COMPARISON OF CONVENTIONAL AND SEROLOGICAL METHODS IN DIAGNOSIS OF CRYPTOCOCCAL MENINGITIS AMONG HIV SEROPOSITIVE INDIVIDUALS IN A SOUTH INDIAN TERTIARY CARE CENTER

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ABSTRACT

BACKGROUND

Cryptococcal meningitis is the life threatening mycosis in HIV patients. Microscopy is poor in sensitivity and Culture is the gold standard in diagnosis. Cryptococcal Antigen detection by Latex agglutination is the rapid serological method. This study is an attempt to throw light on the simple, rapid, most sensitive and specific detection methods.

AIM

Comparative evaluation of diagnosis of Cryptococcal meningitis by using different rapid methods.

METHODS

Adult HIV positive individuals admitted at GHTM from January to December 2011, with clinical presentations suggestive of Meningitis and consenting for perform lumbar puncture were studied. From 100 cases, Cerebrospinal fluid was collected as per the SOP and subjected to India ink staining, Grams staining, Cryptococcal Ag Latex agglutination test (CAALAS) and culture. The diagnostic value was assessed by comparing the sensitivity and specificity of the three rapid methods with culture as a gold standard by using McNemar test.

RESULTS

Of the 100 tested samples, 13 and 19 were positive by India ink and Cryptococcal antigen detection respectively of which 12 samples were positive on both stains. The culture turned positive with 20 samples. Based on these reports, the sensitivity and specificity of Cryptococcal antigen detection was 95.45% and 99.45% respectively and that of India ink was 63.64% and 100% respectively.

CONCLUSION

Cryptococcal antigen detection is found superior to India ink and is a better tool for screening and confirming Cryptococcal meningitis in HIV positives. However, further and larger studies are required to recommend the use of the test in public health programmes.

KEYWORDS

HIV, Cryptococcus, Meningitis, Antigen Detection.

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INTRODUCTION

The encapsulated yeast, *Cryptococcus* has rapidly risen to a worldwide highly recognizable major opportunistic fungal pathogen. Since the epidemic of HIV infections, *C. neoformans* has become a critically important opportunistic infection in individuals in all parts of the world who are infected with HIV.¹ In immunocompromised individuals meningoencephalitis is the most common and serious clinical manifestation of *C. neoformans* infection and can be refractory to antifungal therapy.² Cryptococcal meningitis has occurred at a high frequency in India in the past two decades.³ The tropical climate of the Indian subcontinent offers a suitable

environment for *Cryptococcus neoformans* and the onslaught of the AIDS pandemic since the early 1990s has led to a sharp increase in the number of reported cases of cryptococcosis in the past decade.³

The laboratory diagnosis of *C. neoformans* is established by conventional methods like direct demonstration of the organism by Grams staining, Demonstration of capsule by negative staining, Cultivation of the organism, Biochemical reactions, Serological methods like demonstration of the capsular polysaccharide antigens in the Cerebrospinal Fluid (CSF) and serum and other various molecular methods.

Direct demonstration of organism by India ink staining and Grams staining are rapid and specific, but poor in their sensitivity (50-80%).³ Culture is the "gold standard" for the diagnosis, but it is time consuming and laborious.⁴ Detecting antibodies is an indirect but a rapid means of diagnosis. Cryptococcal antigen detection represents the most immediate way to improve methods for Cryptococcosis serodiagnosis.⁵ Cryptococcal Antigen detection by Latex Agglutination Test (LAT) is a rapid and very simple method to perform. LAT is the most commonly used serological method, which is having 93% to 100% sensitivity and 93% to 98% of specificity.^{6,7} Early

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diagnosis and treatment will reduce the mortality and morbidity. Though CSF is a precious specimen, it is difficult to obtain it in large volume to perform various tests.

This study is an attempt to throw light on simple, rapid most sensitive and specific detection methods in diagnosis of cryptococcal meningitis among HIV seropositive individuals.

AIMS AND OBJECTIVES

- Comparative evaluation of diagnosis of Cryptococcal meningitis by using different rapid methods.
- To diagnose Cryptococcal meningitis in the Cerebrospinal Fluid of HIV seropositive individuals by Microscopy and Serological methods.
- To compare the Microscopy and serological results with Cryptococcal culture.

MATERIALS AND METHODS

One hundred adult HIV positive individuals belonging to both genders admitted at GHTM with clinical presentations suggestive of meningitis and consenting to perform lumbar puncture were included in this prospective study. Exclusion criteria included patients who have already been treated or are under the treatment for Cryptococcal meningitis and those with any contraindications to perform Lumbar puncture.

After obtaining informed written consent from the patient, Cerebrospinal fluid (CSF) was collected by Lumbar puncture under sterile precautions into a sterile container and the specimen was immediately transferred to the laboratory for processing. In the laboratory after the verification of request and macroscopic examination of the specimen, India ink staining, Grams staining, Cryptococcal Ag test (Latex) and culture on Sabouraud's Dextrose Agar (SDA) was performed on the same day of reception. Results were documented along with the basic demography and clinical and laboratory data of the patient. Data was analysed by using Medcalc software.

The diagnostic value of the India ink and Cryptococcal antigen detection by latex agglutination test (CAALAS Kit) was assessed by comparing the sensitivity and specificity of the two rapid methods with culture as a gold standard by using McNemar test. Ethical clearance was obtained in the Institutional Review board of our hospital.

RESULTS

Of the 100 tested samples, 13 and 19 were positive by India ink and Cryptococcal antigen detection respectively of which 12 samples were positive on both tests. The culture turned positive with 20 samples. Among the positives, 16 (80%) were males and 4 (20%) were females [Picture 1].

Pic. 1: Shows the Sex Distribution among the Cryptococcal Meningitis Patients

Pic. 2: Overall Results by India Ink and Cryptococcal Antigen Test Methods

As shown in Picture 2, India ink and CrAg antigen were positive in 13 samples, CrAg alone positive in 6 cases and none of the sample positive for India ink become Negative for CrAg. Based on these reports, the sensitivity and specificity of Cryptococcal antigen detection was 95.45% and 99.45% respectively and that of India ink was 63.64% and 100% respectively.

Pic. 3: Depicts the Age distribution among the Suspected Cases of Meningitis

DISCUSSION

Results of this study indicate males (Picture 1) and adults are (41-50 years) [Picture 3] are more prone to have cryptococcal meningitis. India ink is used as a screening test, but it has low sensitivity. Culture is considered as a gold standard test, but it may take up to 48 hours to grow. Cryptococcal antigen detection is rapid and simple to perform with high sensitivity and specificity as compared to India ink staining. This study results is well supported by Wake et al. 2016.⁶

CONCLUSION

On the basis of the above findings it can be said that Cryptococcal antigen detection is superior to India ink and is a better tool for screening and confirming Cryptococcal meningitis in HIV positives. Cryptococcal antigen detection by latex agglutination test is recommended by WHO as a screening and confirmatory test. However, further and larger studies are required to recommend the use of the test in public health programmes.

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