



COMPARISON OF CONVENTIONAL METHODS WITH LATEX AGGLUTINATION TEST IN THE DIAGNOSIS OF CRYPTOCOCCUS MENINGITIS

Microbiology

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ABSTRACT

Cryptococcus is opportunistic fungi causes meningitis in HIV patients. CSF samples from suspected cases were processed. Negative staining, dark field microscopy, culture, biochemical identification and latex agglutination directly from CSF samples were done. Out of 49 patients 43 (87.7%) patients were positive for HIV antibodies. Cryptococcus was isolated from 13 (26.5%) samples. The result of microscopy and latex agglutination was comparable.

KEYWORDS

Cryptococcus: meningitis

INTRODUCTION

Cryptococcosis is an acute fungal disease caused by encapsulated yeast. Sanfelice first reported this fungus¹. Emmons recovered it from pigeon droppings.

There are many species of this genus prevalent, but neoformans is significant. It is divided into three varieties and five serotypes. It exhibits antigenic variability of muco polysaccharide capsule, shows A,B,C,D and AD.²

This is essentially an opportunistic infection. It primarily involves lungs and disseminates to other sites particularly central nervous system, causing meningo encephalitis.³ It is responsible for high morbidity and mortality among immuno compromised patients. Approximately 5-15% of patients with AIDS suffer from cryptococcal meningitis.⁴ In India var. gatti is also reported from cases of meningitis. Serotypes B is found in tropical areas⁵. The host factors are also important.

This depends upon the cell mediated immunity. The CD4 cells play a important role. Interleukin and interferon have been implicated in host response. The loss of efficient cell mediated response is common in fungal meningitis.

The disease usually occurs after thirty years of age. The respiratory tract is the usual port of entry for Cryptococcus. The incubation period is usually two to three weeks. Cryptococcus is common fungal infections in patients with AIDS⁶. This also depends on the CD4 count. If the count falls below the level of 200 cells/mm, only then infection with the Cryptococcus is seen.⁷

The infection of brain and meninges is the most common presentation. The fever is low grade. The diagnosis is frequently confused with tubercular meningitis.

Cryptococcal meningitis takes more severe course than tubercular The CSF shows numerous organism and few lymphocytes, reduced sugar and elevated proteins.^{2,3}

The laboratory diagnosis include gram stain, India ink staining, dark field microscopy, plating on Sabourauds dextrose agar for growth. The media is incubated at 25^oc, and 37^o c. The yeast like mucoid cream colonies are seen after 2-3 days of inoculation. The growth is further identified by biochemical tests urease, Inositol and Nitrate assimilation.^{2,3}

Immuno diagnosis used for detection of Cryptococcal antigen from serum, and cerebrospinal fluid (CSF). The antigen detection is done with latex agglutination⁸. Other methods like Polymerase chain reaction and molecular techniques have also been introduced for the diagnosis.⁹. The choice of treatment is Amphotericin B, however newer azoles are useful in treatment. Early diagnosis is important in prognosis, even though microscopy is good and culture is the gold standard. Immuno diagnosis is good tool in early stages of meningitis. Comparison of conventional methods and latex agglutination test was

carried out in suspected cases of Cryptococcus meningitis.

MATERIAL AND METHODS

Cerebrospinal fluid of suspected cases of Cryptococcus was analyzed over a period of one year. The CSF was subjected for pathology, biochemistry and microbiology tests. Gram staining, showing yeast cells were taken up for Negative staining, dark field microscopy and culture on Sabourauds dextrose agar, The growth was identified using biochemical tests as per standard methods.

Latex agglutination test was done directly from the cerebrospinal sample. 49 CSF samples were analyzed both for conventional test for fungus and for the latex agglutination test. This test utilizes latex particles coated with anti Cryptococcal poly clonal globulin that reacts with Cryptococcal poly saccharide antigen causing a visible agglutination. The test was performed according to manufactures instructions.

The test for HIV antibody test was done as per guidelines from the patient's serum sample.

RESULTS

Table: 1. shows the number of positive samples and its percentages.

Total sample.	positive	percentage
49	13	26.5%

Table: 2: shows the positive samples by conventional and latex agglutination test.

Total sample	Number of Positive samples	Negative for microscopy and culture but positive for latex
Positive by microscopy	11	--
Positive by culture	11	--
Positive by latex agglutination	10	2

DISCUSSION

The total number of suspected cases of Cryptococcus meningitis was 49. The number of male patients were more than female patients.. 43(87.7%) out of 49 were found to be HIV positive and were Immuno deficient. Out of 49 CSF samples 13(24.4%) were found to be positive for Cryptococcus, Five had tubercular meningitis. Out of positive samples for Cryptococcus, 11(22.4%) was positive by conventional that is Microscopy and culture methods and 10 (20.4%) by latex agglutination tests. The specificity of culture and microscopy was found to be 100%, sensitivity was found to be 84.6%. The specificity of latex agglutination test was found to be 83.3. % and sensitivity was found to be 95.1%. Our results were comparable with other workers¹⁰ Inwidthaya etal, 91%, 100%.¹¹ and Khanna etal reported 87.6%, 100%¹² respectively. Shah Hetal also reported 87.6% and 100%¹³.

The results were comparable. They reported higher sensitivity for LAT. Our study was found to be 95.1%, the reason could be the patients were in early stages. The CD 4 cell counts were less than 200 cells. Our findings 87.7% were HIV positive was comparable with other workers. Shah Hetal reported 93% HIV positive patients in association with Cryptococcus meningitis. To conclude HIV continues to be most important role in morbidity and mortality associated with Cryptococcus meningitis. The clinical picture is very similar to tuberculosis. An evaluation of microbiology for fungal culture and analysis must be emphasized. Early diagnosis is very important for the treatment and response to treatment.^{11,12,13}

LAT is a good tool for diagnosis from serum, and CSF samples. It is early marker and can be used for screening suspected cases. It is easy for technical staff to perform. The sensitivity of the test is good and specificity is comparable. Hence should be recommended in hospitals for screening for Cryptococcus.

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