ORIGINAL RESEARCH PAPER

Volume-7 | Issue-7 | July-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE EVALUATION BETWEEN CONVENTIONAL & SEROLOGICAL METHOD FOR RAPID DIAGNOSIS OF CRYPTOCOCCAL MENINGITIS AT A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

INTRODUCTION- The incidence of Cryptococcal meningitis has risen markedly over the past 20 years as a result of HIV epidemic & increasing use of immunosuppressive therapies. Early and rapid diagnosis is essential to prevent serious complications & deaths associated with the disease.
OBJECTIVE-To evaluate the efficacy of conventional & serological methods for diagnosis of Cryptococcal meningitis.
METHODS- A total of 192 CSF samples of hospitalized patients with history of meningitis were evaluated for Cryptococcus by India ink staining, culture and Cryptococcal antigen detection by Lateral flow immunoassay(LFA) method. The samples which gave the positive growth were also identified by Automated culture system(VITEK-2 compact).
RESULTS- Out of 192 CSF samples, Cryptococcus was detected in 12(6.25%) by India ink, 14(7.29%) by culture, and 18(9.4%) by LFA. Sensitivity of LFA was found to be 100%, followed by culture (77.7%) and then India ink(66.6%) . However, the specificity of India ink & Culture were 100% while that of LFA was found to be 96.7%.
DISCUSSION- LFA is more sensitive than culture followed by India ink test.
CONCLUSION: LFA is a simple, rapid , sensitive and also a less costly test for the early detection of cryptococcal antigen in clinical samples like CSF and may be considered as an aid in establishing diagnosis of Cryptococcal meningitis when culture comes negative.

KEYWORDS

Cryptococcal meningitis, HIV, Lateral flow immunoassay(LFA).

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 also an increase in incidence of cryptococcal meningitis. Though once known to be rare, it has occurred at a high frequency in India in the past two decades[1,2] . Infection may also occur in patients on long-term steroid therapy, diabetes, cancer, renal failure, immunologic diseases, solid organ transplant patients,those suffering from lymphoma, sarcoidosis etc[3,4] . Due to lack of sensitive methods for diagnosis, high morbidity and mortality are associated with the disease. Early diagnosis is essential to prevent serious complications[1].

The laboratory diagnosis of Cryptococcal meningitis is based on direct microscopic demonstration of the organism, culture and antigen detection by different serological methods. Microscopic methods and culture, though specific, show a sensitivity of only 50–80 % (Snow & Dismukes, 1975). Also, culture is time consuming and more labour intensive. Whereas serological methods are more sensitive, less time consuming but suffers from the limitation of false positivity [5].

Keeping in view of the above facts, this study was carried out-
1. To isolate the etiological agent from the CSF samples of clinically suspected cases of Cryptococcal meningitis (CM) and to determine its prevalence & demographic profiles in our set up .

2. To compare and evaluate the serological method with the conventional methods (India ink and culture) for its rapid diagnosis

MATERIALS AND METHODS-

A prospective study was conducted in the Post graduate Department of Microbiology, S.C.B. Medical College & Hospital, Cuttack, Odisha for a period of about 2 years. Patients clinically suspected to be suffering from meningitis were selected for the study. Approval was taken from the Institutional Ethical Committee. Appropriate proceedures were followed to obtain informed consent from the patients. After selection of cases, a detailed clinical history was obtained from the patients. Their physical examination & routine laboratory investigations were also conducted.

192 CSF samples were collected from the patients with meningitis (case group) and 30 CSF samples were collected from the patients with neurological diseases other than meningitis (like suspected subarachnoid hemorrhage , Multiple sclerosis, Guillain-Barré syndrome etc -control group). From each patient 5-7 ml of CSF was collected aseptically by lumbar puncture in a sterile, leakproof container. The samples were subjected for biochemical analysis, cytological examination & microbiological evaluation.

MICROBIOLOGICAL PROCESSINGOF CSF SAMPLE:

The CSF samples were centrifuged at 1000 rpm for 15 minutes. The deposit was processed for direct microscopy and culture, the supernatant was processed for serological tests[1].
Direct microscopy was done by Gram's staining and India ink examination.

For culture, the samples were inoculated on two sets of Sabouraud's dextrose agar (SDA) without Cycloheximide, one tube was incubated at 37° C and another at 25°C in a special biological oxygen demand incubator.The tubes were screened daily for presence of any growth & followed up for 4 weeks.

- Cryptococcus spp. was presumptively identified based on-
- colony morphology on SDA (Cream to buff coloured yeast like mucoid colony)
- Gram positive budding yeast cells on Gram stain done from the colony.
- Urease production
- sensitivity to Cycloheximide 0.5 µg/ml.[6]

As among Cryptococcus spp. the presence of the enzyme phenol oxidase is unique in Cryptococcus neoformans , testing for its presence is a means of accurate identification. Colony from SDA was subcultured on Caffeic acid agar medium, incubated at 37°C and observed for brownish to blackish coloured colony due to production of melanin pigment by Cryptococcus neoformans[6].The colony was also subjected for identification & sensitivity testing by automated

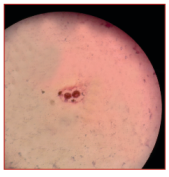
system i.e. VITEK-2 Compact system (BIOMERIUX, North Carolina, USA).

The supernatants of centrifuged CSF samples were used for serological testing i.e. detection of Cryptococcal antigen(Cr-Ag) by Lateral flow immunoassay method (IMMY DIAGNOSTIC, GERMANY).

The subjects were defined as a case of Cryptococcal meningitis (CM) if they were symptomatic and fulfilled any one of the three criteria on CSF analysis:

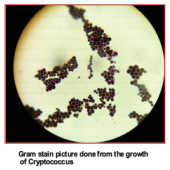
- Direct microscopic examination of CSF by India ink and Gram stain demonstrating Cryptococcus.
- Isolation of Cryptococcus by culture of CSF in Sabouraud Dextrose Agar (SDA) at 37°C.
- A Positive capsular antigen detection test in CSF[7,8]


Growth of Cryptococcus on SDA tube


Cryptococcus in direct Gram stain of CSF sample


Growth of Cryptococcus on SDA plate


Cryptococcus in India ink stain of CSF sample


Gram stain picture done from the growth of Cryptococcus


Cryptococcus neoformans on caffeic acid agar


LFA kit and LFA strip showing positive result for CrAg

Results

Out of 192 clinically diagnosed cases of meningitis, 18 (9.4%) of them were found to be positive for Cryptococcus spp. by one or more of the laboratory test method .Prevalence of Cryptococcal meningitis was found to be 9.4% . Out of 18 laboratory confirmed Cryptococcal meningitis cases,9(50%) were in the age group of 21-40 year . Among all laboratory confirmed Cryptococcal meningitis cases, 12 (6.3%) were male and 6 (3.1%) were female; so, male to female ratio was found to be 2:1 . Majority 16 (88.9%) of cases were HIV seropositive and only 2(11.1%) were HIV sreonegative; among them one was renal transplant recipient and another had tuberculosis along with type 2 diabetes mellitus.

Out of 192 clinically suspected meningitis cases, Cryptococcus spp. was detected in 12(6.25%) by india ink, 14 (7.3%) by culture and 18 (9.4%) by LFA. In the control group, all the cases were negative for Cryptococcus spp. by India ink & Culture; but a single case(3.3%)was positive by LFA. On comparing the three different test methods, LFA had the highest sensitivity (100%), followed by culture (77.7%) and then India ink(66.6%) . However, the specificity of India ink & Culture were 100% while that of LFA was 96.7%.

Out of 14 isolates, most of the isolates 13 (92.8%) were found to be *C. neoformans* except one isolate which was identified to be *C. laurentii* . All the isolates were found sensitive to Amphotericin B and Fluconazole having MIC value of <1 g/ml and MIC value of <2 g/ml respectively. Out of 18 patients having Cryptococcal meningitis, majority 8 (44.4%) patients were found to be surviving after 12 months of follow up therapy with Amphotericin B and Fluconazole.

Discussion

In the present study, the prevalence observed was 9.4% ;this is more or less similar to the study done by **Lakshmi *et al*.(2007)**,**Anupriya Wadhwa *et al*.(2007)** & **Shah et al (2011) ;** who reported the prevalence to be 10.86%, 8.33% & 12.2% respectively. (89,90,10) . In the present study, out of 192 clinically suspected meningitis cases, 12(6.25%) were positive by India Ink , 14(7.3%) by culture and 18(9.4%) were positive by CrAg detection test (LFA) ; which is similar to **Shah Hetal(2011)** who reported positivity of India Ink, culture & CrAg detection test to be 9.5%, 11.1%, 14.3% respectively.

In our study ,the sensitivity of India Ink , culture and CrAg detection test(LFA) was found to be 66.6%, 77.7%, 100% respectively ; which is similar to **Dominic et al(2009)** and **Anuradha et al (2015)** who reported the sensitivity of (60%,60%,100%) and (60%, 64% , 100%) respectively[7,10].

In the present study, the specificity of India ink , Culture and LFA was found to be 100%, 100%, 96.7% respectively which is comparable to the study of **T Kabanda et al (2014)** and **Boulware**

et al(2014); who reported the specificity of (100%, 100%,100%) and (100%, 100%, 99%) respectively[11,12] .

In the present study, on comparing India ink with culture we found, in two cases (1.04%), India ink was negative but culture came positive. This finding is quite similar to the study of **HS Shah et al (2011)**; who reported in 1.5% cases, India ink was negative but culture was positive . The reason behind less positivity of India ink could be due to the low number of yeast cells in those particular cases, which may have been below the detectable level of microscopy in CSF, but above the level of culture positivity[1].

In the present study, on comparing LFA with culture, we found in 4 cases (2.1%), culture was negative but LFA came positive which is similar to the study of **T Kabanda et al(2014)** and **Boulware et al (2014)** ; who reported in 2(1.8%) and 6(2.5%) cases culture was negative , but LFA came positive[11,12].

A large proportion of CM is preventable, because CrAg is present in peripheral blood for an average of 22 days before the onset of CM, and in 10% of cases it may be present for more than 100 days before the onset of meningitis[1,14,15]. The reason behind negative culture but positive serological result may be due to the fact that, culture comes negative in low inoculums of organism or when patient is on treatment ; however such samples give positive result in serological methods as the pick up rate is high. So, usually in very early stage of the disease, culture comes negative but serological test becomes positive[16,17,18].

In our study, all the isolates were found to be sensitive to Amphotericin B (MIC value <1 g/ml) and Fluconazole (MIC value <2 g/ml) which is similar to the study done by **Prasad et al (2003)**; who reported all the strains were sensitive to amphotericin B and fluconazole.

Our study has some limitations. Our study was performed in a single tertiary care hospital and the number of enrolled HIV positive patients with meningitis was small. Further large multicentered studies are required to obtain a more accurate data on cryptococcal meningitis in Indian population.

Conclusion-

The present study clearly indicates that the disease has a high prevalence rate in our tertiary care hospital setting. As the clinical and radiological pictures of CM are often nonpointing , especially in the early stages, routine microbiological evaluation remains the gold standard for early diagnosis of CM which relies on direct microscopic examination, culture & Cryptococcal antigen detection tests. However, every test has its own flaws & advantages. Conventional methods (India ink & culture) are though specific, but less sensitive & time consuming; whereas serological methods of detection are highly sensitive & rapid, but have problems of false positivity. Thus, LFA should be used as a primary test to catch out all suspected CM patients (particularly in HIV positive patients with low CD4 count) and all the positive samples should be further confirmed by culture.

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