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Original Article

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A Comparative Study of Different Diagnostic Modalities, India Ink, Gram Stain & ICT (Rapid Card) for the Detection of Cryptococcal Meningitis among HIV-Infected Patients in a Tertiary Care Hospital at Kanpur

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ABSTRACT

Introduction: *Cryptococcal neoformans* is the most common opportunistic infection of the central nervous system in HIV patients. CNS *Cryptococcosis* commonly present with non-specific manifestations such as headache, fever, seizure and conscious disturbance. Current diagnostic techniques for *Cryptococcosis* include microscopic detection by India ink stain, gramstain techniques. Recently, the new ICT (Immunochromatographic test) is used to screen HIV-infected persons with CD4 counts of less than 100cells/μL .

Aim: A Comparative study of Different Diagnostic Modalities, India Ink, Gram stain & ICT for the Detection of *Cryptococcal* meningitis among HIV - infected patients in a Tertiary Care Hospital at Kanpur.

Material & Methods: This was a prospective cross-sectional study conducted in the Department of Microbiology & Department of Medicine from July 2022-April 2023 in Rama Medical College Hospital and Research Centre Kanpur. 50 CSF samples were collected from the patient's suspected to have HIV with clinical feature of meningitis. The CSF samples were tested for *Cryptococcus neoformans* by India ink, Gram stain, and ICT.

Result: Out of 50 CSF samples 31 were male and 19 were females. 4 (8.0%) were positive for *Cryptococcus neoformans* mostly in the age group of 30-40 years.All 4 were ICT positive, 3 were India ink stain positive, and 2 were gram stain positive. The sensitivity and specificity of ICT, India ink and gram stain were 99.0%,100%;75.0%%,100% and 50.0%,100% respectively. The PPV and NPV of ICT, India ink and gram stain were 99.0%,100%; 75.0%%,100% and 50.0% ,100% respectively.

Conclusion: In our study the incidence of *Cryptococcus neoformans* was 8%. ICT is more effective method when compared to other conventional methods with higher sensitivity and specificity than the gram stain and India ink. Hence, the new ICT kit is rapid and relevant method for detection of *Cryptococcus neoformans.*

Key Words: *Cryptococcus neoformans, immunochromatographic test, human Immunodeficiency virus*

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INTRODUCTION

Cryptococcal meningitis, caused by *Cryptococcus neoformans*, is an opportunistic fungal infection that affects HIV and immunocompromised patients [1]. In an immunocompetent host, infection is caused by inhaling basidiospores and is normally self-limiting and asymptomatic [2].In developing nations, the prevalence of AIDS patients has been estimated to be between 6 and 10%.[3].According to reports, *Cryptococcal* meningitis accounts for 2-7% of all opportunistic illnesses in big cohorts like Mumbai (4-7%), Chennai (2%) and Delhi (3.7%) and is the most prevalent opportunistic infection in individuals with HIV/AIDS in India. [4,5].

The lungs and the central nervous system are where this illness occurs most frequently [6–8]. This organism spreads through the blood and tends to concentrate in the brain, where it can cause meningitis or meningoencephalitis. [9,10]

The clinical signs of meningitis caused by *Cryptococcus* can sometimes be confused with those of other meningitis causes. As a result, a more accurate diagnostic test is required to identify *Cryptococcus* meningitis. [11].

Thus, microscopy continues to be a quick, affordable, and accurate diagnostic technique. The India ink stain and the Gram stain are two microscopy methods for detecting *Cryptococcus* directly from CSF. Currently, the India ink stain is frequently employed to identify cryptococci in CSF at the microscopic level [12].

In order to compare the sensitivity and specificity of the gramstain, India ink, and ICT (Immunochromatographic test) methods for the diagnosis of *Cryptococcal* meningitis at a tertiary care centre in Kanpur, a study was conducted to determine the detection rate of positive *Cryptococcal* meningitis patients among HIV-infected patients presenting with symptoms of meningitis.

MATERIAL AND METHODS:

A prospective cross- sectional study was carried out between July 2022 to April 2023 at Department of Microbiology, Rama Medical College Hospital and Research Centre in Kanpur, Uttar Pradesh India. A total of 50 CSF samples from HIV – positive patients (aged 20 years or above showing sign of meningitis such as headache, fever, vomiting, neck stiffness, photophobia, hearing loss and confusion were included in the study). HIV negative patients and HIV positive patients with other cause of meningitis like bacterial, viral and of unknown etiologies were excluded from the study. After obtaining informed consent from the patients, CSF samples were collected by lumbar puncture with strict aseptic precautions.

Gramstaining, India ink preparation, and ICT detection of *Cryptococcal* antigen were used to process the samples at the microbiology department. A loopful of the centrifuged sediment was combined with India ink on a slide and screened for yeast cells that were encapsulated and in the process of budding. The samples were centrifuged at 1000 rpm for 15 minutes at 37°C. Gram-positive granular inclusions are seen in isolated, budding, thin-walled, oval to spherical cells that form up*Cryptococcus neoformans* when stained. According to the instructions in the manual, the supernatant from the centrifuged CSF sample was utilised for the Dynamiker *Cryptococcal* Antigen Lateral Flow Assay to identify *Cryptococcus neoformans* antigen.

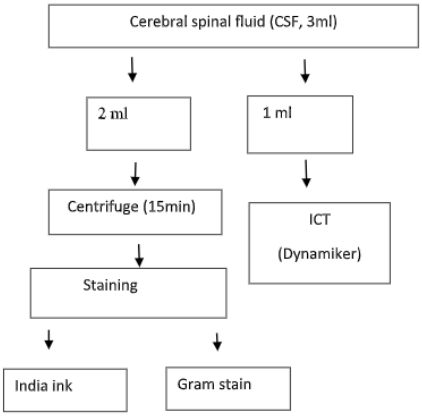


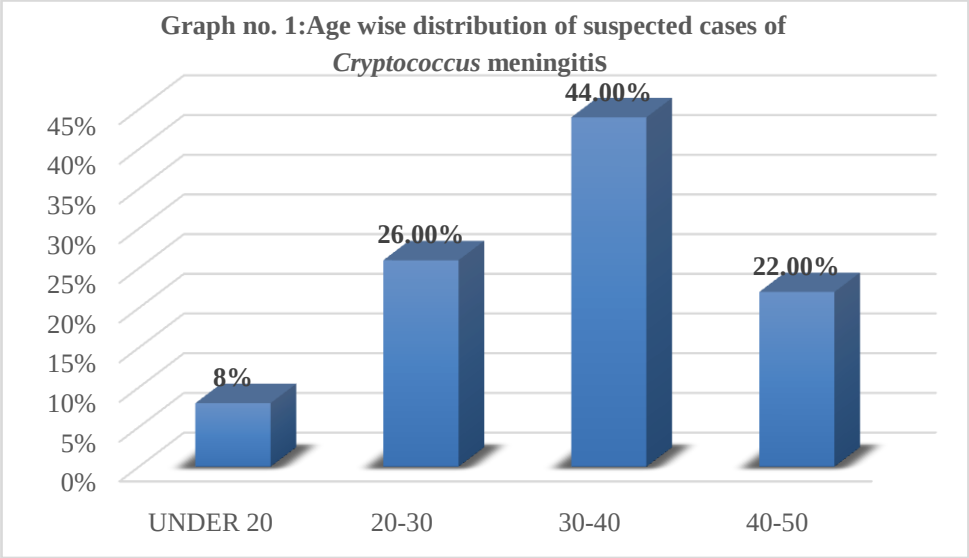
Fig. 1: Sample flow analysis

Statistical analysis:

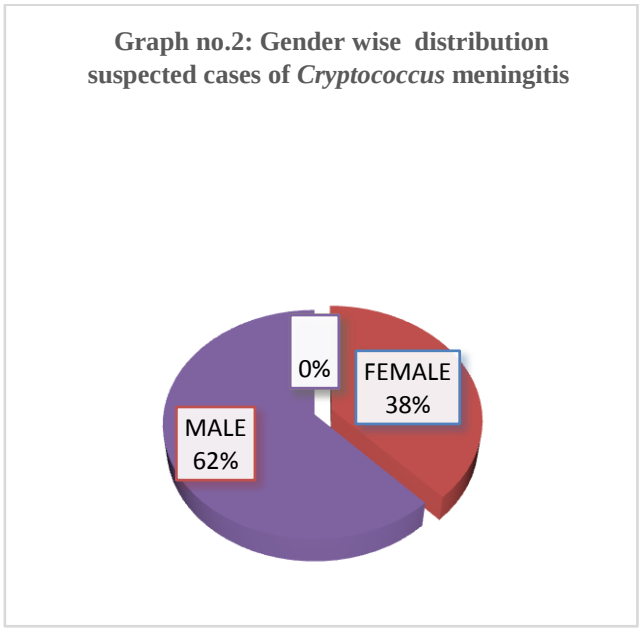
Data recorded on the case report from and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

RESULTS:

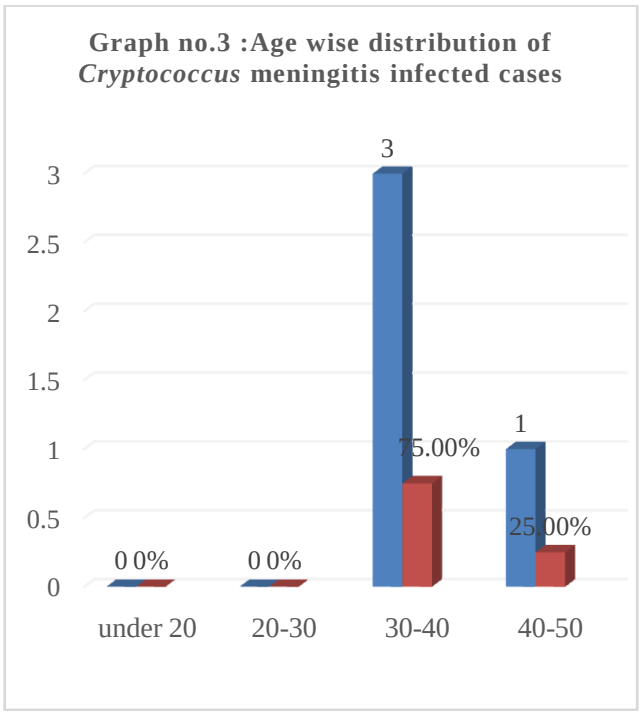
A total of 50 CSF samples were collected from HIV-positive patients in which *Cryptococcal* meningitis suspected cases which were studied, between the age group of under 20 to 50 years with a mean age of 30-40(44%) years.



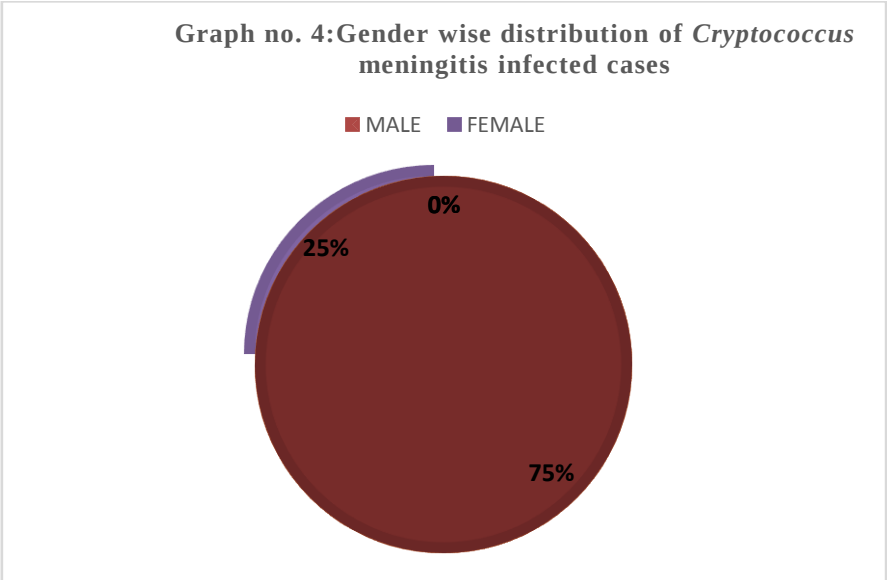
Among there were more male than females (62.0% and 38.0% respectively) in suspected cases.



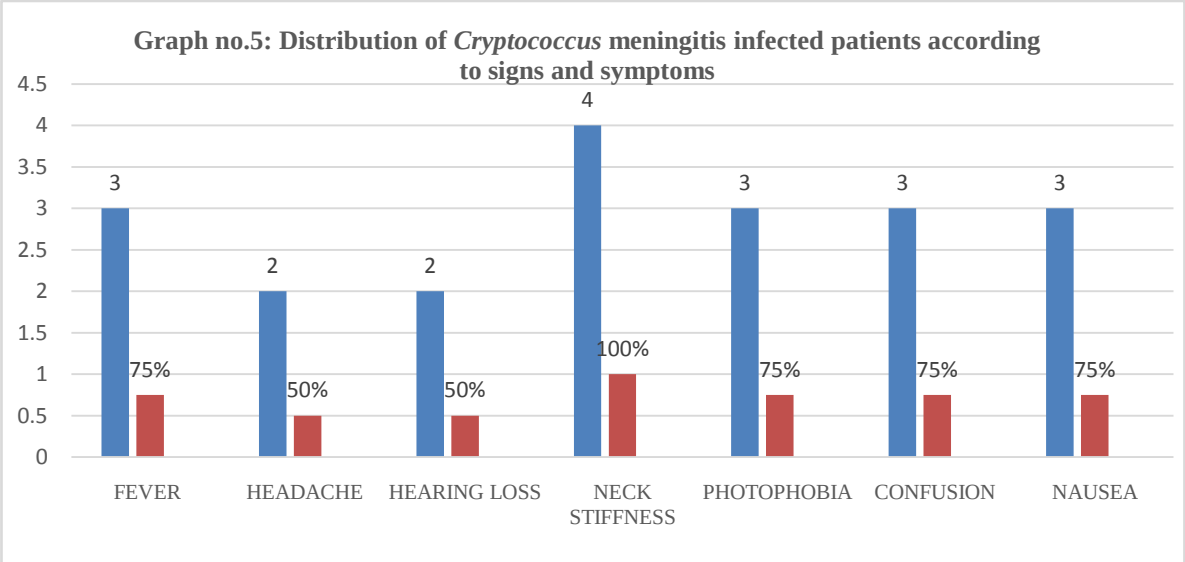
Age wise distribution of *Cryptococcus* meningitis infected cases in which maximum number was found in age group of 30-40 years.



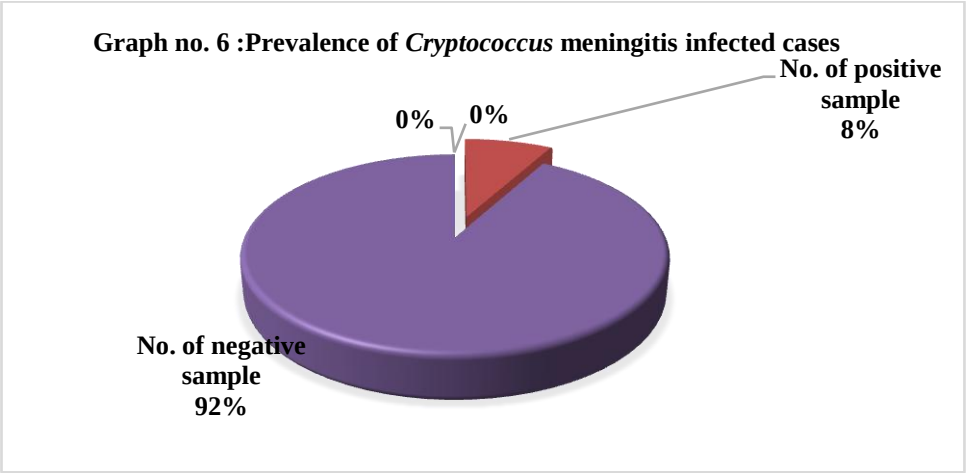
Gender wise distribution of *Cryptococcus* meningitis infected cases in which males were 25% and females were 75%.



Distribution of patients according to signs and symptoms in whichneck stiffness is most common (100%) symptom followed by fever (75%), photophobia (75%), confusion (75%) and nausea (75%).



The prevalence of *Cryptococcus* meningitis which is found to be 4(8%).



In our study Out of 50 samples, 4 were positive by ICT and 3 were found to be positive by India ink preparation [Table 1]. Of these, 4 ICT positive, 2 were found to be positive by gram stain preparation [Table 2].

Table 1. Comparison of result from India ink and ICT.

ICT	INDIA INK STAIN		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	3	1	4
NEGATIVE	0	46	46
TOTAL	3	47	50

Table 2. Comparison of result from Gram stain and ICT.

ICT	GRAM STAIN		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	2	2	4
NEGATIVE	0	46	46
TOTAL	2	48	50

The sensitivity and specificity of ICT, India ink and gram stain is 99.0%,100%; 75.0%%,100% and 50.0%,100% respectively. The PPV and NPV of ICT, India ink and gram stain is 99.0%,100%; 75.0%%,100% and 50.0% ,100% respectively and accuracy of ICT, India ink and gram stain is 100%, 98% and 96%

Table 3: Comparison of the sensitivity, specificity, PPV, NPV and accuracy of three methods for meningitis.

Test methods	Sensitivity	Specificity	PPV	NPV	Accuracy
Gram stain	50.0%	95.8%	50%	100%	96%
India ink	75.0%	100%	75.0%	100%	98%
ICT (Dynamiker LFA)	99.0%	100%	99.0%	100%	100%

DISCUSSION:

In our study, most of the cases belonged to the agegroup is between 30 to 40 year which is similar to the studies conducted by Master R.O et al.,[13] (maximum age 30-39 years) and Swati Vijayet al., [14](maximum age 31-40).

There is a difference in the incidence *Cryptococcal* meningitis in males and females in different regions in the same country. In our study, male (62%) was more affected than female (38%). The finding was in accordance with Swati Vijay with et al.,[14](male- 63% female- 36%).

In our study, the prevalence of *Cryptococcal* meningitis was observed to be 8.0% in HIV patients. The finding was in accordance with MohabawJemel et al showed higher prevalence 11.43% [15] .

The sign and symptoms observed in the present study were – neck stiffness (100%), fever (75%), fluid on the brain (75%), confusion (75%) and nausea (75%)which was comparable to the findings ofBaradkar et al., [16] noted – headache (100%), altered sensorium (100%), terminal neck stiffness (90%).

Our study found that the ICTshowed a sensitivity, specificity, PPV, NPV and accuracy of 99.0%, 100%, 99.0%, 100% and 100%study similar toMaster R. O et al [13] (100%,) andkwizera. R et al (91%) [17].

India ink and gram stain sensitivity, specificity similar to other study show in Table 4.and accuracy of India ink and gram stain were 98%and 96% with other study Master R. O et al [13].(Accuracy of India ink and gram stain were 97.4% and 97.0%).

Table 4: Comparison of India ink and gram stain with other study.

Other study	Year	Sensitivity of India ink	Sensitivity of gram stain	Specificity of India ink	Specificity of gram stain
R. Munivenkataswamy et al[18]	2013	75.00%	58.33%	100%	100%
YM Coovadiaet al [19]	2015	84%	89%	100%	100%
Master R.O et al[13]	2020	54.8%	61.3%	100%	100%
Present study	**2023**	**75%**	**50%**	**100%**	**95%**

CONCLUSION:

Our research found that the ICT was more sensitive than the other methods for the detection of *Cryptococcal* Meningitis. However, ICT was found to be best method for diagnosing *Cryptococcal* Meningitis, and showed the highest levels of sensitivity and specificity.

ETHICAL CLEARANCE:

The ethical committee clearance certificate was taken before starting of study by institutional medical ethical committee.

LIMITATION:

In our study, few numbers of samples were studied due to cost constraints and various antifungal MICs were not studied.

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