Original article

Cryptococcal Meningitis in the Pre ART Era

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Abstract

Introduction : Human Immunodeficiency virus (HIV)infection continues to be the most important risk factor for the development of central nervous system (CNS) cryptococcosis in the pre ART era. This is an important contributor to morbididty and mortality in HIV infected patients. Early diagnosis of such patients is the key to their therapeutic success. The study was done to find out the prevalence of CNS cryptococcosis in patients who have not received antiretroviral therapy and to assess the role of microbiological parameters for its specific diagnosis with meningeal signs in a tertiary care settings.

Materials and Methods : A total of 50 HIV seropositive patients suspected to be suffering from meningitis and not received antiretroviral therapy were subjected to CSF analysis by Gram stain, India ink, culture and latex agglutination test.

Results : Of the 50 CSF samples, 32% (16/50) grew cryptococci. Of the 16 CSF samples 93.75% (15/16) were positive by latex agglutination, 68.75 % (11/16) were positive by india ink and 62.5% (10/16) were positive by culture. Maximum number of patients were seen in the age group of 21-39 years and were predominantly males. Fever and headache were the prominent clinical manifestations

Conclusion : Cryptococcal meningitis should be suspected in all cases of meningitis among HIV infected patients. Early diagnosis and treatment alters the prognosis for these patients. Hence examination of CSF should be considered in all HIV infected persons with symptoms of meningitis.

Key words – Cryptococcal meningitis, latex agglutination, india ink, culture

INTRODUCTION

Cryptococcus neoformans is an opportunistic fungal infection in HIV seropositive patients. [1] It is the fourth most commonly recognized cause of life threatening infection among these patients. Infection of the brain and the meninges is the most common clinical manifestation of cryptococcosis and the most common cause of death from the disease. The clinical signs and symptoms of cryptococcal meningitis are indistinguishable from those of many other causes of meningitis. [2] The infection is fatal without treatment. Therefore early diagnosis of such patients is the key to therapeutic success.[3]

Hence early implementation of appropriate antimicrobial therapy requires prompt identification of the infecting pathogen. Although culture is considered as definitive diagnostic test, microscopic examination provides immediate results but the sensitivity of this technique is just 75%. [4] Hence lab should facilitate and aid in the diagnosis through rapid methods. Therefore the present study was carried out to compare the results of microscopy,

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culture and latex agglutination test for diagnosis of cryptococcal meningitis.

MATERIAL AND METHODS

The study was conducted in the Department of Microbiology of a tertiary care hospital in the year 2002. A total of 50 CSF samples of HIV serpositive suspected of cryptococcal meningitis and who have not received antiretroviral treatment were included in the study. The CSF sample collected from each patient was centrifuged at 2000 rpm and the deposit was used for direct microscopy and culture and the supernatant for antigen detection.

Direct microscopy

The centrifuged deposit of CSF were used to detect Cryptococcus neoformans by modified India Ink stain. In this procedure a small drop of CSF was placed on a clean glass slide with a drop of 2% chromium mercury and mixed. Immediately a drop of India ink was added and a cover slip was placed. The preparation was observed under light microscope at low power objective (10 X) and high power objective (10 X)

Culture

The CSF deposits were cultured on Sabouraud dextrose agar (SDA), bird seed agar and were incubated at 25 deg C for 4 weeks. The suspected colonies on SDA and bird seed agar were identified as Cryptococcus neoformans based on Gram stain and urease production as per standard methods and black coloured colonies on bird seed agar.

Antigen detection

The CSF samples were inactivated in a water bath at 100 deg C for 5 minutes and cooled to room temperature before testing. The cryptococcal antigen in the CSF was detected by latex agglutination test (CALAS by Meridian Biosciences) as per manufacturer's instructions.

RESULTS

A total of 16 out of 50 CSF samples (32 %) were positive for Cryptococcus by Microscopy, Culture and latex agglutination tests. (Table 1)The sensitivity of antigen detection was found to be the highest 93.75% (15/16) while that of India Ink and culture was 68.75 % (11/16) and 62.5% (10/16)respectively. One sample (6.25%) was both culture and latex agglutination positive. 5 (31.25 %) CSF samples were India Ink and culture negative. Maximum number of patients with cryptococcal meningitis were seen in the age group of 21-39 years (75%)and predominantly males. Headache (87.5 %), Fever (62.5 %) , and vomiting (50 %)were the prominent clinical manifestations.

Table 1

Comparison of Direct Microscopy, culture and latex agglutination test.

No of CSF samples	India Ink	Culture	Latex agglutination test
1	+	-	-
10	+	+	+
5	-	-	+
Percentage of Positivity	68.75%	62.5 %	93.75 %

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DISCUSSION

Cryptococcal meningitis remains an important presenting illness in HIV infected patients in India. Its prevalence varies from place to place. In our study the prevalence observed was 32 % which is comparable with Manoharan G et al in 2001 who have reported 34.8% prevalence among HIV seropositive cases. [1] Studies have shown the prevalence rate varying between 19.8 % - 45.8%.[5] Over the years three successive studies spanning over a period of 12 years (1992-2004) in AIIMS, it revealed that parallel to increase in number of HIV cases, HIV cryptococcosis coinfection increased from 20 % in 1992-1996 to 30 % , 1996-2000, 37 % and 49 % in 2000-2004. [6]

Our study showed headache (87.5%) followed by fever (62.5 %) and vomiting (50%) were the common presenting features in patients with cryptococcal meningitis. Similar clinical presentation was also reported by Patel et al where headache (96.29 %) was the commonest presenting symptom followed by vomiting (77.77 %) and fever (66.66 %). [7] Our study showed 75 % of the patients belonging to the age group of 20-39 years with predominance of males. Numerous studies have reported a predominance of male patients among individual with cryptococcal meningitis. This was probable due to outdoor activities. [1,8]

The negative staining by India ink is a useful diagnostic tool for cryptococcal meningitis, as results are ready within few minutes. Sensitivity of India ink is variable as it depends on the skill of personnel involved in microscopy. Various workers have reported varying sensitivity of India Ink ranging from 35.5 % to 82 % . [1,9] In the present study, of the 16 CSF samples 93.75% (15/16) were positive by latex agglutination, 68.75 % (11/16) were positive by India ink and 62.5% (10/16) were positive by culture. One sample (6.25 %) was both culture and latex agglutination negative but India ink positive. 5 samples (31.25 %) were India Ink and culture negative but latex agglutination positive. Table 1.In the study by Chakrabarti et al in 2000, out of 58 India Ink positive samples, 1 sample was culture negative. 8 But in the study done by Aquinas et al, out of 7 culture positive CSF samples 1 sample was culture positive and India Ink negative. [9,10] Literature mentions two clinical situations due to which CSF culture is negative. It is either due to low burden of organism or the patient may have received antifungal treatment. In our study latex kit could could detect 5 patients with crptococcal meningitis where India Ink and culture was negative. But 1 sample positive by India Ink turned out to be negative by latex agglutination. Eng et all used CALS kit for the diagnosis of cryptococcal meningitis where they stressed the fact that after initiation of antifungal treatment the culture becomes negative but latex agglutination remains positive. [11] Khyriem et al showed a sensitivity of latex agglutination as 92.3 % which is correlating with our study. [12] Out of 16 patients, 4(25%) expired whereas remaining 12 patients responded well to antifungal therapy.

CONCLUSION

Cryptococcal meningitis should be suspected in all cases of meningitis among HIV infected patients. Early diagnosis and treatment alters the prognosis for these patients. Hence examination of CSF should be considered in all HIV infected persons with symptoms of meningitis. Latex agglutination is a useful adjunct to direct microscopy and culture for diagnosis of cryptococcal meningitis.

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