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## Cryptococcal Meningitis among AIDS Patients in Jakarta

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### Abstract

Cryptococcosis is a common opportunistic infections in AIDS and caused by the encapsulated yeast *Cryptococcus neoformans*. The predominant clinical manifestation in AIDS patients is meningitis. For the establishment of diagnosis, India ink test and culture were done. Serology test to detect capsular antigen *glucoronoxylomannan* (GXM) was done to support the diagnosis of cryptococcosis. The aim of this study is to know the prevalence of cryptococcosis among AIDS patients with CNS involvement in Jakarta and its surrounding places. The study was conducted from 2005 – 2007 at the Mycology laboratory Department of Parasitology, University of Indonesia, Faculty of Medicine. Spinal fluid from 154 AIDS patients with CNS involvement from Cipto Mangunkusumo hospital and other hospitals around Jakarta were tested using India ink test and cultured on sabouraud dextrose agar (SDA) and bird seed agar (BSA) and 48 were tested by latex agglutination test to detect GXM antigen. Out of 154 spinal fluids tested by india ink and culture, 32 (20,77%) samples contained *Cryptococcus*. GXM antigen was detected in 29 out of 48 samples. From 29 samples with GXM antigen, eight samples were also positive after mycology examination. The prevalence of cryptococcosis among AIDS patients with CNS involvement in Jakarta is 20.77%.

**Key words:** spinal fluid, *Cryptococcus neoformans*, prevalence, GXM antigen

## Meningitis *Cryptococcus* pada Penderita AIDS di Jakarta

### Abstrak

Kriptokokosis yang disebabkan oleh khamir *Cryptococcus neoformans* merupakan infeksi oportunistik pada AIDS, dan meningitis adalah manifestasi klinis yang paling sering ditemukan. Untuk menegakkan diagnosis digunakan pemeriksaan tinta India dan kultur pada media agar. Uji serologi untuk deteksi antigen kapsular *glucoronoxylomannan* (GXM) dapat digunakan untuk mendukung penegakan diagnosis. Penelitian ini bertujuan untuk mengetahui prevalensi kriptokokosis pada penderita AIDS dengan gangguan serebral yang dilaksanakan sepanjang April 2005 – February 2007. Sebanyak 154 cairan otak penderita AIDS dari daerah Jakarta dan sekitarnya diperiksa dengan sediaan tinta India, dan kultur pada agar sabouraud dekstroze (ASD) dan *bird seed agar* (BSA). Dari 154 sampel, hanya 48 sampel yang menjalani pemeriksaan antigen GXM dengan metode agglutinasi lateks. Dari 154 cairan otak yang diperiksa dengan tinta india dan kultur 21 (20,77%) sampel mengandung *Cryptococcus*. Antigen GXM terdeteksi pada 29 dari 48 sampel. Dari 29 sampel yang positif antigen GXM, delapan positif mengandung *Cryptococcus*. Prevalensi kriptokokosis pada penderita AIDS dengan gangguan SSP di Jakarta sebesar 20,77%.

**Kata kunci:** cairan otak, *Cryptococcus neoformans*, prevalensi, antigen GXM

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## Introduction

The *acquired immunodeficiency syndrome* (AIDS) is a syndrome that occurs due to the damage of immune system because of HIV infection.<sup>1,2</sup> The impaired immune system results in loss of capability to cope with infections, thus AIDS patients are easily infected by bacteria, virus or fungi or so called opportunistic infections (OI). One of the most important opportunistic infection in AIDS is cryptococcosis, caused by the encapsulated yeast *Cryptococcus neoformans*.<sup>2-5</sup>

*Cryptococcus* enters the human body via inhalation into the lung. Due to AIDS the fungus easily disseminates and infects many organs, but the most preferred is the brain where it causes meningitis. Cryptococcosis is distributed world wide and its prevalence in the world is ranged between 2.09% - 72% of HIV infected patients. Cryptococcal meningitis is the main cause of death among AIDS patients and its mortality rate is 13-44%.<sup>3-10</sup> According to Park *et al.*,<sup>10</sup> Southeast Asia after sub-Saharan Africa, is number two in the number of AIDS patients with cryptococcal meningitis i.e. 120 000 cases; range 24 000–216 000 with mortality rate 1.2 in 1000 cases.

India ink test and culture, both are laboratory methods for the diagnosis of cryptococcal meningitis. In addition, the glucuronoxylomannan (GXM) cryptococcal antigen may support the diagnosis.<sup>3</sup>

Currently, in Indonesia the number of HIV infected patients shows a steep rise, but the data on opportunistic infections including cryptococcosis are rare. This report will be the first report on cryptococcal meningitis among AIDS patients in Jakarta.

## Materials and Method

This study is part of the public service of the Mycology Laboratory in the Department

of Parasitology, Fakultas Kedokteran Universitas Indonesia, Jakarta during the years 2005-2007. The samples investigated were spinal fluid sent to our laboratory for the diagnosis of fungal infections. All samples were obtained from AIDS patients with cerebral involvement and they were examined by India ink test and culture. Most of the samples came from Cipto Mangunkusumo Hospital and only a small portion from other hospitals in Jakarta. All samples were tested by India ink and culture on sabouraud dextrose agar (SDA) and bird seed agar (BSA). Inoculation on BSA was conducted for easy identification, since *Cryptococcus* produces melanine and grow as dark brown colony. The cultures were incubated at room temperature (29-30°C) for 2-10 days, and observed every day. The culture was considered negative when after 10 days no fungal occurred on both media. Glucuronoxylomannan antigen detection was done using the latex agglutination method (Pastorex-BIORAD Kit, cat. number 7EM2093, France), according to the manufacturer. To obtain a more comprehensive results, demographic data, age and sex were also recorded.

## Results

During the study, 154 samples from 34 females and 120 males were investigated. Based on mycology investigations (India ink and culture both on SDA and BSA) 32 samples were found to be positive for *Cryptococcus*, consisting of 27 males and five females. Thus the prevalence of cryptococcosis in this patient population was 20.78% (Table). The ratio between male and female was 5.4:1, and the age ranged between 22-38 years.

All isolates studied confirmed as *Cryptococcus* by the melanine production, which shown as dark brown colonies when grown on bird seed's agar.

**Table: Results of Mycology Investigation in Spinal Fluid**

year	n	Positive <i>Cryptococcus</i>		Percentage
		Male	Female	
<b>2005</b>	13			
male	11	3	-	3/13
female	2			
<b>2006</b>	52			
male	42	8	3	21.15
female	10			
<b>2007</b>	89			
male	67	16	2	20.22
female	22			
Total	154	27	5	20.78

Note: the positive result presented in the table is the result of India ink test and culture

Antigen detection was done on 48 samples, and gave a positive result for 29 samples. Out of 29 positive samples, eight also gave positive results in both India ink and culture. The rest were only antigen positive and no fungus could be found in mycology examination.

## Discussion

Cryptococcosis is one of the leading causes of death in HIV infected patients. It was estimated that every year cryptococcal meningitis causes 504 000 deaths in Sub Saharan Africa only and South East Asia comes second.<sup>10</sup> In Indonesia, data on cryptococcosis are rare, so the result of this study will be the first report on the occurrence of cryptococcosis in Jakarta.

Before 2005, cryptococcosis was rarely reported in Indonesia. Epidemiological changes started after the arrival of AIDS pandemic and the number of patients with HIV increased. Some of them entered the phase of AIDS and they became susceptible to opportunistic infections such as cryptococcosis. Mostly, patients in this study visited the hospital in a bad condition, not knowing their health status, particularly

those associated with HIV infection. In most patients cryptococcosis is AIDS *defining illness*.

Based on mycology examination, the prevalence of cryptococcosis of AIDS patients in Jakarta is 20.8% (Table). Thus, the prevalence of cryptococcosis is high in Jakarta when compared to other countries such as India and Thailand.<sup>11-14</sup> In South India, Kumarasamy *et al.*,<sup>11</sup> reported that cryptococcosis occurred in 5% of the HIV infected population at a tertiary care, while Vajpayee *et al.*,<sup>12</sup> reported in northern India prevalence of cryptococcosis of only 1.7%. Laksmi *et al.*,<sup>13</sup> studied 1,863 HIV infected hospitalized patients in India that were suspected to suffer from cryptococcal meningitis. Here the prevalence was 10.86%. In Thailand the prevalence was 20%, which is higher than India but lower than Jakarta.<sup>14</sup> In a two-year study in Malaysia, 96 cryptococcosis cases were reported and most of them suffered from cryptococcal meningitis.<sup>15</sup> A study by Espie'E *et al.*,<sup>16</sup> in Cambodia reported a prevalence of cryptococcal meningitis of 12%.

The prevalence obtained in this study is higher than some western European countries, which ranges between 2-10%.<sup>5,17</sup>

Compared with African and Latin American cryptococcal meningitis prevalence in Jakarta is lower. In Uganda for example, the prevalence reached 53.5%, in South Africa 63% with 18% of them were relapse.<sup>14,18-21</sup> Is climate has effect on the prevalence of cryptococcosis? The weather was very different, Europe with four seasons while Africa possess dry weather and Indonesia is a wet and humid tropical country, which accomodates the grow of the fungus in nature.

The high prevalence of cryptococcosis in Jakarta compared to other Asian countries seem related to the patients studied that were used. In our study, HIV-infected patients showing signs of meningitis, while the results of other studies analyzed the perevalence to the HIV-infected population, except for research.<sup>14</sup> The high prevalence of cryptococcal meningitis in Jakarta is in line with the high number of HIV-infected patients that reached the phase of AIDS and are vulnerable to opportunistic infections. Anti-retroviral drugs (ARVs) can reduce viral load and as a consequence immunity is improved. As result in many parts of the world, especially in developed countries, opportunistic infections in AIDS, including cryptococcosis, are decreasing. However, in developing countries, including Indonesia, most patients do not know their health status especially with regard to HIV infection. Generally they only become aware of this after being infected with opportunistic pathogens. Even though ARV in Jakarta is available, but the burden of cryptococcal meningitis must come into consideration (Table). Furthermore, reports from Africa showed that many patients become clinically manifested for cryptococcal meningitis after taking ARV related to immune function restoration or the immune reconstitution inflammatory syndrome (IRIS).<sup>22-25</sup>

Estimation of global burden of cryptococcal meningitis shows that in

Southeast Asia the burden is 120 cases per year.<sup>10</sup> The number of cryptococcosis in Indonesia may be high, but the data are not yet available. This study was the first report on cryptococcosis and conducted in Jakarta, where diagnosis of fungal infections eventually easily done. Thus, the prevalence of cryptococcosis in Jakarta does not represent the prevalence in Indonesia as a whole.

Differences in prevalence in various countries may related to the source of infection in nature, because cryptococcosis is not transmitted from person to person. In Jakarta pigeon droppings were reported as source of cryptococcal infection.<sup>14,15</sup> Other source of infection may be trees such as *Eucalyptus camaldulensis*, *Terminalia catappa* as already reported in Australia and Brazil.<sup>4,26</sup> The high prevalence of cryptococcosis in Jakarta represent the environment as potential source of infection.

This study showed that the prevalence of cryptococcosis in males is higher than females with a ratio 5.4:1. Similar result were obtained by other studies conducted in Asia and Europe.<sup>5,14,16,17</sup> Fundamental evidence was obtained from a study of Lothorlory *et al*,<sup>27</sup> who found that female mice showed a higher immune activity then male. Female mice produced anti inflamtory cytokines 2.3 times more compared with male. Another study on the influence of the hormones diethylstilbestrol and estradiol on the growth of *Cryptococcus*, revealed that both are able to inhibit its grow.<sup>28</sup> Another study done by McClelland *et al*,<sup>29</sup> showed that *Cryptococcus* isolated from male patients release higher concentration of GXM when incubated with testosteron, while strains isolated from female patients release less GXM. The greater ability to release GXM suggest the greater virulence of the fungus. Further study from the same researcher showed that female macrophages is more efficient in inhibiting of *Cryptococcus*

replication. This may also explain why cryptococcosis is more prevalent in male than female.

The age of patient with cryptococcal meningitis in this study ranged between 22-38 year. Dromer *et al.*<sup>5</sup> conducted a nine-year survey on cryptococcosis in France where the mean age in non-HIV patients was 49 years, while in HIV-infected patients the mean age was younger than 36 year.

For the diagnosis of cryptococcal meningitis, India ink staining and isolation of the fungus from spinal fluid have the highest clinical significance, but antigen detection from the same clinical material can also be used to establish the diagnosis. Out of 48 samples tested for cryptococcal antigen, 29 were positive for GXM antigen and only eight of these 29 samples gave a positive result after mycology investigation. According to De Pauw *et al.*<sup>7</sup>, GXM antigen detection in spinal fluid is sufficient for the diagnosis of cryptococcal meningitis.

When the results of antigen detection and mycology investigations are combined, the number of patients with cryptococcal meningitis becomes 53 out of 154 samples or 34,42%. According to Casadeval and Perfect,<sup>3</sup> the threshold of the yeast concentration approximately circa  $10^3$  colony forming unit (CFU)/ ml to be detected by India ink investigation. Negative result on India ink test and positive result on antigen detection most likely correspond to low concentration of the yeast in the spinal fluid. Culture, even though specific, also has a sensitivity of only 50-80%.<sup>30</sup>

This study revealed the importance of antigen detection for early diagnosis of meningeal cryptococcosis. The existence of antigens in clinical material precedes the appearance of the yeast in traditional mycology investigation, and can be used as early diagnosis. The occurrence of the fungus in the cerebrospinal fluid is usually

indicating advanced and severe infections, resulting in death. Early diagnosis using antigen detection can reduce mortality. World Health Organization,<sup>31</sup> recommend two methods for cryptococcus antigen detection. The previous method is latex agglutination assay and the newest one is a chromatography based method called lateral flow assay (LFA). Both methods can be done in a limited setting laboratory, but LFA is easier and gave faster result.

## Conclusion

This study is the first report on cryptococcosis among HIV infected patients showing signs of meningitis in Jakarta. The prevalence of cryptococcosis based on the result of mycology examination is lower than the prevalence based on antigen detection. Antigen detection seems promising for the diagnosis of cryptococcal meningitis. It seems important for doing antigen detection because of a greater sensitivity which compared to microscopic detection or isolation of the fungus from spinal fluid.

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