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Research Report

CRYPTOCOCCAL ANTIGENEMIA IN HIV/AIDS PATIENTS USING LATERAL FLOW IMMUNOASSAY DETECTION AT DR. SOETOMO GENERAL HOSPITAL SURABAYA

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ABSTRACT

Cryptococcus infection in HIV / AIDS patients results in cryptococcal meningitis, a major cause of subacute meningitis with 100% mortality if not receiving appropriate antifungal therapy. An examination of cryptococcal antigen will provide risk information for patients who will experience cryptococcal meningitis. Better diagnosis in asymptomatic and symptomatic phases of cryptococcosis are key components to reduce morbidity and mortality. This study aims to determine the proportion of cryptococcal antigenemia in HIV / AIDS patients treated at Intermediate Treatment-Infectious Diseases Unit of Dr. Soetomo General Hospital Surabaya. Cryptococcal antigenemia was examined in HIV / AIDS patients with suspected Cryptococcus infection and CD4⁺ T cell lymphocyte count <200 cell /µl. The examination used a lateral flow assay diagnostic tool, a simple FDA(Food and Drug Administration)-approved immunochromatographic test system for detection of capsular polysccharide antigens of Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) in blood. This test meets all of the World Health Organization ASSURED criteria (affordable, sensitive, specific, user friendly, rapid/robust, equipment-free, and delivered). Sensitivity and specifiticy of this method from serum are both 100%. There were 3 positive cryptococcal antigenemia from 41 serum HIV / AIDS patients with suspected cryptococcus infection at Intermediate Treatment- Infectious Diseases Unit of Dr. Soetomo General Hospital Surabaya. All of these patients were male aged over 36 years, had CD4⁺ T cell lymphocytes <100 cell /µl and had never received antiretroviral therapy before. The proportion of cryptococcal antigenemia in HIV / AIDS patients with suspected Cryptococcus infection at Intermediate Treatment-Infectious Diseases Unit of Dr. Soetomo General Hospital Surabaya was 7.32%.

Keywords: *cryptococcal antigenemia, AIDS, HIV, Dr. Soetomo Hospital, Surabaya*

ABSTRAK

Infeksi jamur Cryptococcus pada pasien HIV/AIDS mengakibatkan cryptococcal meningitis, yang merupakan penyebab utama meningitis subakut dengan mortalitas sebesar 100% bila tidak mendapatkan terapi antijamur yang tepat. Pemeriksaan antigen cryptococcal akan memberikan informasi risiko pasien yang akan mengalami cryptococcal meningitis. Semakin baik dan cepat diagnosis Cryptococcus antigenemia baik dilakukan pada fase simptomatik maupun asimptomatik cryptococcosis merupakan kunci penting dalam mengurangi morbiditas dan mortalitas. Penelitian ini bertujuan mengetahui proporsi cryptococcal antigenemia pada pasien HIV/AIDS yang dirawat di Unit Perawatan Intermediate Penyakit Infeksi Rumah Sakit Dr. Soetomo Surabaya. Dilakukan pemeriksaan cryptococcal antigenemia pada pasien HIV/AIDS dengan kecurigaan infeksi Cryptococcus dan hitung limfosit T CD4⁺<200sel/µl. Pemeriksaan menggunakan alat diagnostik lateral flow assay yang merupakan teknik imunokromatografi yang telah disetujui oleh FDA (Food and Drug Administration) dan dapat mendeteksi antigen kapsul polisakarida dari kompleks spesies cryptococcus (Cryptococcus neoformans dan Cryptococcus gattii) dari darah. Pemeriksaan ini memenuhi kriteria dari WHO (World Health Organization) antara lain mudah dijangkau, sensitif, spesifik, mudah digunakan, cepat, tidak memerlukan peralatan yang sulit didapat, dan mudah dibawa. Sensitifitas dan spesifisitas pemeriksaan ini dari serum adalah sebesar 100%. Didapatkan hasil penelitian yaitu 3 positif cryptococcal antigenemia dari 41 serum pasien HIV/AIDS dengan kecurigaan infeksi cryptococcus di Unit Perawatan Intermediate Penyakit Infeksi Rumah Sakit

Dr. Soetomo Surabaya. Semua pasien tersebut adalah laki-laki, berusia diatas 36 tahun , memiliki hitung limfosit T CD4+<100 sel/µl dan belum pernah mendapat terapi ARV sebelumnya. Proporsi cryptococcal antigenemia pada pasien HIV/AIDS dengan kecurigaan infeksi Cryptococcus di Unit Perawatan Intermediate Penyakit Infeksi Rumah Sakit Dr. Soetomo Surabaya adalah sebesar 7.32%.

Kata kunci: *cryptococcal antigenemia, AIDS, HIV, RSUD Dr. Soetomo, Surabaya*

INTRODUCTION

HIV/AIDS has been a cause of death for mostly humans in their productive times. Death is mainly due to opportunistic infection. Opportunistic infection is an immune system will not cause illness but is fatal in people with decreased immunity, as in HIV/AIDS patients.[1]

Cryptococcal meningitis is a major cause of subacute meningitis and death in patients with advanced HIV infection, affecting an estimated one million people each year, especially in sub Saharan Africa.[2,3]

Cryptococcal antigenemia testing will provide risk information for patients who will experience cryptococcal meningitis and death. Therefore, appropriate antifungal administration in patients with positive cryptococcal antigenemia (CrAg) is highly recommended. Rapid diagnostic testing using LFA (lateral flow immunoassay) method recommended by WHO is also recommended as a screening diagnostic method especially in HIV/AIDS patients with mild symptoms (no neurological disorders) or asympomatic. LFA examination is inexpensive and easy to perform, giving a sensitivity and specificity of more than 95% within 10 minutes.[4]

More than 80% positive CrAg was obtained in HIV/AIDS patients with $CD4^+$ T cell lymphocyte count <100 cells/µl. In other African and American studies, it was also found in HIV/AIDS patients with $CD4^+$ T cell lymphocyte count between 100-200 cells/µl.[5,6]

This study aim to determine the proportion of Cryptococcal antigenemia in HIV/AIDS patients treated at Dr. Soetomo Hospital Surabaya.

MATERIAL AND METHOD

Study Design and Patients

This was an observational study with cross sectional study design to study the proportion of Cryptococcal antigenemia. HIV/AIDS patients ≥21 years old confirmed by three methods of HIV examination, $CD4^+$ count <200 cells/µl with suspected cryptococcal antigenemia (had fever with or without headache) were enrolled. Study participants were not required to be ART naive. Patients treated for cryptococcal infection in the last three months or currently taking an antifungal agent were excluded from study participation.

Ethics Statement

The study was conducted according to the principles of the Declaration of Helsinki. Written informed consent was required from all study participants and the study was approved by Ethical Committee of Dr. Soetomo Hospital, Surabaya, Indonesia (No. 382/Panke.KKE/V/2017).

Procedures

A blood sample was obtained from each study participant to perform cryptococcal antigen Lateral Flow Assay (IMMY, USA), a simple FDA-approved immunochromatographic test system for detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in blood. Serum was separated from the blood sample. According to IMMY manufacturer's recommendation one drop of lateral flow specimen was added to microtube, and then 40µl of serum was added to microtube. After that, lateral strip was immersed to the mixture and stayed for 10 minutes. The test is positive if there were 2 line on strip, negative test if only 1 line on strip. Step by step of IMMY CrAg LFA can be seen in Figure 1.

Data Analysis

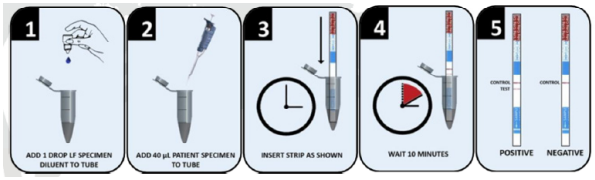

Figure 1. Step by step of IMMY CrAg LFA.[7]

All analyses were performed using SPSS 16. Chi square was used to determine factors associated with positive cryptococcal antigenemia. A p value ≤0.05 was considered significant.

RESULT AND DISCUSSION

The diagnostic use for detection of cryptococcal capsular polysacchride antigen (CrAg) in serum by latex agglutination test (CrAg-latex) or enzym-linked immunoassay (EIA) has been available for over decades. Better diagnostics in asymptomatic and symptomatic phases of cryptococcosis are key components to reduce mortality.[8]

Cryptococcal antigen lateral flow assay (CrAg LFA) was included in the armamentarium for diagnosis. Unlike the other tests, the CrAg LFA is a dipstick immunochromatographic assay, in a format similar to the home pregnancy test, and requires little or no lab infrastructure. This test meets all of the World Health Organization ASSURED criteria (affordable, sensitive,

specific, user friendly, rapid/robust, equipment-free, and delivered).[8]

CrAg LFA has better analytical sensitivity for *Cryptococcus gattii* than CrAg-latex or EIA. Sensitivity and specificity of CrAg LFA from serum are both 100%.[7]

A total of 41 HIV/AIDS patients were enrolled into the study. The mean age of those enrolled was 36.37 years and 90.24% were male. The prevalence of male against female can be explained by the higher incidence of HIV in men.

The prevalence of cryptococcal antigenemia in Soetomo Hospital is 7.32%. The mean global prevalence of cryptococcal antigenemia is 6%.[4] Positive CrAg LFA were 3 males from 41 patient. A 3% prevalence of cryptococcal antigenemia is the point at which the cost of treating cryptococcal meningitis with amphotericin B is greater than the cost of screening for CrAg examination. In Uganda, screening to prevent a case of cryptococcal meningitis requires a cost of $28, whereas to prevent a single death due to cryptococcal meningitis costs $40.[9] The gold standard treatment for cryptococcal meningitis is to use amphotericin B which are known to be expensive and difficult to obtain especially by low-income countries, so more often fluconazole is used.[10] Other analyses reported that CrAg screening in areas with prevalence of <1% remained "cost-effective" due to lower LFA screening costs when compared with CrAg latex.[9,11]

The host gender also plays a role in the pathogenesis of cryptococcal, which estrogen can inhibit the growth of cryptococcus in vitro. Male immune responses are less efficient in controlling cryptococcus infections, because male macrophages tend to be killed by cryptococcus rather than phagocytosis of cryptococcus.[12,13] In this study there is no correlation between male and female with positivity of cryptococcal antigenemia (p:1.0). There is also no correlation between male and female with positivity of cryptococcal antigenemia in another studies.

All of the positive patients are >36 years old, but there is no correlation between age with positivity of cryptococcal antigenemia (p:0.091) in this study. It is the same with another studies.[7,11] One patient is 42 years old, another 54 years old and 71 years old. Old patient (71 years old) was died, but the others were alive. The prognosis of cryptococcosis occuring in patients >60 years is generally worse.[14] Baseline characteristics of patients according to cryptococcal antigenemia status can be observed in Table 1. The positive test of cryptococcal antigen lateral flow assay can be seen in Figure 2.

There were 15 patients (36.6%) that already take ARV, 96% from that have CD4+ T cells lymphocyte count <100 cells/µl. In this study, all of CrAg positive patients were naive ARV, but there is no significant correlation (p:0.287), the same as studies that have already done by Vidal and Manga.[6,12]

There is no correlation within symptoms and CrAg positive antigenemia in this study (p:0.143 for fever, and p:0.539 for headache). In Manga's study, fever present in 82.5% of cases, was more frequent in patients with positive

Table 1. Baseline characteristics of patients according to cryptococcal antigenemia status.

Parameter	CrAg-Positive	CrAg-Negative	P
Age (years)			
<36	0	22	0.091
>36	3	16	
Sex			
Male	3	34	1.000
Female	0	4	
Fever			
Yes	2	37	0.143
No	1	1	
Headache			
Yes	3	25	0.539
No	0	13	
CD4⁺ cells			
<100 cells/µl	3	36	1.000
>100 cells/µl	0	2	
ARV			
Yes	0	15	0.287
No	3	23	

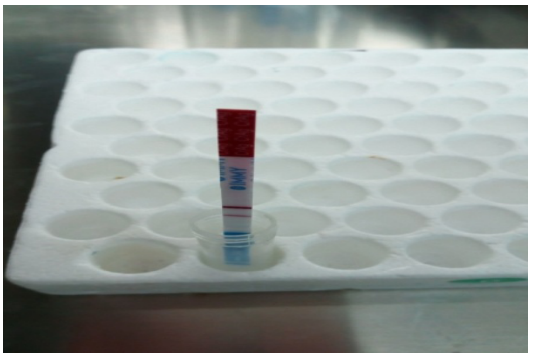

Figure 2. Positive test of Cryptococcal antigen lateral flow assay

antigenemia, but also have no significant corellation,[7] but presenting headaches has significantly associated with positive cryptococcal antigenemia (p:0.000008).[12]

From total patients enrolled in this study, 95% have CD4⁺ T cell lymphocyte count <100 cells/µl, mean CD4⁺ T cell lymphocyte count is 31.5 cels/µl. CrAg positive patients in this study have CD4⁺ T cell lymphocyte count <100 cells/µl. But there is no significant correlation between CD4⁺ T cell lymphocyte count with positivity of CrAg LFA. Research conducted by Manga, Alemu and Ganiem also get the value of p>0.05.[5,12,13]

More opportunistic infections will occur in HIV/AIDS patients that have CD4+ lymphocyte count <100 cells/µl. The prevalence of opportunistic infections varies between regions.[15] Opportunistic infections encountered in this study include pulmonary tuberculosis, cerebral toxoplasmosis, oropharyngeal candidiasis, PCP. The most common types of opportunistic infections in this study are pulmonary tuberculosis. Research conducted by Andama[16] and Jarvis[17]states that tuberculosis is an infection that is often encountered in conjunction with cryptococcus antigenemia.

Indonesia, China and India are known to be a country with high tuberculosis burden.[18]

Several studies suggest that tuberculosis alone may result in a decrease in cellular immune function (a decrease in $CD4^+$ T cell lymphocyte count), and that concurrent tuberculosis infection with HIV will result in significantly lower $CD4^+$ T cell lymphocyte count compared to HIV monoinfection or Tuberculosis monoinfection.[19–21]

Our study is subject to several limitations. These include absence of lumbar punctures, which would help define the proportion of patients with cryptococcal meningitis among those with a positive cryptococcal antigen test, and no long-term clinical follow up of our patient cohort, also the high difference between $CD4^+$<100 cells/µl and $CD4^+$ 100-200 cells/µl.

CONCLUSION

The prevalence of Cryptococcal antigenemia in Soetomo Hospital's HIV/AIDS patients is 7.32%. It means that CrAg screening in Soetomo Hospital will be "cost-effective". Future studies should be conducted to optimize screening and pre-emptive treatment of cryptococcosis.

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