

**ORIGINAL ARTICLE**

# EVALUATION OF CONVENTIONAL AND SEROLOGICAL METHODS FOR RAPID DIAGNOSIS OF CRYPTOCOCCAL MENINGITIS IN HIV SEROPOSITIVE PATIENTS AT TERTIARY CARE HOSPITAL

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**ABSTRACT**

**Introduction:** With the increase in the incidence of HIV infection, there is an increase in incidence of cryptococcal meningitis. Due to lack of sensitive methods for diagnosis, high morbidity and mortality are associated with the disease. Early and rapid diagnosis is essential to prevent serious complications.

**Objective:** To know the prevalence of *Cryptococcosis* in HIV positive patients and to evaluate conventional methods with rapid serological diagnostic method.

**Methods :** A total of 63 CSF samples of HIV seropositive hospitalized patients with history of meningitis were evaluated for *Cryptococcus* by India ink staining, culture and *Cryptococcal* antigen latex agglutination test(LAT) by CALAS.

**Results:** Out of 63 CSF samples, prevalence of cryptococcosis were 9 (14.28%) by LAT, 7 (11.11%) by India ink preparation and 6 (9.5%) by culture. Sensitivity and specificity of India Ink is 83.3 % and 96.49 % and of latex agglutination test is 100 % and 94.7 % respectively considering culture as a gold standard.

**Discussion:** Latex agglutination test is more sensitive than India ink test followed by culture.

**Conclusion:** LAT is a simple, rapid and sensitive test for the early detection of cryptococcal antigen in clinical samples like CSF and may be considered as an aid in establishing diagnosis when culture is negative.

**Key words:** Cryptococcal meningitis, HIV, cryptococcosis.

**INTRODUCTION**

Cryptococcal meningitis is a common opportunistic infection and AIDS-defining illness in patients with late stage HIV infection, particularly in South-east Asia and Southern and East Africa. With the increase in the incidence of HIV infection, there is an increase in incidence of cryptococcal meningitis. Cryptococcal meningitis is the leading cause of meningitis in patients with AIDS.<sup>1</sup>

*Cryptococcus neoformans* is the second most common fungal opportunist after *Candida albicans*, causing symptomatic cryptococcosis in up to 8.5% of HIV-infected individuals. *Cryptococcus* is the commonest central nervous system (CNS) fungal pathogen in immunocompromized patients, particularly among those with AIDS.<sup>2</sup>

The morbidity and mortality in cryptococcal meningitis is 10-30 % in developed countries

and 50-100% in developing countries, where medical facilities are less accessible.<sup>3</sup>

The rising incidence of cryptococcosis in India is posing a serious threat. Due to lack sensitive methods for diagnosis, high morbidity and mortality are associated with the disease. Early diagnosis is essential to prevent serious complications.<sup>4</sup>

Though once known to be rare, cryptococcosis has occurred at a high frequency in India in the past two decades.<sup>5</sup>

Diagnosis of cryptococcal infections is often missed or delayed, with damaging and sometimes fatal consequences, on account of either unawareness or defects in available diagnostic procedures.<sup>6</sup> Unless diagnosed early and specific treatment instituted it can be fatal. There is an urgent need for a rapid and specific diagnostic tool for better management of the patients.<sup>5</sup>

So, this study is undertaken to know prevalence of *Cryptococcus* and to compare and evaluate conventional methods (India ink and culture) with serological method (LAT) for detection of cryptococcal meningitis in the CSF in our setup.

## MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology from July 2009 to December 2010. A total of 63 HIV seropositive, suspected of Cryptococcal meningitis (headache, altered sensorium, meningitis etc.) were included in the study. Medical records of these patients were reviewed and data was collected clinically.

The cerebrospinal fluid (CSF) samples were centrifuged and deposit was processed for fungal culture, negative staining with 10% Nigrosin, Gram's staining and culture. The deposit of CSF was inoculated on two sets of Sabouraud's Dextrose agar (SDA), one incubated at 25°C and other at 37°C. Sample is also inoculated on Bird seed agar at 37°C, and on Blood Agar. Fungal cultures were observed for growth, for appearance of suggestive of *Cryptococcus neoformans* were followed for four weeks. The colony morphology was noted. *Cryptococcus neoformans* was identified base on yeast like mucoid cream to buff coloured colony on SDA, urease test, brownish colonies on Niger seed agar.<sup>7,8</sup>

Supernatant of the CSF sample was used for the LAT. LAT assays were performed with CALAS (Meridian Bioscience, Inc., Cincinnati, Ohio). It is a qualitative and semi quantitative test system for detection of capsular polysaccharide antigens. This test utilizes latex particles coated with anti-cryptococcal polyclonal globulin that reacts with the cryptococcal polysaccharide antigen causing a visible agglutination. The test was performed according to manufacturer's instructions. CSF specimens were inactivated by placing in boiling water bath for 5 min prior to each test to limit non specific interference. A titre of >8 was considered to be positive for cryptococcal infection; however, a final antigen was not determined in all cases. CD<sub>4</sub> count was done by using FACS caliber (Becton and Dickinson) system.

## RESULT

Prevalence of *Cryptococcosis* was 14.28% (9/63) by Latex agglutination test, 11.11% (7/63) by culture and 9.5% (6/63) by India Ink preparation.

Out of total 63 samples 44(69.84%) were male and 19(30.15%) were female. Out of 44 male sample for CSF 5 were positive and out of 19 CSF samples, 2 were positive. Out of 7 positive cryptococcosis by culture, prevalence rate in men is 5(71.42%) and in women is 2(28.57%). The age ranging from 3 yrs to 82 years with a mean of 33.52 years. In all suspected patient CD<sub>4</sub> count was < 200 µg/ml. In cryptococcal positive case, CD<sub>4</sub> count ranges from 24 to 143 µg/ml.

Comparative evaluation of the various diagnostic tests was done in 63 CSF samples by taking culture as gold standard.

**Table 1:** Comparison of LAT with culture

	Culture positive	Culture negative	Total
Latex Agglutination test Positive	7	2	9
Latex Agglutination test Negative	0	54	54
<b>Total</b>	<b>7</b>	<b>56</b>	<b>63</b>

Sensitivity: 100%, Specificity: 96.42%, Chisquare = 39.7, P<0.0001

Table 1 shows comparison of latex agglutination test with the culture. It shows that sensitivity and specificity of Latex agglutination test is 100 % and 96.42 % respectively.

Table 2 shows Comparison of India ink with culture. Sensitivity and specificity of India Ink is 85.7 % and 100 % respectively.

**Table 2:** Comparison of India ink with culture

	Culture positive	Culture Negative	Total
India Ink Positive	6	0	6
India Ink Negative	1	56	57
Total	7	56	63

Sensitivity: 85.7%, Specificity: 100%, Chi-square = 43.6,  $P < 0.0001$

**Table 3:** Comparison of our study with other study

	Imwidthhaya et al	Khanna et al	Present study
CSF India Ink positivity	91 %	87.36%	85.7%
CSF cryptococcal antigen positivity	100%	98.81%	100%
CSF cryptococcal culture positivity	100%	100%	100%

Table 3 shows comparison of our study with the other studies.

## DISCUSSION

Cryptococcosis is the most common systemic fungal infection in AIDS.<sup>9</sup> and its incidence is increasing with the rapid spread of AIDS. The CSF may appear normal in these patients with cryptococcal meningitis and many of them have normal levels of protein and glucose in CSF. Classical meningeal symptoms occur in only about a quarter or one-third of the patients that cause delay in the diagnosis. Asymptomatic patients may have a positive culture of CSF with no other abnormality of the fluid.<sup>10</sup> As infection with HIV is widespread in India and cryptococcal meningitis is a common problem in those with AIDS. Recent data indicate that incidence of *C. neoformans* infection is high in developing countries like in India.<sup>2</sup>

Cryptococcosis, one of the AIDS defining infections, considered as "sleeping

disease"...became an "awakening giant" within a couple of years and has been now been predicted as the "Mycosis of the future," with a predilection that for every million patients with AIDS, 50,000–100,000 will contract cryptococcosis. Its prevalence varies from place to place.<sup>10</sup> In our study prevalence of cryptococcosis was 11.11% considering culture as a Gold standard and is well correlated with study done by Meena G et al in Western India (The overall prevalence remained between 9 and 27%).<sup>11</sup> Culture is considered to be the "gold standard" method of diagnosis for *Cryptococcus*, but it takes at least 3 days to a maximum time of a month for growth cumbersome, labour intensive, time consuming.

Our study shows that prevalence of cryptococcal meningitis by direct microscopy was 9.52% & by LAT was 14.29%. Positivity of India Ink, LAT and culture in our study was 85.7%, 100%, 100% respectively which is comparable to study carried out by Imwidthhaya et al (91%, 100%, 100%)<sup>12</sup> and Khanna et al (87.36, 98.81%, 100%).<sup>13</sup>

Comparing LAT with culture showed that sensitivity of LAT is 100% and specificity of LAT is 96.42%. In this study, two samples gave false positive results with the LAT. The reasons behind it were: one patient was on antifungal treatment (Amphotericin B) hence gave positive in LAT but negative in culture and the other patient had gram negative infection giving positive result with LAT due to cross reactivity with it though the patient was not suffering with *Cryptococcus*. Antigen detection represents the most immediate and rapid way to enhance methods for diagnosis of cryptococcosis. It is a highly sensitive as well as specific and rapid test, and the antigen can remain detectable for several months after infection.<sup>14</sup>

Comparing India Ink with culture, the sensitivity of India Ink was 85.7% and specificity was 100%. The reason behind low sensitivity could be due to the low number of yeast cells, which may have been below the detectable level by microscopy in CSF.

In our study, it was observed that males were involved slightly more 44 (69.84%) than females 19 (30.15 %), which may reflect a difference of exposure rather than a difference in host susceptibility, as it was noted earlier and low number of females were also due to social stigma, because of that they were not came up to the hospital for the diagnosis as well as treatment though they were suffering from the

disease. The age group involved in this study were from 3 to 82 with mean age group of 33.52 years that is well correlated with study of V Lakshmi showed mean age of 31 years and study of P. Imwidthaya showed mean age of 32.1 years.<sup>2,12</sup> CD4 count were 24 to 143\_ with mean of 89.77 that is higher than the study of P. Imwidthaya showed mean CD4 count 45 mm<sup>3</sup> and the study of Shaikh M S A mean CD4 count was 60.27.<sup>12,1</sup>

To Conclude, Infection with HIV continues to be more important risk factor for development of CNS cryptococcosis and is an important contributor to morbidity and mortality in HIV-infected patients. As clinical picture may be confusing with viral or tubercular meningitis, a high index of suspicion and routine mycological surveillance is required to help in an early diagnosis and appropriate therapy, as majority of patients responded to therapy. LAT is a simple, rapid and sensitive test for the early detection of Cryptococcal antigen in clinical samples like CSF and antigen can remain detectable for several months after infection, so that treatment can be instituted immediately. It is therefore a suitable choice of laboratory test for screening and quantitative analysis of antigen has prognostic value and it also helps in guiding chemo therapy and period of hospitalization. Thus LAT should be used as a primary test to catch out all suspected cryptococcal meningitis and all positive samples should be further confirmed by culture.

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