Thesis Title Strain Typing by PCR Fingerprinting and Drug Sensitivity of *Cryptococcus neoformans*

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ABSTRACT

Cryptococcosis, an infection caused by *Cryptococcus neoformans* has been recognized for many years. The incidence of cryptococcal meningitis has increased along with the AIDS epidemic. At present, cryptococcosis is the most common opportunistic fungal infection of AIDS patients in Thailand and worldwide. In 1994 to 1995 at Siriraj hospital in Bangkok, Thailand, the number of cryptococcosis cases increased to 86, of which 77 were diagnosed as having AIDS. The strains typing of *C.neoformans*, the etiologic agent of cryptococcosis should provide important information for epidemiological study.

The objectives of this study were to assess the RAPD technique for typing strains of *C.neoformans* by comparing with serotyping, and to undertake *in vitro* susceptibility test of *C.neoformans* with four antifungal agents (amphotericin B, ketoconazole, fluconazole and itraconazole) by macrodilution technique.

One-hundred and six strains of *C.neoformans* were cultured from CSF recieved from 86 patients. The random single-oligonucleotide primer $(GACA)_4$ and the phage M 13 core sequence (5'GAGGGTGGXGGXTCT 3') were selected to amplify hypervariable and repetitive sequence from all serotypes of *C.neoformans* (A, B, C and D). The patterns of PCR fingerprinting method produced by these primers could clearly distinguish variation among serotypes of *C.neoformans*. There were 104 isolates of serotype A (98.1%), 1 isolate of serotype B (0.94%) and 1 isolates revealed serotype D (0.94%). All *C.neoformans* serotype A strains could not be distinguished from each other by this PCR fingerprinting.

The *in vitro* susceptibility test was performed with all 106 strains of *C.neoformans* by broth macrodilution method. Four antifungal agents such as amphotericin B, ketoconazole, fluconazole and itraconazole were tested. The MIC of each strain and the mean MIC of four antifungal agents against *C.neoformans* were recorded after incubation at 37°C for 24 and 48 hour. The mean MIC of amphotericin B, itraconazole, ketoconazole and fluconazole at 24 hour were 0.67, 0.38, 13.13 and 7.11 μg/ml respectively, these were correspond very well with results from other groups.

The results of our study showed that PCR fingerprinting method can distinguished *C.neoformans* in different serotypes . The most common serotype of *C. neoformans* found was serotype A which was the same as in many areas of USA, Germany and Japan. The PCR fingerprint could be used as a tool for epidemiological study of cryptococcosis. Moreover, the MIC of four antifungal agents against *C.neoformans* could be used as a supporting data for cryptococcosis treatment.