

Cryptococcal Meningitis in HIV Patients from a Tertiary Care Centre in Northern India

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ABSTRACT

Introduction: Cryptococcal meningitis, caused by *Cryptococcus neoformans*, an opportunistic fungal infection that affects immunocompromised Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) patients. In recent years, the incidence of cryptococcal meningitis has increased in both the HIV-positive and HIV-negative patients; therefore there is a need for development of efficient methods for early diagnosis and treatment to reduce mortality and morbidity.

Aim: To determine the incidence of cryptococcal meningitis in HIV/AIDS patients and to compare the results of latex agglutination test and India ink preparation with fungal culture examination.

Materials and Methods: A total of 204 Cerebrospinal fluid (CSF) samples from HIV-positive patients (aged >18 years) showing signs and symptoms of meningitis were collected. The samples were cultured on Sabouraud's Dextrose Agar (SDA),

and analysed using India ink preparation and cryptococcal antigen detection by the rapid Latex Agglutination assay.

Results: Mean age of the patients was 32.45 years. The male:female ratio was observed to be 10:1. Of these, 30 (14.70%) cases were culture positive, 35 (17.15%) were positive for cryptococcal antigen and 16 (7.84%) patients were found to be positive by India ink preparation. Incidence of cryptococcal meningitis was observed to be 14.70% in the study population. Considering culture as gold standard, the sensitivity and specificity of India ink was observed to be 53.33% and 100% respectively. The antigen detection test showed the sensitivity and specificity of 100% and 97.12%, respectively.

Conclusion: Rapid and early diagnosis of cryptococcal infection by detection of the fungal antigen in CSF of patients by latex agglutination when compared to culture and India ink can alter the course of management of cryptococcal meningitis patients.

Keywords: *Cryptococcus neoformans*, Cryptococcus antigen, Human immunodeficiency virus, Incidence, India ink

INTRODUCTION

Cryptococcal meningitis, caused by *Cryptococcus neoformans*, is an opportunistic fungal infection that mainly affects HIV, immunocompromised patients. *C. neoformans*, often found in soil, is a free-living environmental microbe that does not require a host to reproduce or survive. Its key virulence factors include the polysaccharide capsule and cell wall associated melanin [1].

Over the past decade, the incidence of cryptococcal meningitis has increased in both immuno-compromised and immuno-competent patients. Recent study by Rajasingham R et al., estimated an average global cryptococcal antigenemia prevalence of 6.0% among people with a CD4+ cell count of less than 100 cells per μL and the disease was responsible for 15% of AIDS-related deaths [2]. Cryptococcosis is the second most common fungal infection after candidiasis in HIV-patients and *cryptococcal* meningitis is the fourth most commonly recognised cause of life-threatening infection among AIDS patients [3]. Thus, the magnitude of the disease is worth a concern. Diagnosing cryptococcal meningitis is convoluted, as the meningeal signs are not predominantly revealed in HIV patients and the CSF may appear normal with glucose and protein levels within range.

Conventional method such as fungal culture is specific, but time consuming and is limited by culture conditions and amount of CSF [4]. India ink staining is rapid but lacks sensitivity and is subjective to technician skill set, and latex agglutination test is highly sensitive and specific and helps in evaluating both severity and prognosis of illness. Rapid diagnosis is essential for prompt therapy to manage fatality rates.

Therefore, the study was carried out to find the incidence of cryptococcal meningitis in HIV/AIDS patients attending OPD of a Tertiary Care Hospital in Delhi and to compare the results of rapid latex agglutination test and India ink examination with fungal culture.

MATERIALS AND METHODS

The present prospective study was carried out between January 2018 to December 2018 at Department of Microbiology, PGIMER, Dr. RML Hospital, a Tertiary Care Centre, in Delhi, India. A total of 204 consecutive CSF samples from HIV-positive patients (aged 18 years or above), showing signs of meningitis such as headache, fever, convulsions, nuchal rigidity, and altered sensorium were tested for *Cryptococcus neoformans*. HIV-negative patients and HIV-positive patients with other fungal or bacterial infections were excluded from the study. After obtaining informed consent from the patients, CSF samples were collected by the clinician by performing lumbar puncture with strict aseptic precautions. For further processing, collected samples were immediately sent to Microbiology Department. The samples were processed using India ink preparation, culture on SDA and cryptococcal antigen detection by the rapid latex agglutination kit available in the department [5].

The samples were centrifuged at 1000 g for 15 minutes at 37°C and a loopful of centrifuged sediment was mixed with India ink on a slide and screened for encapsulated, budding yeast cells. The supernatant from the centrifuged CSF sample was used for the detection of *Cryptococcus neoformans* antigen by latex agglutination test using Cryptococcal Antigen Latex Agglutination Systems (CALAS™) from Meridian Bioscience as per the manual's instruction. For fungal culture, the centrifuged deposit was inoculated onto two SDA slants, supplemented with antibiotic chloramphenicol (0.05mg/ml) and incubated at 25°C and 37°C separately for two weeks and were checked for growth at regular intervals. Yeast like colonies on SDA were further identified as *Cryptococcus* species as per the standard mycological procedures using Gram stain, germ tube test, Dalmou technique, urease test, carbohydrate fermentation and carbohydrate assimilation test [5].

STATISTICAL ANALYSIS

Statistical analysis was done by calculating the sensitivity and specificity with the help of 2*2 tables. Data was represented in the form of frequency and percentages.

RESULTS

A total of 204 CSF samples were collected from HIV-positive patients, aged 18 to 65 years with a mean age of 32.45 years. The male: female ratio was observed to be 10:1. Of these, 30 (14.70%) cases were culture positive, 35 (17.15%) were positive for cryptococcal antigen and 16 (7.84%) patients were found to be positive by India ink preparation [Table/Fig-1]. All the India ink positive samples were also positive by culture and latex agglutination test. It was also observed that in 5 (2.45%) patients, cryptococcal antigen was detected but no fungal growth observed. Also, in 14 India ink negative patients, positive cultures were obtained for *Cryptococcus* [Table/Fig-2]. Considering culture as gold standard, the sensitivity and specificity of India ink was observed to be 53.33% and 100%, respectively. The antigen detection test showed the sensitivity and specificity of 100% and 97.12%, respectively.

	Culture	Latex Agglutination	India Ink Preparation
Positive	30 (14.70%)	35 (17.15%)	16 (7.84%)
Negative	174 (85.29%)	169 (82.84%)	188 (92.15%)
Total	204	204	204

[Table/Fig-1]: Number of positive and negative cases of cryptococcal meningitis diagnosed using various tests.

Test		Culture	
		Positive	Negative
India Ink (n= 204)	Positive	16	0
	Negative	14	174
	Total	30	174
Latex Agglutination (n= 204)	Positive	30	5
	Negative	0	169
	Total	30	174

[Table/Fig-2]: Comparison of culture with latex agglutination and India ink preparation.

DISCUSSION

Worldwide cryptococcal meningitis affects 220,000 HIV/AIDS patients annually and results in nearly 181,000 deaths [2]. The fungus enters the human body by inhalation and invades the host lungs, where it results in either a local subclinical infection or an invasive disease that frequently leads to development of lethal meningitis [6]. Animal models have demonstrated the importance of cell-mediated immunity, which is hampered in AIDS patients, and therefore makes the host susceptible to *C. neoformans* infection [7].

In the present study, considering culture as gold standard, the incidence of cryptococcal meningitis was observed to be 14.70% in the patient group. The findings are in accordance with published data from North India and were significantly higher as compared to south India population [8-10]. A possible explanation for persisting high incidence of cryptococcal meningitis could be poor adherence to Anti-retroviral therapy (ART). A recent meta-analysis reported an ART adherence rate of 70% in Indian population [11]. A difference in the incidence of cryptococcal meningitis in men and women in different countries and in different regions in the same country has been observed. In the present study, the ratio of incidence among men and women was 10:1 proving male preponderance, which is comparable to the findings of various other studies [8,10,12,13]. A possible explanation for this could be higher exposure of the infection in men as opposed to varying susceptibilities towards the infection [10].

A comparison of the three methods in this study showed that India ink staining though rapid and specific (100%) was relatively insensitive (sensitivity: 53.33%). Only 16 (7.84%) of the cases were diagnosed with the help of India ink. In 14 patients India ink test was observed to be negative while their culture was positive. This emphasises on the limited sensitivity of India ink as a screening test, which depends upon both fungal burden and capsule production and even small discrepancies often lead to false negative results [9].

On the other hand, latex agglutination, a rapid and easy to perform and read test, correctly diagnosed 35 (17.15%) patients out of 204. Studies have shown that the capsular polysaccharide antigen of *Cryptococcus* can remain detectable in CSF for several months after infection and remains positive in patients undergoing treatment [14]. The high sensitivity (100%) and specificity (97.12%) highlights the accuracy and reproducibility of antigen detection test.

Culture was positive in 30 (14.70%) samples. All culture positive cases were also positive by latex agglutination test with the sensitivity of 100%. Five cases were obtained which were positive by latex agglutination test but no growth was observed on SDA slants. This could be explained by the reason that empirical Amphotericin B was started on strong clinical suspicion of cryptococcal meningitis immediately on admission as the patients presented with altered sensorium and culture can be falsely negative when fungal burden is low. Similar findings were observed by the other studies [8-10,15,16].

Overall the present study justifies the use of rapid cryptococcal antigen detection methods as a screening tool for suspected cases of cryptococcal meningitis followed by a fungal culture for the confirmation of diagnosis.

LIMITATION

In the present study, the speciation of *Cryptococcus* and the various antifungal MICs were not studied. Also, the HIV negative patients were not enrolled in the study group. The persisting high prevalence of cryptococcal meningitis in north India warrants further studies.

CONCLUSION

Due to the high mortality of the disease, early diagnosis of cryptococcal meningitis is of utmost importance for the management of patients. The latex agglutination for detection of cryptococcal antigen is more sensitive and efficient as compared to conventional culture and India ink preparation analysis.

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Date of Submission: **Mar 14, 2019**

Date of Peer Review: **Apr 12, 2019**

Date of Acceptance: **Jul 12, 2019**

Date of Publishing: **Oct 01, 2019**

FINANCIAL OR OTHER COMPETING INTERESTS: None.