Early Detection Of Cryptococcus *neoformans* in Suspected Cases of Fungal Meningitis Using Point of Care Laboratory Testing.

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Abstract

Background: Cryptococcal meningitis is an opportunistic infection caused by Cryptococcus *neoformans* is potentially life threatening. Cryptococcal meningitis remains a problem in terms of its treatment because of the late diagnosis. Early initiation of the patient on oral fluconazole can reduce the incidence of cryptococcal meningitis.

Material and Methods: This cross-sectional study was conducted over the period of 12 months (June 2024 May 2025) in Mycology Laboratory of NIMS&R, Jaipur. 37 CSF samples collected from immunocompromised patients who were suspected to have meningitis. Cryptococcal antigen detection in all the samples involved LFA and culture on Sabouraud Dextrose Agar and Bird Seed Agar. The values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined with the use of SPSS.

Results: In this study, out of 37 CSF samples, 02 (5.4%) samples showed strong positive result in Lateral Flow Assay which gave results within 10 minutes and same 02 samples showed growth in culture after 3 to 5 days of incubation. The sensitivity, specificity, were found to be 100%, PPV and NPV of both culture and LFA were also found to be 100%.

Conclusion: Availability of Lateral Flow assay as a point of care testing not only in tertiary care, but even in remote locations would result in a profound effect on cryptococcal detection, and contribute to early treatment and consequently reduce the morbidity and mortality rate. The synergistic approach could maximize the accuracy and speed of diagnosis, which will eventually increase patient management and outcomes.

Keywords: Cryptococcus neoformans, Point of Care Testing, Cryptococcal Meningitis, Lateral Flow Assay, CSF.

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1. Introduction

Cryptococcal meningitis has become the major infection in AIDS patients. The second most important opportunistic neuro-infection is cryptococcal meningitis in a human immunodeficiency virus (HIV)-seropositive group. The cryptococcal meningitis also observed in non-HIV immunodeficiency state such as diabetes, cancer, solid organ transplant recipient, chemotherapeutic agent, hematologic malignancies etc. In some instances, it is also practised in normal individuals without any obvious predisposing factors. There are a number of pathogens that can cause chronic meningitis.[1] The most prevalent of all is tuberculous meningitis, however with the emergence of the pandemic of the acquired Immunodeficiency Syndrome (AIDS), fungal meningitis, especially cryptococcal meningitis, is on the rise. Cryptococcal meningitis is the most prevalent type of fungus meningitis and it is caused by Cryptococcus neoformans. C. neoformans is a hetero-basidiomycetous fungus which is encapsulated. Cryptococcus neoformans traditionally exists in two varieties and five serotypes (A, B, C, D, AD) depending on its capsule structure.[2]

Cryptococcus causes over 1,80,000 deaths in the world every year.[3] Most incidences of cryptococcal meningitis are in sub-Saharan Africa where diagnostic services, availability of best antifungal drugs, and availability of best hospital-based treatments are inadequate.[4] Disease due to Cryptococcus is treatable and preventable too, but due to the time lag in the diagnosis, the disease continues to present significant morbidity and mortality.[5]

The infection caused by the Cryptococcus is known as Cryptococcosis, which is one of the major health issues in the globe as the Human Immunodeficiency Virus (HIV) pandemic surfaced in the year 1980.[6] Cryptococcus leads to pulmonary cryptococcosis, which gets into the body by means of breathing in the spores of Cryptococcus organism. This can be seen mostly in immunocompetent patients. The infection reaches the brain in the immunocompromised patients resulting in cryptococcal meningitis. The Cryptococcal meningitis predominantly is caused by the main species of Cryptococcus, which is Cryptococcus neoformans,[7] Diagnosis of cryptococcosis in the laboratory is largely based on microscopic, culture as well as serological results of Cryptococcal Antigen (CrAg). Culture, being considered as gold standard, is time consuming.[8]

Serological tests are found to be more sensitive and specific as compared to other tests.[9,10] The CrAg LFA approved by the U.S. Food and Drug Administration (FDA) in the year 2011 (Immy, Norman, OK) is an immunochromatographic dipstick assay, that is able to detect the antigen qualitatively/semiquantitative outcomes.[11] In case the drop of the CSF sample has the Cryptococcal antigen, it combines with the gold-conjugate, anticryptococcal antibodies on the test strip and forms a visible line. A FDA-approved point-of-care dipstick test has transformed the diagnosis of cryptococcal meningitis because the test does not demand the advanced laboratory facilities. This test does not need refrigeration and the result is available within 10 minutes,[12] Lateral Flow Assay (LFA) can be conducted even by semiskilled health care personnel in both the clinics or in the bedsides of the patient. This test has significantly improved the prevention and diagnosis of this deadly disease in the past 10 years.[13]

Therefore, the objective of this research was to compare the lateral flow assay performance in the diagnosis of cryptococcal meningitis against the results of the gold standard i.e. Culture.

2. Materials and Methods

This hospital based observational cross-sectional study was conducted in the Mycology Laboratory, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, over a period of 12 months (June 2024-May 2025). The study was started after obtaining clearance from Institutional Ethics Committee (REF: NIMSUR/IEC/2024/1128).

3. Inclusion Criteria:

1. The CSF samples from all immunocompromised patients of suspected meningitis which was sent to the laboratory in 12 months were included in the study.
2. All genders with age group 18 years to 80 years
3. All patients who are willing to give informed consent.

4. Exclusion Criteria:

1. Patients who have taken antifungal medications for past two weeks.
2. Specimens with obvious signs of contamination were excluded from the study.

5. Study Procedure:

5.1. Sample Collection and Culture Preservation:

37 CSF samples from the suspected meningitis immunocompromised patients were included in the study. All of the samples were subjected to Lateral flow assay and culture. Isolates that tested positive in the culture were stored in Sabouraud Dextrose Agar (SDA) slope at room temperature.

5.2. Serological Tests:

Serological test such as Lateral Flow Assay was performed for all the samples using IMMY CrAg® Lateral Flow Assay kit supplied by Norman, Oklahoma, USA, approved by the U.S. Food and Drug Administration (FDA) in 2011, (Ref. no: CR2003), following the manufacturer's guidelines. This is an immunochromatographic dipstick assay for the qualitative or semiquantitative detection of cryptococcal antigen. The IMMY CrAg® Lateral Flow Assay is the most sensitive diagnostic test for cryptococcal infection available in the market today, this test is much more sensitive compared to microscopy, for the diagnosis of cryptococcal meningitis. No pre-treatment required for the samples. First one drop of sample diluent added in a sterile tube, and then 40 microlitres of CSF was added to the same tube containing the diluent. The test strip was inserted into the tube and incubated for 10 minutes and result was noted.

5.3. Culture:

First gram stain was used for microscopical examination of all the 37 samples because of standard protocols. All the samples were cultured on basal fungal culture media (Sabouraud Dextrose Agar) and special media (bird seed agar), Incubated at 37°C for next three to five days for SDA and at 30°C for next five days for Bird Seed Agar respectively. All the inoculation and media preparation work were strictly performed under the laminar flow cabinet to prevent contamination.

5.4. Phenotypic Identification:

After three to five days of incubation period, Visual growth in the inoculated tubes was observed and Colony characteristics were noted. Identification was done on the basis of Colony characteristics and gram staining.

6. Statistical Analysis:

The sensitivity, specificity, Positive Predictive Value and Negative Predictive

Value were calculated using Statistical Package for the Social Sciences (SPSS).

7. Results:

7.1. Serological Tests: Lateral Flow Assay:

Out of total CSF samples screened by lateral flow assay, 02 (5.4%) samples showed strong positive result by forming two distinct coloured band (C & T) (fig.1) while the remaining 35/37 (94.6%) were found negative by LFA method. Results of LFA were compared with culture.(Table:1)

Fig. 1 Positive LFA (CrAg) Test strip

7.2. Culture:

A total of 37 CSF samples of in-patient were received and processed in the microbiology laboratory of NIMS Hospital in Jaipur, Rajasthan. Out of total CSF samples, 2 were found positive for Cryptococcus neoformans as identified on the basis of microscopic observation of grams stain and Colony characteristics.

7.2.1. Basal Media:

Out of total CSF samples, 2 (5.4%) were grow in basal culture media (SDA), showed cream colored mucoid colonies after 03 days of incubation at 37°C.(Fig.2)

Fig.2 Smooth, creamy white mucoid colonies on SDA

7.2.2. Special Media: (Bird seed agar):

Out of 37 CSF samples, 2 (5.4%) were grow brown coloured colonies on Bird Seed Agar after 4-5 days of incubation at 28°C owing to utilization of phenolic compounds in the media. Cryptococcus species utilize the phenolic (Caffeic acid extracted from Niger seed) and polyphenolic substances contained in the special media to synthesize melanin that got absorbed by yeast cell wall to develop brown pigmented Colonies. Colony characterstics were noted.(Fig. 3)

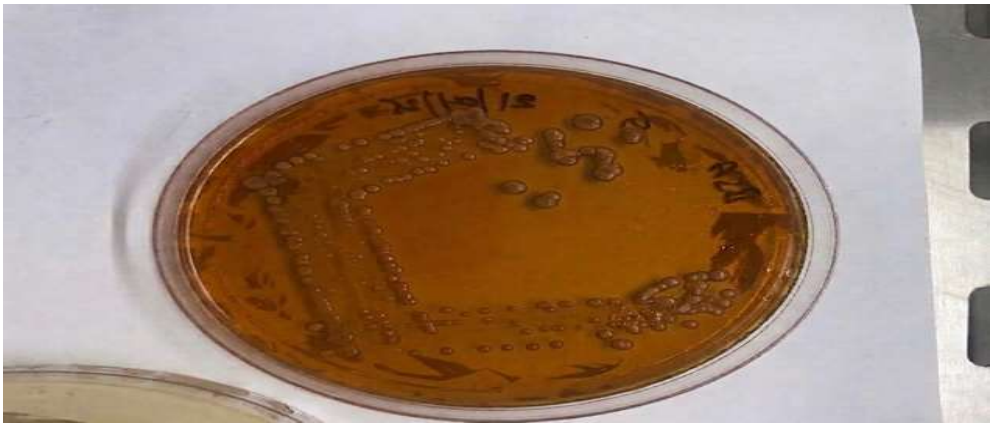

Fig.3 Brown pigmented colonies on Bird Seed Agar

Tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture	100	100	100	100
LFA	100	100	100	100
PPV: Positive predictive value. NPV: Negative predictive value.				

Table:1: Sensitivity and specificity of different diagnostic tests in cryptococcal Meningitis (CM) (n=37).

8. Discussion:

Cryptococcal meningitis remains one of the most critical opportunistic infections that have a high morbidity and mortality rate especially in immunocompromised patients, including those with HIV. A major issue is the delayed diagnosis, which in most cases ends in poor clinical outcomes. Thus, timely diagnosis and early intervention by the use of quick and precise methods is the key to the effective treatment of the patient with antifungal agents.[14,15]

In the current research, culture detected 5.4% of cerebrospinal fluid samples with Cryptococcus neoformans. Culture is consider a gold standard test because it can finds viable organisms and helps in the identification of the species. however, it is not fast because it may take days to even weeks and it requires specific laboratory infrastructures and expertise.[16] **Boulware DR et al. (2014)** also made similar observations, indicating that culture, however accurate, is inappropriate in making fast clinical decisions since they take a long time.[17]

It is also observed that lateral flow assay is more sensitive and specific, and it remains stable at room temperature, with a short turn-around time of (<10 min), and is easy to operate.[18] A diagnostic test validation study of cryptococcal meningitis was done through a large scale study in South Africa and Uganda, involving 832 HIV-positive patients. The most suitable diagnostic test of cryptococcal meningitis out of them was CrAg LFA, which had sensitivity of 99.3 and specificity 99.1 respectively, in cerebrospinal fluid sample.[12] The cryptococcal antigen lateral flow assay (CrAg LFA) was found to be sensitive and specific by 100 percent in the current study and the results were totally concordant with the culture data. These results have been consistent with an extensive multi-centric study performed by **Jarvis JN et al. (2011)**, which showed sensitivity and specificity of LFA in cerebrospinal fluid samples 99.3 and 99.1 respectively in HIV-infected patients.[19] In the same manner, another study by **Lindsley MD et al. (2011)** showed that CrAg LFA is highly sensitive relative to latex agglutination and enzyme immunoassays, particularly in low fungal burden samples.[20]

Such quality of LFA is explained by the fact that it is able to identify the cryptococcal capsular polysaccharide antigen even in the initial stages of infection. This is especially helpful in the situations where culture can lead to false-negative outcome. The use of LFA as a very sensitive and specific test to identify the presence of cryptococcal infection was also supported by a study done by **Binnicker MJ et al. (2012)** especially in the early diagnosis of the disease.[21]

Besides being highly sensitive and specific, LFA has a number of practical benefits such as a quick turn-around time (around 10 minutes), low level of technical set up, and user-friendliness. These properties render it extremely appropriate to point-of-care testing particularly in resource constrained environments. World Health Organization states that diagnostic tools that fulfill the ASSURED requirements (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, and Deliverable) are necessarily needed to enhance the healthcare outcomes in the low-resource settings. LFA satisfies all these requirements hence making it a perfect diagnostic tool that can be used widely.[22]

Moreover, in sub-Saharan Africa where the incidence of cryptococcal meningitis is high, research work has shown that CrAg screening programs with LFA have a significant effect on mortality reduction. As a study of **Meya DB et al. (2010)** stated that the identification of the cryptococcal antigenemia early and subsequent preventive use of the antifungal therapy decreased the cases of cryptococcal meningitis and better prognosis.[23]

LFA has some limitations even though it has these much advantages. It does not provide information of species differentiation and antifungal susceptibility, which is important in targeted therapy. It is thus true that culture is still essential in diagnosing and epidemiological studies. Therefore, LFA ought to be viewed as a supportive diagnostic tool, but not a substitute of culture.

To sum it up, cryptococcal antigen lateral flow assay is a very effective, fast and convenient diagnosing method of cryptococcal meningitis. It can be successfully implemented in everyday clinical practice and lead to outstanding patient outcomes and better early diagnosis, especially in the resource-limited environment.

9. Conclusion:

Availability of Lateral Flow assay as a point of care testing not only in tertiary care, but even in remote locations would result in a profound effect on cryptococcal detection, and contribute to early treatment and consequently reduce the morbidity and mortality rate. This paper has shown that the LFA has the same diagnostic capacity as traditional culture and that both systems have the same sensitivity and specificity. Although culture implies the gold standard, by virtue of the fact that it allows specious identification and additional characterisation, culture is time consuming and might slow down the process of making clinical decisions.

On the other hand, LFA have a result in 10 minutes, which is a fast and convenient diagnostic instrument to use in early detection. Even though it cannot identify species, it is very useful in initial screening and timely intervention especially in source limited or high throughout environment because of its speed and easy to use nature. Thus, LFA may be a good first line diagnostic test, whereas culture may be an auxiliary tool in confirmatory testing and specific identification of microorganisms.

The synergistic approach could maximize the accuracy and speed of diagnosis, which will eventually increase patient management and outcomes.

10. Limitations:

The current research has some limitations. The study might be limited in the applicability of the findings due to a relatively small sample size (n=37), and the single-centre design. More comprehensive multicentric studies need to be performed to establish the accuracy of present study findings and to check diagnostic ability of LFA in a variety of populations.

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