The clinical records of recruited patients for this review were retrieved from the records office and analyzed. The clinical information inclusive of demographic data; clinical manifestations; investigation results; choice and duration of antifungal therapy and the history of opportunistic infections (OI) was obtained. Data were processed with the Statistical Package for the Social Sciences (Windows version 13.0; SPSS Inc, Chicago [IL], US). Continuous data were expressed as mean (standard deviation [SD]) or median (interquartile range [IQR]), and categorical data were reported as numbers and percentages.

RESULTS

A total of twenty patients with advanced HIV (male: 17, female: 3) with *P marneffei* infections who has been successfully treated were identified in 2008. There were ten Chinese, five Indians, three Malays, one Bidayuh and one Indonesian. Their mean age was 39 (SD, 8) years. Majority of the patients had acquired HIV via heterosexual transmission (45%), followed by intravenous drug use (40%) and homosexual transmission (15%). Their median CD4 cell count when *P marneffei* infection was diagnosed was 10.0 cells/mm^3 (IQR, 1.0-55.0 cells/mm^3). Eleven (55%) of patients had deranged liver function, with a mean alanine aminotransferase (ALT) level of 48 IU/L (SD, 37.5 IU/L). [normal range, 20-5 IU/L] . The alkaline phosphatase (ALP) level was elevated in nine patients with a mean of 259 IU/L (SD, 295 IU/L) [normal range, 40 - 150 IU/L]. The diagnosis of penicilliosis in all of these patients was established from blood cultures. In four of the patients, the organism was also isolated from culture of the skin biopsy. Eleven (55%) patients were first diagnosed to have HIV infection at the time of diagnosis of penicilliosis. Nine patients were aware of their HIV sero-status at diagnosis of penicilliosis and five of them have already had other OI previously. Only four (17.4%) of these patients were receiving HAART at diagnosis

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of penicilliosis and all of them had records of poor compliance to HAART and defaulting HIV care. Only one patient did not have any concurrent OI. Eight patients had more than one concurrent OI. The commonest concurrent OI were oral candidiasis and tuberculosis. [Table I].

The characteristic umbilicated papular skin lesion with central necrosis [Fig 1], fever, anaemia and hepatomegaly were the commonest presenting features (80%) [Table II]. More than half of the patients also presented with lymphadenopathy and malaise (75%), loss of appetite, diarrhoea (65%), splenomegaly and loss of weight (55%). The median length of their hospital stay was 19 (SD, 9) days.

All patients received intravenous amphotericin B as the induction therapy. The mean duration of amphotericin B induction therapy was 14 (SD, 5) days. All patients who received amphotericin B as an induction therapy were subsequently switched to oral itraconazole (70%) and oral fluconazole (30%) as the maintenance therapy. RVD patients, who were not on HAART, were started on such therapy upon completion of the induction phase for the penicilliosis. There was no reported relapse or mortality from Penicillium marneffei in our patients following completion of the scheduled therapy and at 3 months follow-up.

Table I: Clinical characteristics of HIV patients who has been successfully treated for Penicillium marneffei infection (n= 20)

Characteristics	Patients No. (%)
Gender	
Male	17 (85)
Female	3 (15)
Age	39 ± 8 years
Race	
Chinese	10 (50)
Indian	5 (25)
Malay	3 (15)
Others	2 (10)
HIV risk factors	
Heterosexuals	9 (45)
IVDU	8 (40)
Homosexuals	3 (15)
Median length of hospital stay	19 ± 9 days
Median CD4 cell count	10.0 ± (IQR, 1.0-55.0) cells /mm³
Median Haemoglobin (Hb) count	9.4±2.6 g/dL
Median Alanine Aminotransferase (ALT) level	48 ± 37.5 IU/L
Median Alkaline Phosphatase (ALP) level	259 ± 295 IU/L
Concurrent infections	
Oral Candidiasis	19 (95%)
Tuberculosis	6 (30%)
PCP	1(5%)
Toxoplasmosis	1 (5%)

Table II: Clinical manifestations of HIV patients who has been successfully treated for Penicillium marneffei infection (n= 20)

Symptoms/Signs	Patients No. (%)
Skin lesions	16 (80)
Fever	16 (80)
Anaemia	
Hepatomegaly	
Malaise	16 (80)
16 (80)	
15 (75)	
Lymphadenopathy	15 (75)
LOA	13 (65)
Diarrhoea	13 (65)
Splenomegaly	11 (55)
LOW	11 (55)

DISCUSSION

Penicillium marneffei infection has been reported as an endemic infectious disease in immunocompromised population in Thailand, China, Vietnam, Taiwan, Singapore and India [1-6]. The first case of *Penicillium marneffei* infection in HIV patient in Malaysia was reported in 1995 [7]. However, up to this date there had been no publish case series of *Penicillium marneffei* infection in Malaysia. Hence, the true incidence of this infection in our population has not yet been determined. To our knowledge, this present study is the first retrospective review on the characteristic of a group of advanced HIV patients with *Penicillium marneffei* infection who has been successfully managed in Malaysia. In western countries, most *Penicillium marneffei* infections have been described in patients with travel history to Asia [8-10].

At present, the natural reservoir, mode of transmission, and natural history of *Penicillium marneffei* infections is not clearly defined and is still a subject of an on-going research. The only known natural hosts are bamboo rats (Rhizomys and Cannomys spp.) and humans [11-12]. *P. marneffei* can be isolated from soil but rarely from other environmental sources. In Thailand, human infection is reported to be a seasonal incident and is associated with soil exposure, especially during the rainy seasons [13]. It is presumed that human became infected via inhalation of the fungal pathogen and unlikely from direct contact with the rodents [11-12]. Inhalation of the conidia (spores) is subsequently phagocytised by the pulmonary histiocytes which later invades and disseminates throughout the host's body leading to systemic infection [11-12]. To this date, there is no reported evidence of person-to-person spread. However, the retrospective nature of this review has limited our ability to explore the risk factors of Penicillium marneffei infection in our patients as the medical notes were lacked in the documentation regarding the patient's occupational history and travel history to other endemic area. We cannot conclude whether or not the Penicillium marneffei infection in our patients is seasonally related or associated with soil exposure.

Penicilliosis is frequently reported as a late manifestation of a HIV infection [1-10]. Correspondingly, we found that the CD4+ cell count of our patients at the time of the diagnosis with *Penicillium marneffei* infection was consistently less than 50 cells/ml. Similar to other reports, [14-18], our patients also presented with non-specific symptoms such as fever, malaise, anaemia, hepatomegaly, lymphadenopathy, loss of appetite, diarrhea, splenomegaly and loss of weight. These symptoms are not diagnostic of *Penicillium marneffei* infection as it could be due to the underlying advanced immunosupression state of these patients. The skin lesion characterized as umbilicated papular lesions with central necrosis was the commonest presenting feature in our observation. Some

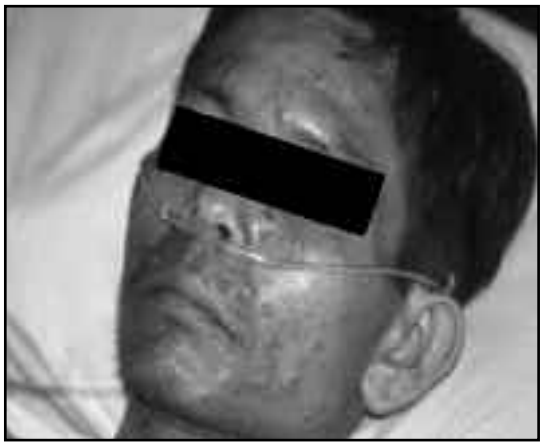

Fig. 1: Characteristic umbilicated papular skin lesion with central necrosis on the face of a HIV patient with *Penicillium marneffei* infection.

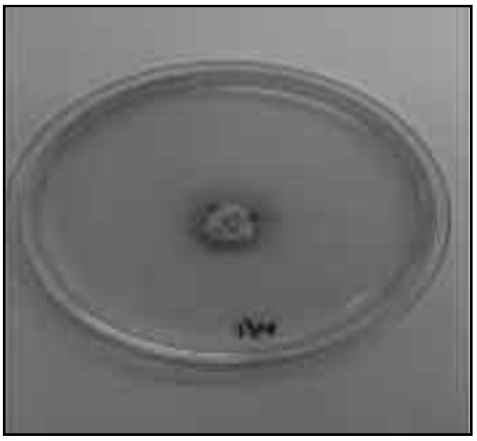

Fig. 2: *P. marneffei* on SDA after 2 days of incubation.

Fig. 3: *P. marneffei* on SDA after 4 days of incubation with diffusible red pigment seen.

authors have also commented that these characteristic skin lesions are also very similar to those lesions observed in disseminated cryptococcosis or histoplasmosis [1-17]. From our experience, we have considered these lesions as rather a specific finding to support a preliminary diagnosis of *Penicillium marneffei* infection clinically. At least, one third of our patients with advanced HIV were commenced on systemic antifungal therapy empirically solely based on their clinical presentation before the results of fungal culture could confirm the diagnosis. However, we are not able to propose a definite scoring system of diagnosis for Penicillium marneffei infection solely on the basis of this review considering a small number of our patient.

In parallel with the other reports [1-18], we also found that the rate of concurrent OI were very high in our patients. At least 40% of our patient had more than one concurrent OI. Candidiasis and tuberculosis were the commonest concurrent OI among our patients. There was no laboratory confirmation of concurrent disseminated cryptococcosis or histoplasmosis in our patients.

Definite diagnosis of Penicillium marneffei infection is based on mycological culture. Studies have shown high sensitivity from bone marrow (100%), blood (76%) and skin biopsy (90%) cultures [11]. This was also observed in our patients whereby all of them had positive blood cultures. Only four of our patients had skin biopsy done and none of them had bone marrow aspiration performed.

Identification of Penicillium marneffei is based upon its colony and microscopic morphology, as well as its mold-to-yeast conversion. Penicillium marneffei is a dimorphic fungus. It exhibits a mold phase at 25-30°C and a yeast phase at 37°C. This phase transition is a reversible process and controllable solely by temperature [19]. Penicillium sp colonies are usually fast growing and often mature within 4 days at 25°C on Sabouraud Dextrose agar (SDA). It produces filamentous with initially flat and subsequently radially sulcate colonies. These colonies are yellowish-green at centre and white at periphery [Figure 2]. As it matures, it becomes reddish yellow in colour and produces a typical red soluble pigment, rapidly diffusing into the medium [Figure 3]. Microscopically, the conidiophores usually posses biverticilliate or univerticilliate penicilli. Each penicillus is composed of three to five metullae, bearing four to seven flask-shaped phialides. Attached to the phialides is a short chain of single-celled, round, smooth-walled to echinulated conidia [20-21]. On the other hand, at 37°C by culturing onto BHIA, Penicillium marneffei colonies are cream to slightly pink in colour and glaborous to convolute in texture (yeast-like colonies) and do not produce the diffusible red pigment as seen in the mold phase [20-21].

Histologically, it is most readily demonstrated by fungal stains such as periodic acid-Schiff and Grocott silver methenamine stains [11,19-21]. The cells of *Penicillium marneffei* are spherical to oval, 3 to 5 micrometer in diameter [19], and resemble those of Histoplasma capsulatum. This may account for early cases being misdiagnosed as histoplasmosis. However, detection of non budding yeast cells with characteristic central transverse septum would give a presumptive diagnosis of *Penicillium marneffei* and this should be confirmed by culture.

Various types of antigen and antibody testing specific to *Penicillium marneffei* have been described but they are not widely available [22]. Galactomannan assays for Aspergillus species was recently approved by US Food and Drug Administration (FDA) to facilitate early diagnosis of invasive aspergillosis. However it is also known to detect the galactomannan of other fungal e.g Penicillium and Paecilomyces species. In addition, Galactomannan is also detected in several drugs that originated from fungal organisms, including amoxicillin-clavulanic acid, piperacillin and piperacillin/tazobactam [23]. Its usage for early detection of penicilliosis in newly diagnosed HIV-patients is not conclusive and need to be elucidated by larger studies. Due to this issues, our centre do not routinely use galactomannan testing for penicilliosis screening among our HIV patients.

On the other hand, an antigenic cell wall mannoprotein (Mp1p) of Penicillium marneffei appears to be promising focus of diagnosis, offering good sensitivity (82%) and specificity (100%) in detection of the infection [24]. PCR assay specific for Penicillium marneffei has been developed in research setting and is not currently available for routine clinical use.

Penicillium marneffei infection in patient with advanced retroviral disease has repeatedly been reported as a fatal disease if left untreated [1-6, 8, 14-18]. Unfortunately, we are not able to report the case fatality rate of *Penicillium marneffei* infection in our institution as this is beyond the scope of this review.

In vitro study has shown that *P. marneffei* is highly sensitive to itraconazole, voriconazole, ketoconazole, miconazole, terbinafine and 5-fluorocytosine, intermediately sensitive to amphotericin B and least sensitive to fluconazole [25]. The recommended therapy for *Penicillium marneffei* infection is two weeks of induction therapy with intravenous amphoteracin B at a dose of 0.6 mg/kg/day, followed by secondary prophylaxis with ten weeks of oral itraconazole at a dose of 400 mg/day and subsequent chronic maintenance therapy with oral itraconazole at 200 mg/day [26-28]. In addition, the role of newer antifungals against penicilliosis is promising and their uses in combination with amphotericin B have been recommended. In vitro combinations of echinocandins such as caspofungin or micafungin with amphotericin B and triazoles showed synergistic antifungal activity [29-30]. Another study has also shown that combination therapy with micafungin would allow for the use of lower doses of amphotericin B and itraconazole without the loss of a clinical response [31]. The secondary prophylaxis can be considered for discontinuation if the CD4 cell count increases to more than 100 cells /ml³ for at least 6 months after the patient has been started on HAART [28, 32]. A combination of appropriate and adequate antifungal therapy with HAART to reconstitute the patients' immune system is crucial to ensure a successful outcome.

In this review, all patients diagnosed with penicilliosis in our centre were given intravenous amphotericin B as the induction therapy. Oral itraconazole was given as a maintenance therapy in one third of our patients. The remaining two third of our patients who are also receiving rifampicin for their pulmonary tuberculosis co-infection, received oral fluconazole for their *Penicillium marneffei* maintenance therapy in view of the fact that itraconazole is contraindicated with rifampicin. The patients who were not yet on HAART were started on HAART upon completion of the induction phase of the penicilliosis.

CONCLUSION

In conclusion, clinicians should make themselves familiar with penicilliosis clinical presentations, investigations and treatment. The key to successful treatment of *Penicillium marneffei* infection is thinking about the diagnosis, sending the appropriate investigations and immediate institution of appropriate antifungal drugs. Our study is limited by the fact that it is only a review on the clinical characteristics of advanced HIV patients who had been successfully treated for *Peniccilium marneffei*. A proportion of patient with advanced HIV who was admitted at the very late stage of their illness with overwhelming sepsis and multiorgan failure could have been infected with *Penicillium marneffei* but were overlooked from this review due to missed opportunity of the attending clinician to make the correct diagnosis and sending an appropriate investigation at the time of the patient's presentation. Nonetheless, our study does shed some light that the incidence of *Penicillium marneffei* infection among HIV population in Malaysia is on the rise since it was first reported in 1995. We believed that a continuous surveillance study to determine *Penicillium marneffei* infection incidence in Malaysia is very appropriate in order to verify the endemicity of this infection in our country. Such knowledge is very useful to improve our understanding towards better control of this emerging infectious disease. A prospective study of *Peniccillium marneffei* infection involving a large number of immunocompromised patients may give us an opportunity to assess our ability to make early diagnosis based on our observed clinical characteristics, attest the various available diagnostic methods and explore the patient's long term outcome subsequent to reconstitution of their immune systems. The findings from such study may permit us to recommend a scoring system for *Penicillium marneffei* infection.

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