

CASE REPORT

India Ink Staining, a Rapid and Affordable Test for Diagnosis of Cryptococcal Meningitis

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ABSTRAK

Cryptococcal Meningitis meningkat seiring dengan peningkatan insiden HIV-AIDS. Infeksi ini dapat meningkatkan morbiditas dan mortalitas pasien. Penegakan diagnosis yang cepat memegang peranan penting. Penegakan etiologi dengan deteksi *Cryptococcal polysaccharide antigen* cepat tetapi mahal, sedangkan kultur membutuhkan waktu relatif lama.

Seorang laki-laki berusia 47 tahun dirawat di rumah sakit dengan nyeri kepala, demam, mual, dan muntah. Riwayat HIV sejak 6 tahun yang lalu. Pemeriksaan kesadaran kompos mentis, didapat tanda rangsang meningeal, sedangkan pemeriksaan *nn craniales*, motorik dan sensibilitas dalam batas normal. Darah rutin dalam batas normal, CD4 60 sel/mm³. Analisis LCS agak keruh, kadar glukosa menurun (glukosa LCS 43 mg/dl vs glukosa darah 293 mg/dl), protein meningkat (137,1 mg/dl), dan pleositosis dengan peningkatan sel polimorfonuklear. Hasil pengecatan tinta india pada sampel LCS didapatkan gambaran yeast berkapsul.

Cryptococcus sp merupakan satu satunya yeast berkapsul, sedangkan *C. neoformans* merupakan penyebab terbesar Cryptococcosis pada pasien dengan HIV-AIDS. Pasien ini didiagnosis meningitis oleh *Cryptococcus neoformans* dari hasil pemeriksaan pengecatan tinta india, dan segera mendapat terapi anti fungal.

Kata Kunci : Tinta india, *Cryptococcal meningitis*, HIV-AIDS

ABSTRACT

Cryptococcal meningitis incidence has increased along with an increase in incidence of HIV-AIDS. This infection causes increased morbidity and mortality in patients with HIV-AIDS. A rapid diagnosis plays an important role to ensure a prompt therapy of the disease. The cryptococcal polysaccharide antigen test for diagnosis of meningitis is rapid but relatively expensive while culture is time consuming.

A 47-year man was admitted to hospital with a headache, fever, nausea, and vomiting and a HIV history for the last 6 months. On physical examination, he was compos mentis, meningeal's stimuli signs (+), where as on examination of craniales nerves, motor and sensibility was in a normal range. Routine blood was normal, 60 CD4 cells/mm³. Laboratory finding included a cloudy/turbid Cerebrospinal fluid (CSF), low glucose level (CSF glucose 43 mg / dl vs. blood glucose 293 mg / dl), elevated protein concentration (137.1 mg / dl), and polymorphonuclear pleocytosis. India ink stain showed encapsulated yeasts.

Cryptococcus sp is the only encapsulated yeast, while C. neoformans is the most common cause of Cryptococcosis in patients with HIV-AIDS. The patient was diagnosed with Cryptococcal meningitis by indian ink staining, and immediately given anti-fungal therapy.

Keywords: india ink, *cryptococcal meningitis*, HIV-AIDS

INTRODUCTION

Incidence of meningitis caused by *Cryptococcus sp* (cryptococcal meningitis) has increased along with the increase in the prevalence of HIV-AIDS (Lortholarya et al., 2006), mostly caused by *Cryptococcus neoformans* (Bencurova et al., 2011, Tortora et al., 2010). The mortality rate of patients with cryptococcal meningitis in HIV-AIDS accounted for 76% (Vida et al., 2013). An early diagnosis can reduce mortality because it can lead to an appropriate therapy (Mc Mullan et al., 2012).

Cryptococcus sp can be diagnosed by direct examination of clinical specimen with india ink staining (Fisher and Cook 2006, Tille, 2014), serological methods including Latex agglutination for cryptococcal

antigen detection latex agglutination test for detection of cryptococcal capsular polysaccharide, enzyme immunoassay (EIA), cryptococcal lateral flow assay (Mc Mullan et al., 2012), and culture on Sabouraud dextrose agar medium (Fisher and Cook, 2006). Biochemical examination includes tests of assimilation, fermentation of sugars, the production of urea, nitrate assimilation and test niger seed (Murray et al., 2007). Detection of cryptococcal polysaccharide antigen is a quick way to perform screening in patients with suspected presence of cryptococcal meningitis (McMullan et al., 2012), but this method is relatively expensive. The most in expensive and rapid methods is india ink staining to detect a typical encapsulated yeast. The report will

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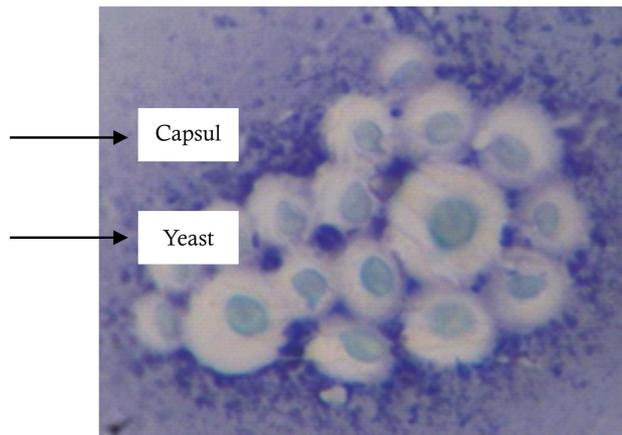


Fig 1. Capsulated yeast on India ink staining



Fig 2. Colorless and slightly cloudy CSF

discuss the case of a man with cryptococcal meningitis and HIV-AIDS diagnosed by the simple technique of India ink staining.

CASE PRESENTATION

A 47 year old man was admitted in a hospital for headache especially affecting the back of his head. He also complained of nausea, vomiting, and fever. He had a HIV history since the last 6 years without ever taking *anti-retro viral* (ARV) drugs and history of diabetes (diabetes mellitus) type II since the 10 years. The physical examination of consciousness showed compos mentis, isokor eyes, a stiff neck, while nn.craniales examination, motor, sensibility within a normal range. Laboratory Hb 14.1 g%, leukocytes 4.700/mm³, leukocyte count of eosinophils/ basophils/ stab/ segment/ lymphocyte/ monocyte by 2/0/1/79/15/3, platelets 270 000/mm³, as blood sugar (GDS) of 293 mg/dl, urea 15 mg/dl, creatinine 1 mg/dl, CD4 60/mm³. Radiological examination resulted in the crucible posterior infarction lacunar on the right and left internal capsule, demand deposits enhancement in the temporal region of the right and left with the impression of meningitis, with no sign of intra-cranial pressure elevation.

Cerebrospinal fluid (CSF) microbiology laboratory examination found no bacteria by both on Gram staining or acid-fast bacilli on Ziehl Neelsen staining, but in india ink staining showed encapsulated yeast (Figure 1), and negative bacterial culture. Chemical examination of the CSF resulted in colorless but slightly cloudy CSF specimen (Figure 2), CSF glucose 43 mg/dl, protein (137.1 mg/dl), polimorfonuclear leukocytes (PMN) 84/mm³, where as mononuclear leukocytes (MN) 25/mm³.

Patient was treated with Anti Retro Viral (ARV) and anti-fungal therapy of fluconazol during his hospitalization, but died after 9 days of treatment.

DISCUSSION

The patient was diagnosed with Cryptococcal meningitis, due to having signs of meningitis, while in india ink painting in CSF samples found encapsulated yeast (Mandell et al., 2000, Murray et al., 2007). India ink staining is a method of using negative stain resulting in a better look of capsule of fungi. *Cryptococcus sp* is an encapsulated yeast which can be identified by a variety of methods other than staining, which were serological method for detection of Cryptococcal polysaccharide antigen (Mc Mullan et al., 2012) and culture in sabouraud dextrose agar medium at a temperature of 250C-370C by forming colonies of round, convex, white to yellow or brownish (Fisher and Cook, 2006).

Gram staining, acid-fast bacilli staining, and india ink staining were conducted on the sample of CSF in this case. Grams staining found no germs, where as india ink staining showed encapsulated yeast. This patient was diagnosed with Cryptococcal meningitis caused by neoformans species without fungal culture and species identification through biochemical examination (assimilation tests, fermentation of sugars, the production of urea, nitrate assimilation and niger seed test). Reasons can be put forward are encapsulated yeast is an illustration of pathognomonic genus *Cryptococcus*, with the consideration that the *C. neoformans* is the most common cause of *Cryptococcus* infections in immuno compromised patients other than any species such as *C. gatii*, *C. albidus*, *C.luteolus*, *C.laurentii*, *C.terreus*, and *C. Gastricus* (Mahon et al., 2014).

The characteristics of bacterial or fungal infection includes a cloudy CSF, decreased CSF glucose compared to blood glucose, elevated CSF protein and pleocytosis with a of PMN cell predominance (Youssef et al., 2006, Tille, 2014). This is consistent with the results of the analysis of CSF in the patient. A decreased glucose is

caused by the utilization of glucose by microorganisms and metabolism by leukocytes. In the *central nervous system* (CNS) there is a *blood brain barrier* (BBB) which does not allow glucose to be easily distributed to the central nervous system. That explain why in meningitis either by bacterial, fungal, as well as TB infection, a hipoglycorrachia is common. Elevated protein is caused by the degradation of cell proteins resulted from a damage caused by a bacterial, tuberculosis, fungal and viral infection (Venkatesh et al., 2003). These results can vary because it depends on several factors, among others, are the duration of sample processing. A study showed that less than 30 minute-delay in the sample processing resulting in difference in the level of significance (Youssef et al., 2006). Centrifugation with high speed processing performed on CSF samples can improve the positive results, but on the other hand can damage the capsule of *Cryptococcus* (Fisher and Cook, 2006).

The mortality rate of HIV-AIDS patients with *Cryptococcosis* coinfection was high (Vida et al., 2013), accounting for 55-70% in developing countries in patients with HIV-AIDS, and 15-20% in HIV patients with *highly active antiretroviral therapy* (HAART). A rapid diagnosis can play a significant role in reducing mortality by ensuring a prompt institution of antifungal therapy. (Mc Mullan et al., 2012). Besides rapid, India ink staining is relatively inexpensive compared with other tests such as *cryptococcal polysaccharide antigen*, enzyme immunoassay (EIA), *cryptococcal lateral flow*. This examination in accordance developed in developing countries with simple laboratory facilities.

The patient died after 9 days of treatment despite adequate antifungal therap. This might have been due to the prognostic factors of mortality of patients with HIV-AIDS with *Cryptococcosis* coinfection test include CD4 lymphocytes cell count, the number of HIV RNA (viral load), ARV therapy prior to *Cryptococcosis*, and the severity of meningitis (Lortholarya et al., 2006). The patient's prognostic factors of mortality included a low CD4 lymphocyte cell count (60/ mmk), ARV inadequate ARV before *Cryptococcosis* (6 years of HIV history without taking ARVs regularly).

CONCLUSION

Diagnosis of meningitis by india ink staining is cost effective, easy to perform, and rapid diagnosis to detect encapsulated yeast of *Criptococcus*. Rapid, effective diagnosis and a therapy improves prognosis and reduce mortality assosisted with meningitis. Rapid, accurate diagnosis of infection by india Ink staining may improve prognosis and reduce mortality.

India Ink Staining, a Rapid and Affordable Test for Diagnosis ...

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