



Full Length Research Article

**A STUDY OF PENICILLIOSIS MARNEFFEI IN AIDS PATIENTS AND ITS CO-RELATION
WITH CD4 COUNT**

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ABSTRACT

Objectives: *Penicillium marneffeii*, the causative agent of Penicilliosis is a common opportunistic fungal infection in AIDS patients in Manipur. A preliminary study was conducted to determine the prevalence of *P. marneffeii* and to study its co- relation with CD4 count from AIDS patients in Manipur.

Methods: A total of 200 samples from 161 patients – 17 HIV positive and 144 AIDS patients attending the Microbiology Department of Regional Institute of Medical Sciences (RIMS), Imphal for CD4 and CD8 count and from the different wards of RIMS hospital were studied for *P. marneffeii* infection. The samples included skin scrapings (60), sputum induced (10), sputum coughed out (100), corneal swabs (9), pleural fluid (7) and lymph node aspirates (5). They were processed by observing under 20% KOH mount for the presence of round oval or sausage shaped yeast cells with characteristic septation. Culture was done on two Sabouraud's Dextrose agar (SDA) with chloramphenicol and incubated at 37°C and 25°C for two weeks. The isolates were identified by the conventional method. CD4 / CD8 count was estimated by using FACS count system (Becton Dickinson).

Results: *P. marneffeii* was isolated from 18(11.18%) AIDS patients. Mixed infection of *P. marneffeii* with one or more fungi was observed in 9 (5.5%). CD4 cell counts of patients were in the range of (6 – 182 cells/ μ l).

Conclusion: This study provides supportive evidence of the prevalence of *P. marneffeii* which was 11.18% amongst the AIDS patients of Manipur. Moreover, the fungus was isolated mainly from full blown AIDS patients with low CD4 count.

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INTRODUCTION

Penicilliosis caused by the dimorphic fungus *Penicillium marneffeii* is an AIDS indicator disease in the South East Asia and the third most common systemic infection in AIDS. The common clinical features are fever, anorexia, weight loss, anaemia, and hepatosplenomegaly and skin lesions resembling molluscum contagiosum. In India, Manipur, a northeastern border state is the only endemic focus for this fungus. The disease has been reported from AIDS patients in Manipur, one of the six high prevalent states of HIV/AIDS in India. The present study aims at finding out the prevalence of *Penicillium marneffeii* amongst HIV/AIDS patients and its co-relation with CD4 count.

MATERIALS AND METHODS

The study included 161(17 HIV positive and 144 AIDS) patients of which 60 were inpatients admitted in the different

wards of RIMS hospital, Imphal and 101 were outpatients attending the Microbiology Department for CD4 and CD8 lymphocyte count during September 2003 – August 2004. Approval was taken from the ethical committee of RIMS and informed consents of the patients were obtained in the prescribed proforma. A total of 200 samples from 161 patients were collected. The samples were taken depending on the clinical suspicion. The inclusion criteria for selecting the sample were patients having suspected signs and symptoms of systemic infection and exclusion criteria were the asymptomatic HIV positive patients with no signs and symptoms.

They were skin scrapings (60), sputum induced (10), sputum coughed out (100), corneal swabs (9), blood (9), pleural fluid (7), lymph node aspirate (5). All the specimens were processed in the Microbiology Department by doing a direct microscopic examination using 20% KOH, Gram stain for sputum, Giemsa stain for skin scraping, lymph node aspirate etc. They were cultured on Sabouraud's dextrose agar with chloramphenicol and incubated each at 37°C and 25°C. Brain heart infusion

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agar (biphasic medium) was used for blood culture. All the cultures (Sabouraud's Dextrose agar and Brain heart infusion agar) were prepared in house using commercially available dehydrated media (Hi Media Laboratories, Mumbai, India) according to the manufactures' recommendation, checked for sterility and store at 2-8^o C till use. Slide cultures were done whenever necessary. Repeated cultures were also done on subsequent days to rule out contamination except for three cases – skin lesions where in patients were not available for follow up.

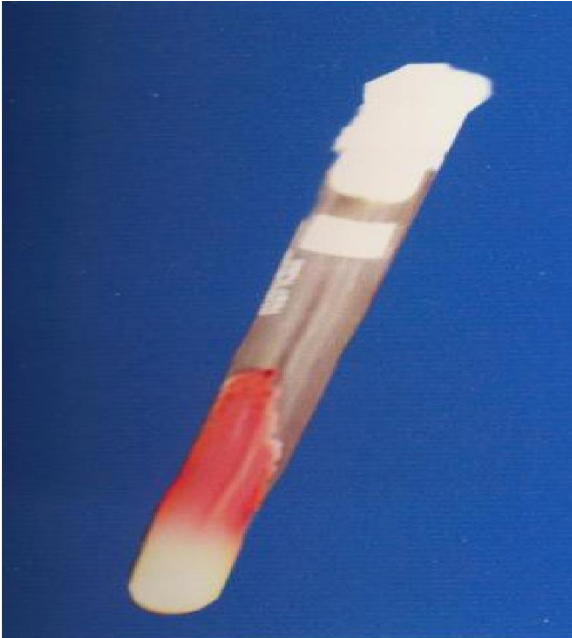


Fig. 1. Penicillium marneffeii – culture at 25^oC showing diffusible red pigment

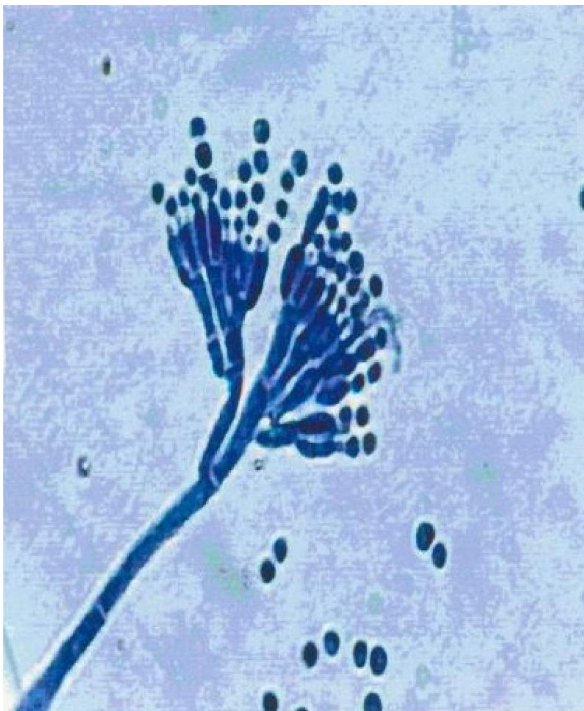


Fig. 2. Penicillium marneffeii – LCB mount from SDA/25^oC showing conidiophores with phialides singly or in groups; giving rise to branched metulae with conidia in chains- brush-like appearance (40x)

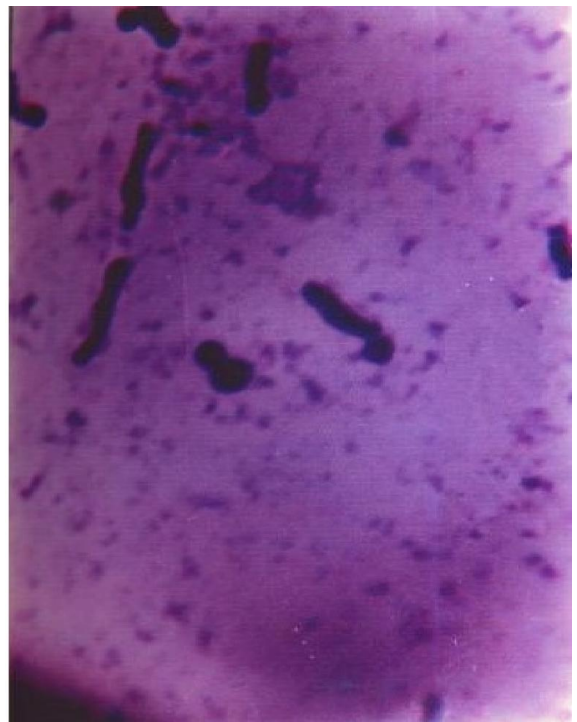


Fig. 3. Penicillium marneffeii – Gram stain showing dividing yeast cells, sausage- shaped and pleomorphic forms from SDA culture at 37^oC(100x)



Fig. 4. Molluscum – Contagiosum like skin lesions of disseminated Penicilliosis

Cultures were identified by the conventional methods, i.e., by direct microscopy using lacto phenol cotton blue (LCB) mount and confirmation was done by conversion of mould form to the yeast form by growing on enriched media like Brain Heart in fusion agar incubated at 37^oC Penicillium was identified by its characteristic colony morphology with red diffusible pigment at 25^o C and microscopic examination of LCB mount of the culture showing conidiophores with conidia

characteristics of *Penicillium spp.* Microscopic morphology of the fungus at 37°C was intra or extra cellular pleomorphic, round, oval or sausage shaped yeast like cells dividing at the central septum by fission. Estimation of CD4 and CD8 lymphocyte count was done by the FACS count system (Becton – Dickinson) using whole blood as per manufacturer's instructions.

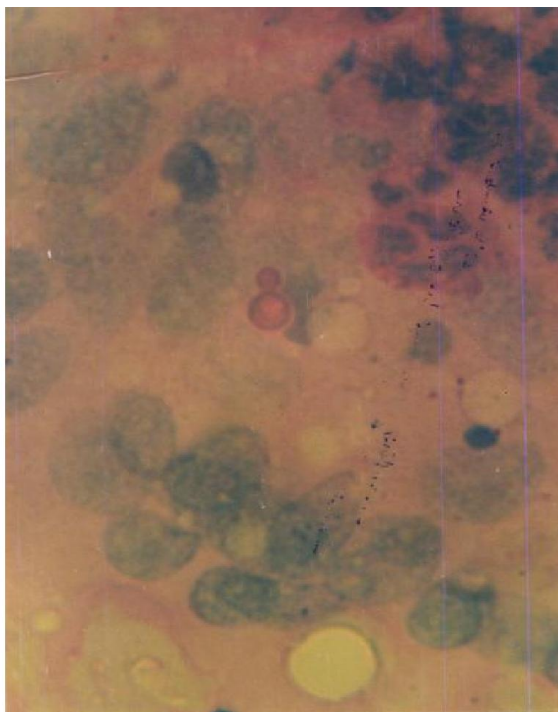


Fig. 5. *Penicillium marneffei* – budding yeast from tissue extract stained by Giemsa stain (40x)

RESULTS

Maximum number of patients (132) belonged to the age group of 21 – 40 years with (male) 108 preponderance (Table 1). Characteristic septate yeast cells could be seen in 1 sputum and 2 skins scraping by Gram stain and Giemsa stain.

Table 1. Age and sex distribution in the study group

Age group	Male	Female
0 – 20	5	8
21 – 40	108	24
41 – 60	10	6
Total	123	38

Table 2. Clinical specimens from where *P. marneffei* isolated

Specimen type	Total no. of specimens	<i>P. marneffei</i> Positive %
Skin scraping	60	10 (16.6%)
Sputum (induced)	10	5 (50%)
Sputum (coughed out)	100	7 (7%)
Blood	9	Nil
Corneal swab	9	Nil
Pleural fluid	7	Nil
Lymph node aspirate	5	Nil
Total	200	22

Table 3 shows that weight loss of more than 10% body weight was the most frequent complaint of these patients – 96 (60%), followed by cough / chest pain – 90 (56%) and anaemia 78

(47%) was the third most common finding. In 6 patients there were mixed infection of *P. marneffei* with *Candida* (33%) 2 of them were isolated from skin scrapings, 2 from sputum (induced) and 2 from sputum (coughed out).

Table 3. Signs and symptoms of patients with *P. marneffei* infections

Symptoms/Signs	Number	Percentage (%)
Fever	48	30
Weight loss >10% body weight	96	60
Night sweats	35	22
Anaemia	78	47
Skin lesions	40	25
Cough / Chest pain	90	56
Diarrhoea	13	8
Hepatosplenomegaly	19	12
Lymphadenopathy	16	10

Table 4. Mixed isolation of *P. marneffei* with other fungus

Fungal Isolation	Number	Percentage (%)
<i>Candida spp.</i> And <i>P. marneffei</i>	6	33
<i>Cr. neoformans</i> and <i>P. marneffei</i>	3	16.6

Table 5. Distribution of CD4 + T lymphocytes and *P. marneffei* isolated in the study group

CD4/ μ l	No. of patients	<i>P. marneffei</i> isolated
≥ 500	17	Nil
200 – 499	30	Nil
100 – 199	21	3
6 – 99	93	15*
Total	161	18

* Mixed infection seen

6 with *Candida spp.*

3 with *Cryptococcus neoformans*.

Mixed infection of *P. marneffei* with *Cryptococcus neoformans* was seen in 3 (16.6%) isolated 2 from skin scrapings and 1 from sputum (coughed out) as shown in Table 4. The CD4 count of the 161 patients is shown in Table 5. Fungal infection were found in patients whose CD4 count is below 200 and the frequency of *P. marneffei* infection was much more in patients with CD4 count <100. Three of these patients had CD4 count as low as 6 (1.4%). The CD4/CD8 ratio ranged from (0.02 – 0.04) as shown in Table 6.

Table 6. CD4, CD8 + T lymphocyte count and CD4/CD8 ratio in *P. marneffei* isolated patients

CD4	CD8	CD4:CD8
43	1041	0.04
6	392	0.02
97	901	0.10
28	1015	0.03
141	1027	0.14
23	205	0.11
	1433	0.13
8	79	0.10
172	847	0.20
10	260	0.04
27	1011	0.02
180	1401	0.12
147	364	0.40
54	235	0.23
90	893	0.10
40	1002	0.03
153	1216	0.13
43	1077	0.03

DISCUSSION

P. marneffeii, the causative agent of penicilliosis is classified under the class Ascomycetes, Genus *Penicillium* and Sub-Genus *Biverticillium*. It was first discovered by Capponi *et al.*, from the liver of a bamboo rat (*Rhizomys sinensis*) in South Vietnam (Capponi *et al.*, 1956). *P. marneffeii* is the only pathogenic species in this genus. The first human infection was reported from a 61 years old missionary from Thailand with Hodgkin's disease who underwent splenectomy within a year of touring South East Asia and *P. marneffeii* was isolated from the necrotic material of the spleen (Peto *et al.*, 1988). Primarily, the disease is geographically restricted to South East Asia and far East including Myanmar, Cambodia, Southern China, Laos, Malaysia, Thailand, Vietnam and now extending far West up to Manipur.

Manipur is small border state, which is ecologically and culturally different from the main land India. Geographically, climatically, ecologically and culturally Manipur resembles more closely with the South East Asian countries where *P. marneffeii* is prevalent than with India. This fungus has been stated to be commonly found in the environment (Yuen *et al.*, 1986). The route of entry of *P. marneffeii* into the body is still unknown although the respiratory route has been suggested (Hilmarsdottir *et al.*, 1993). The clinical manifestations of penicilliosis include fever, weight loss, night sweats, anaemia, skin lesions, cough, diarrhoea, hepatosplenomegaly, and lymphadenopathy and lung involvement⁷. Skin lesions are a common sign of disseminated penicilliosis. The lesions are papular with central necrotic umbilications like that of molluscum contagiosum. Septate yeast like organisms seen in skin biopsy/scraping is diagnostic of *P. marneffeii*.

Penicilliosis is an emerging fungal infection amongst AIDS patients in Manipur (Singh *et al.*, 1999). The endemicity of *P. marneffeii* in Manipur has been thought to have spread from Myanmar with Manipur sharing its eastern and southeastern borders. It stands second amongst the opportunistic fungal infections; the first being oropharyngeal candidiasis and third being cryptococcosis (Usharani *et al.*, 2005). But, in endemic areas like South East Asia, Penicilliosis stands third amongst the common systemic infections in AIDS patients after *Mycobacterium tuberculosis* and *Cryptococcus neoformans* in prevalence and incidence (Siham, 2004). Its infection increases when the CD4 count decreases below 100 (Helen *et al.*, 1998). Disseminated penicilliosis and multiple fungal infections occur when the CD4 count is <50 cells/ μ l (Deng *et al.*, 1988). In the present study also multiple fungal infections was found when the CD4 count was <100 cells/ μ l *Penicillium marneffeii* infection in HIV-infected patients has been considered an AIDS defining event (Sathapatayavongs *et al.*, 1989).

An infection with *P. marneffeii* causes serious infections in AIDS patients which may eventually lead to death if left untreated. Cell mediated immunity plays a major role in eradicating *P. marneffeii* infection; dissemination being more common in AIDS patient with low CD4 count. A co-relation between increase in AIDS and penicilliosis has been suggested (Supparatpinyo *et al.*, 1994). Penicilliosis has been reported in immunocompetent patients from China, Thailand and Hong

Kong (Wong *et al.*, 2001), but in Manipur, *P. marneffeii* infections have not been reported from immunocompetent patients. The clinical symptoms and signs of *P. marneffeii* infections are non-specific as are the haematological and biological findings and may evoke various disseminated infections. As a result microbiological investigations should include a search for fungal agents by direct examination and culture of tissues and body fluid specimens (Baveja and Sokhey, 1999). In many instances *P. marneffeii* can be isolated from samples such as mucocutaneous scrapings, sputum, and oral mucosa indicating that invasive procedures are not always necessary to establish the diagnosis. Cutaneous involvement is common in disseminated penicilliosis and when recognized permits rapid identification of the causative agent. The development of a specific skin test or serological test would be useful to establish the precise geographic distribution of *P. marneffeii* and to evaluate latent and sub clinical infections in this part of the country.

Conclusion

P. marneffeii infection is found in 11.18% AIDS patients in this part of the country. Association of *P. marneffeii* infection was found when the CD4 count was <100 cells/ μ l. Mixed fungal infection was found when the CD4 count was <50 cells/ μ l.

REFERENCES

- Baveja, U.K. and Sokhey, J. 1999. Editors. Manual on Laboratory Diagnosis of common opportunistic infections associated with HIV/AIDS. Government of India, p76.
- Capponi, M.P., G. Sureau, P. and Segretain, G., Penicilliosis de *Rhizomys sinensis*. 1956. *Bull. Soc. Pathol. Exot. Filiales* 49:418 – 421.
- Deng, Z., Ribas, J.L., Gibson, D.W. and Connor, D.H. 1988. Infections caused by *Penicillium marneffeii* in China and Southeast Asia. Review of eighteen cases and report of four more Chinese cases *Rev. Infec. Dis.*, 10: 640 – 52.
- Duong, T.A. 1996. Infection due to *Penicillium marneffeii* as emerging pathogen. Review of 155 reported cases. *Clin. Infect Dis.*, 23: 125 – 30.
- Helen Mc. Shane, Christoph M. Tang and Christopher P. Conton, 1998. Disseminated *Penicillium marneffeii* infection presenting as a right upper lobe mass in an HIV positive patient. *Thorax*, 53: 905 – 906.
- Hilmarsdottir, I., Meynard, J.L., Rogeaux, O., Guermonprez, G. and Detry, A., Katlama, C. *et al.* 1993. Disseminated *Penicillium marneffeii* infection Associated with Human Immunodeficiency Virus: A report to two cases and a review of 35 published cases. *J. Acquir Immuno Defic. Syndr.*, 6(5), 466 – 471.
- Peto, T.E.A., Bull, R., Millard, P.R., Mackenzie, D.W.R., Campbell, C.K. and Haines, M.E. *et al.* 1988. Systemic Mycosis due to *Penicillium marneffeii* in a patient with antibody to Human Immunodeficiency Virus. *J. Infect.* 16:285 – 290.
- Sathapatayavongs, B., Damrongkitchaiporn, S., Saengditha, P., Kiatboonsri, S. and Jayanetra, P. 1989. Disseminated Penicilliosis associated with HIV infection. *J. Infect.* 19: 84 – 5.

- Singh, P.N., Ranjana, K, Singh ,Y.I., Singh, K.P., Sharma, S.S., Singh, M.K. *et al.* 1999. Indigenous disseminated *Penicillium marneffeii* infection in the state of Manipur, India: report of four autochthonous cases. *J. Clin. Microbiol.*, 1999 Aug; 37 (8): 2699 – 702.
- Supparatpinyo, K., Khamwan, C., Baosoung, V., Nelson, K.E. and Sirisanthana, T. 1994. Disseminated *Penicillium marneffeii* infection in Southeast Asia. *Lancet*, 344:110-3.
- Usharani, M., Sulochana, Kh., Lokhendro, H. and Brajachand, Ng. 2005. A preliminary study on CD4 counts on AIDS patients with fungal infection. *J. Commun. Dis.*, 37 (2): 139 – 43. *SIHAM Newsletter*, Issue 03, June 2004, p4.
- Wong, S.S., Wong, K.H., Hui, W.T., Lee, S.S., Lo, J.Y, Cao, L. *et al.* 2001. Difference in clinical and laboratory diagnostic characteristics of *Penicillium marneffeii* in Human Immuno Deficiency Virus (HIV) and non – HIV infected patients. *J. Clin. Microbiol.*, 39(12): 4535 – 40.
- Yuen, W.C., Chan, Y.F., Loke, S.L., Seto, W.H., Poon, G.P., and Wong, K.K. 1986. Chronic lymphadenopathy caused by *Penicillium marneffeii*; a condition mimicking tuberculous lymphadenopathy. *Br. J. Surg.*, 73: 1007 – 1008.
