PNEUMOCYSTIS CARINII INFECTION AMONG HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED MYANMAR PATIENTS

Kay-Thwe-Han[1], Rai-Mra[2], Htin-Aung-Saw[3], Ye-Htut[1] and Wynn-Naing[2]

[1]Parasitology Research Division, Department of Medical Research (Lower Myanmar); [2]Clinical Research Unit (HIV); [3]Specialist Hospital, Waibargi, Myanmar

Abstract. A total of 60 HIV infected patients complaining of dry cough for at least two weeks and attending the Out-patient Department of the Specialist Hospital, Waibargi, were screened for *Pneumocystis carinii*. Induced sputum samples were examined with Giemsa and Gomori silver methenamine stains. *P. carinii* were detected in 18 patients (30%) with silver stain and 13 patients (21.7%) with Giemsa stain. The sensitivity and specificity of the Giemsa stain were 72.2% and 95.2%, respectively. The range of CD4 counts in *P. carinii*-positive patients was found to be 0-562/µl, and the mean CD4 count was 132.3/µl. Out of 18 *P. carinii*-positive cases, CD4 counts of 15 cases (83.3%) were <200/µl and those of 3 cases were >200/µl. Clinically, *P. carinii*-positive cases were associated with fever in 55.5%, with tightness of the chest in 38.9%, and with cyanosis and tightness of the chest in 11.1%. Co-infection with tuberculosis was found in 16.7%. Anti-pneumocystic prophylaxis is recommended for those patients with a CD4 count <200/µl. Giemsa staining could be used as an alternative diagnostic method for detecting *P. carinii*. This study documented the existing prevalence of *P. carinii* among HIV-infected Myanmar patients.

INTRODUCTION

P. carinii was originally classified as a protozoan parasite because of its morphologic similarities with parasites and its response to antiprotozoal agents. However, the ultrastructure and staining characteristics are consistent with fungi (Santamauro and Stover, 1997). *P. carinii* is responsible for the most common and life-threatening pulmonary complication: *Pneumocystis carinii* pneumonia (PCP) in HIV patients. It is usually nonpathogenic and its replication takes place when T helper cells are deficient. Early in the AIDS epidemic, 75% of patients developed PCP at some point in their illness. With initiation of anti-retroviral therapy and prophylaxis against PCP, the incidence of PCP declined in the late 1980s in Europe (Santamauro and Stover, 1997). The incidence of PCP in HIV patients was assumed to be low in developing countries (Bernad *et al*, 1992). However, PCP is still the leading complication in HIV patients today (Armengol, 1995). Diagnosis of *P. carinii* depends on the type of sample collected and the experience of the technician. The induced sputum sample has been reported as the most sensitive non-invasive sample (Santamauro and Stover, 1997; Masur *et al*, 1989). Silver staining is the gold standard for diagnosing *P. carinii* (Cregan *et al*, 1990). Therefore, this study aims to explore the prevalence of *P. carinii* in HIV-infected Myanmar patients, the sensitivity of Giemsa stain compared with silver stain in detecting *P. carinii*, and the relationship between PCP and CD4 counts of patients, and the survival of PCP patients.

Correspondence: Dr Kay-Thwe-Han, Parasitology Research Division and Clinical Research Unit (HIV), Department of Medical Research (Lower Myanmar), Waibargi, Myanmar.
Tel: +95 01 251508 ext 153

MATERIALS AND METHODS

The study was conducted at the Specialist Hospital, Waibargi, from July 2000 to October 2001. A total of 60 HIV patients presenting with nonproductive (dry) cough for at least 2 weeks, with no history of PCP prophylaxis, were enrolled into the study after obtaining informed consent. The detailed histories and CD4 counts of the enrolled patients were also noted. Induced sputum was collected following deep inhalation of nebulized hypertonic saline by patients. After centrifugation, the sample was made into smears to be stained with Gomori silver methenamine stain and Giemsa stain. The sensitivity and specificity of the Giemsa stain were analyzed in comparison with the silver stain. The prevalence of PCP was correlated with CD4 counts of the host and finally survival of PCP was studied.

RESULTS

Prevalence rate of *P. carinii*

Among 60 induced sputum samples, *P. carinii* were detected in 18 samples (30%) by Gomori silver stain and 13 samples (21.7%) by Giemsa stain (Table 1 and Fig 1). The sensitivity and specificity of Giemsa staining were 72.2% and 95.2%, respectively, compared with Gomori silver methenamine staining.

PCP and CD4 counts

The range of CD4 count of all enrolled patients was 0-876/µl and that of *P. carinii* positive patients was 0-562/µl. Out of 18 *P. carinii*-positive patients, the CD4 counts of 15 cases (83.3%) were <200/µl and those of 3 cases (16.7%) were >200/µl. The mean CD4 count of *P. carinii*-positive cases was 132.3/µl (SD=157.6).

Clinical presentation

Among 18 *P. carinii* positive cases, ten patients (55.5%) had cough with fever, seven cases (33.3%) had cough with tightness of the chest and two patients (11.1%) had cough with tightness of the chest and cyanosis. Three patients were co-infected with pulmonary tuberculosis.

Survival of the *P. carinii*-positive patients

The survival rates of the HIV-infected patients with PCP were compared with those of the HIV-infected patients without PCP using the Kaplan-Meier survival curve (Fig 2). HIV infected patients with PCP were found to have less chance of survival, especially four months after being diagnosed with PCP (p=0.086).

DISCUSSION

PCP in HIV patients has been assumed to be a disease of developed countries, since it has been epidemic together with AIDS in most European countries. Initiation of anti-pneumocystic prophylaxis, early diagnosis and prompt treatment caused PCP prevalence to decline in those countries, while developing countries were confronting PCP with limited facilities. The fact that the diagnosis of *P. carinii* is costly, time consuming and difficult, might cause lower apparent prevalence than the actual situation in the developing world. This study shows that PCP does exist among HIV-infected Myanmar patients, at a rate of 30%. That prevalence rate is comparable to those of Zimbabwe; 22% (McLeod *et al*, 1989) and 33% (Malin *et al*, 1995; and Malaysia, 22.7% (Cheong *et al*, 1997).

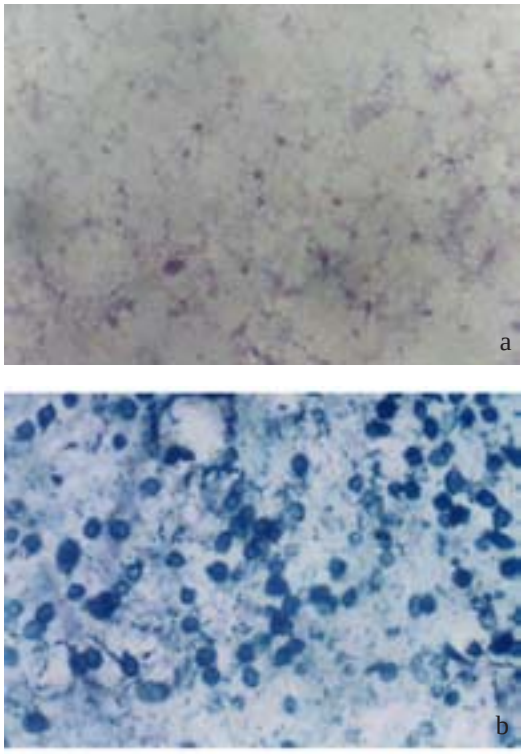

Fig 1–*Pneumocystis carinii* cysts in (a) Giemsa stain, (b) Silver stain.

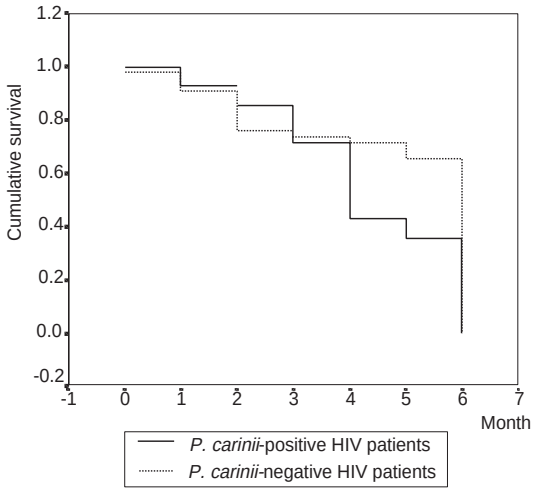

Fig 2–Survival of *Pneumocystis carinii*-positive HIV patients.

For detecting *P. carinii*, the silver staining method was found to be superior to Giemsa staining in this study. Since it stains the wall of the *P. carinii* cyst without staining background materials, the silver staining method is also more sensitive and specific. Another advantage is that it can

Table 1
Sensitivity and specificity of Giemsa stain.

Diagnostic test	Silver stain		Total
	Positive	Negative	
Giemsa stain			
Positive	13	2	15
Negative	5	40	45
Total	18	42	60

differentiate *P. carinii* from *Candida* species, which is the most commonly seen fungus in sputum samples. The cyst wall of *P. carinii* is stained brownish black without staining intracystic ingredients, whereas the whole fungus is uniformly stained. Therefore, interpretation of a silver-stained smear does not need expertise. Giemsa stain is readily available and inexpensive. The staining method is simple, traditional and quick. It stains both intracystic ingredients of *P. carinii* (without staining the cyst wall) and background materials. Therefore, it could cause false negative results if the yield of organisms is very few. However, it should be recommended as an alternative diagnostic method where silver stain is not available.

The mean CD4 count of *P. carinii*-positive cases was found to be 132.3/μl, and the range was 0-562/μl. Out of 18 *P. carinii* positive cases, 83.3% had CD4 counts <200/μl. It is not surprising that PCP is encountered at this stage. Three *P. carinii*-positive cases had CD4 counts >200/μl. This indicated that PCP could set in, in high CD4 counts (the highest one was a CD4 count of 562/μl). Therefore, a high CD4 count does not exclude the occurrence of PCP, and HIV patients with dry cough and CD4 count <200/μl should be investigated for *P. carinii* as a routine procedure. Those *P. carinii*-positive should be treated promptly, while those *P. carinii*-negative should be treated with anti-pneumocystic (cotrimoxazole) prophylaxis. In this way, PCP prevalence would decline to some extent and the morbidity and mortality due to PCP would also be reduced.

By studying the Kaplan-Meier survival curve, HIV patients with PCP were found to have less chance of survival compared with those without PCP. Since achieving better quality and longer survival is the mainstay in managing HIV-infected patients, prevention, postponement and reduction of frequency of opportunistic infection is essential. Therefore PCP, an easily preventable and treatable opportunistic infection, should not be ignored.

Conclusion

The study shows the existing prevalence of *Pneumocystis carinii* among HIV-infected Myanmar patients. Giemsa staining, a readily available, inexpensive, simple and quick method, could be used as an alternative diagnostic method to detect *P. carinii*. A high CD4 count does not exclude the occurrence of PCP in HIV patients. Since it is a preventable and treatable opportunistic infection, the study provides evidence to recommend anti-pneumocystic prophylaxis (cotrimoxazole) in HIV patients.

ACKNOWLEDGEMENTS

This study was funded by the Department of Medical Research (Lower Myanmar). The authors would like to express their gratitude to the Medical Superintendent of the Specialist Hospital, Waibargi and also to Dr Maung Maung Toe for expert opinions on statistics. Thanks are also due to staff of the Specialist Hospital and staff of the Parasitology Research Division, Department of Medical Research (Lower Myanmar).

REFERENCES

Armengol CE. A historical review of *Pneumocystis carinii*. *JAMA* 1995; 273: 747-51.

Bernad EM, Sepkowitz KA, Telzak EE, Armstrong D. Pneumocystosis. *Med Clin North Am* 1992: 76; 107-19.

Cheong I, Lim A, Lee C, Ibrahim Z, Sarvanathan K. Epidemiology and clinical characteristics of HIV-infected patients in Kuala Lumpur. *Med J Malaysia* 1997: 52; 313-7.

Cregan P,Yamamoto A, Lum A, VanDerHeide T, MacDonald M, Pulliam L. Comparison of four methods for rapid detection of *Pneumocystis carinii* in respiratory specimens. *J Clin Microbiol* 1990; 28: 2432-6.

Malin AS, Gwanzura LK, Klein S, *et al. Pneumocystis carinii* pneumonia in Zimbabwe. *Lancet* 1995; 346: 1258-61.

Masur H, Lane HC, Kovacs JA, Allegra CJ, Edman JC. NIH Conference on Pneumocystis Pneumonia: from bench to clinic. *Ann Intern Med* 1989; 111: 813-26.

McLeod DT, Neill P, Robertson VJ, *et al.* Pulmonary diseases in patients infected with the human immunodeficiency virus in Zimbabwe, Central Africa. *Trans R Soc Trop Med Hyg* 1989; 83: 694-7.

Santamauro JT, Stover DE. *Pneumocystis carinii* pneumonia. *Med Clin North Am* 1997; 81: 299-319.