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Isolation And Clinical Characteristics Of Cryptococcus Isolates From Hiv Positive Patients

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Abstract

Cryptococcal meningitis is a dangerous fungal infection in both of HIV and non-HIV patients. Accordingly, from the recent taxonomy the fungus is divided into two types of species namely Cryptococcusneoformans and C. gattii). The infection of HIV is accepted globally as a major disease that for the evolution of Cryptococcal meningitis for cases of 80-90% and it is an abandon disease. Totally 150 HIV patients with meningitis were selected in this study. From them, 13 have Cryptococcal meningitis of which 10 were found to have primary episode and 3 have relapse episode. The ubiquity of Cryptococcal meningitis was noted as 10.58% and most of the patients were males with ratio of 81.25% and female in the ratio of 5.3:1. In this study the mostly affected persons were in the age of 41-50. The chief symptom found was headache in 89.47% next vomiting in 63.16% and fever in 42.11% patients. It has increased protein levels in 73.68% and also cell counts in 78.94%. Lymphocyte was the principal cell type and absence of CSF cells in two patients. Between these patients, the primary episode of CM, preparation of Indian ink was noted as positive 10 cases, positive LAT was found in 1 case and culture in two patients. In relapse patients Indian ink preparation was positive in 66.67%), LAT in 100% and culture in 33.33% respectively.

Key words: AIDS, meningitis, fungal infection, Indian ink stain.

Introduction

Cryptococcus spp., is a microscopic single celled fungus, pathogenic to human and it was first observed in juice of peach in 1894 (Emmons, 1951). The first identified clinical isolate was in the same period from lesions of sarcoma on the tibia of a 31 aged old lady (Jarvis et al., 2010). The name Cryptococcus means 'hidden seed', earlier less known pathogen came into public with the arrival of pandemic AIDS. The Cryptococcus genus composed of above 70 species but infection in human is rarely formed by species other than C.neoformans and C.gattii. Periodic cases of infection caused by C.laurentii (Lynch et al, 1981)59, C.albidus (Horowitz et al, 1993) and C.adeliensis (Rimeket al, 2004) have been isolated but the other cryptococcal species isolated from clinical samples need both culture and cytological proof of conquering before attribution of disease to them. Commonly C.neoformans correlated with

immunocompromised host infection where the association of C.gattii with immunocompetent host infections.

Cryptococcus spp., for many decades were classified into two chief species: C. neoformans var. neoformans and C. neoformans var. gattii with antigenic heterogeneity having five existing serotypes. Cryptococcosis human spectrum differs from colonization of respiratory tract to spreading infection. Cryptococcal meningoencephalitis (CM) is the severe, dangerous clinical symptoms so the affected patients required the immediate antifungal treatment. AIDS defining condition is CM that patients have HIV infection and caused the reduced count of CD4 lowers 100 cells /µl. At 1990s about 5-15% of AIDS patients affected by CM that had 2/3rd cases had presence of meningitis (Pfeiffer and Ellis, 1991).

Beginning accretion of childhood Cryptococcus spp. (Satish chandraet al., 2007) by inhalation from environment deplete airborne yeast cells into lungs. The small amount of inhaled aerosols reachthe airways caused the interaction of primary immune with alveolar macrophages or dendritic cells (DC). The in vitro studies showed that the involvement of DC in detecting, binding, phagocytosis, antigen presentation and activation of T-cells (Zimmer and Roberts, 1979). In 1990s the spreading of AIDS as pandemic, the cryptococcosis was considered as AIDS defining illness and reduced antiretroviral therapy (ART).

But in some countries, HIV infection is the main cause for depression of immune system about 90% of CM with HIV correlated. After the effort of ART, over 19 – 26% of patients had HIV-care with < 100 CD4 cell/ml, a chief risk factor for HIV correlated CM (Forbes et al., 2007).AT disease of cryptococcal, the components of polysaccharide capsule called as cryptococcal antigen (CrAg) are hut into biological fluids and onset of symptoms. Finding of CrAg in HIV patients are linked with 25% of patients had <100 CD4 cell/ml at high risk of CM and represents the immune depressed patients about 6%. Therefore, to contribute to the integrated management of HIV and its related opportunistic infections, we proposed to carry out this work.

Materials and method

Study Design

This study work was approved in the Government hospital and Institutional Review Board, Thiruchurapalli was acquired by the study commencement. All of the patients were fulfilling with criteria of inclusion and the permission was got from the concerned patients or their relatives and a questionnaire section was worn to get the patient's details. This research work was mainly depends on the hospital-based descriptive study.

Place of study

This work was accompanied on patient with HIV positive that they were admitted in the Government hospital, Thiruchurapalli, medicinal wards.

Study Period

The period of this study was lead from October 2020 to September 2021.

Samples collection

The samples were collected with precautions of aseptic from the patients and transferred to the laboratory as soon as possible. From the patient's the blood and Cerebrospinal fluid (CSF) were collected. The other samples were collected with the presence of extra meningeal apart from meningitis from the patients.

Processing of samples

The CSF was centrifuged for 10 to 15 minutes at1500-2000 rpm and sediment was used to perform Gram staining and culture and its supernatant were used for LAT. If the process was delay then the CSF was incubated at 37^0C for future use. For all the selected patients, the laboratory analysis including biochemical, gram staining, Indian ink stain, CD4 count and latex agglutination test.

Statistical analysis

It was measured by the usage of Statistical Package for Social Sciences (SPSS) version 20.0. This study proportional data was recorded by analysis of Pearson's Chi square test $\chi 2$.

Results and discussion

A total of 150 CSF samples were collected during the study period from HIV positive patients presenting with features of meningitis. The age of the patients varied from 14 to 65 years with a mean age of 38 yrs. Only 30 (18.86%) of the 150 patients were females and the male patients were 120 (Table-1). Among these HIV patients, 13 patients were diagnosed with Cryptococcal meningitis (CM), of which, 10 were primary episodes and 3 were symptomatic relapse episodes.Prior to the arrival of pandemic AIDS, few patients had infections because of C.neoformanswere described and the higher occurrence of cryptococcosis resembled the increase in infection of HIV (Franzotet al., 1999). The study executed in Chandigarh – PGIMER, the yearly occurrence of CM increased in 15 fold in 1995-99 compared to 1970-82 with the spreading of HIV patients in India (Land et al., 1975). The other research work described by AIIMS, New Delhi (1992-2004) reported that Cryptococcus co-infection HIV increased in 1992 at 20% to 49% in 2004. In this study, the prevalence of CM in HIV positive patients was analyzed to be 10.86%. It was reduced that compared to 12.9% in a report of Nayak et al (2010) researched among 2009 to 2012 at BHU, Varnasi.

Table-1: Biochemical analysis of CS Fin Cryptococcal meningitis (N=13)

CSF	No. of Patients	Percentage
GLUCOSE	2	31.57
Reduced	10	63.15
Normal	1	5.26
Elevated		

PROTEIN Reduced Normal Elevated	Nil 0 10	- 26.31 73.68
Cell count* Normal Elevated	2 11	10.52 78.94

*****complete absence of cells in the CSF (n=2)

Among the 10 patients who presented with primary meningitis, only 3 were females (18.75%). The relapse episode does not noted in none of the female during this study. For HIV patients, the headache was the main symptoms with meningitis symptoms. When compared to women the man was most frequently affected by Cryptococcal meningitis (Kalraet al., 1999) and considered before the susceptibility (Ellis and Pfeiffer, 1990).

The CSF culture on SDA observed the creamy coloured mucoid yeast like colonies in 48 hours in two samples and in 9 samples they found yeast like colonies in 48-72 hours. The balance two samples growth were noted on 5^{th} and 7^{th} day (Table-2). Cryptococcus delayed growth differ from 5-14 days and it was noted in earlier studies (Chuck and Sande, 1989).

Table-2: Biochemical identification of culture positive Cryptococcal isolates

Biochemical test	**No. of isolates**	
	Positive	**Negative**
Hydrolysis of urea (n=13)	13	13
Brown colonies on CFA medium(n=13)	13	13
Growth on CGB medium(n=13)	1	12
Assimilation of D-proline(n=13)	1	12
Growth on CDBT medium(n=12)	0	12

CFA- Caffeic acid ferric citrate agar, CGB-Canavanine Glycine Bromothymol blue, medium, CDBT-Creatinine Dextrose Bromothymol blue Thymine medium

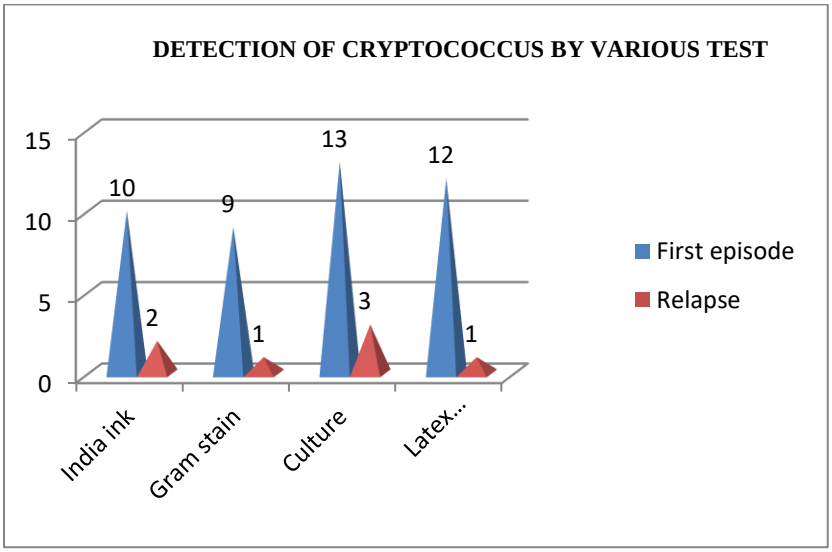
The predominant symptoms of CM was the headache (89.47%) were observed in this study and this was compared to the study by Bicanicet al., 2006 where 90% of patients had headache and another research by Chakrabarti et al., 2010 had 92.31% of cases had headache. Next the symptom noticed was vomiting in 63.16% cases then the fever was the next symptoms for 42.11% of cases. 36.84% of patients

had changed sensorium and stiffness of neck but in the study by Khanna et al., 1996 had changed sensorium in 45% and 71.79% respectively.

The CSF biochemical analysis in CM patients was showed in the table-1 that has levels of normal glucose in 63.15%. The increased range of protein were observed in 73.68% of patients and 78.94% of patients have increased levels of cell counts. Lymphocytes were the leading cell type andabsence of cells in the CSF was noticed in 2 patients. The normal ranges of glucose for adult is 40-70 mg/dl, 15-40 mg/dl of protein and <5 cells/µl of cell counts. The CSF patients had normal glucose levels but increased protein and cell counts. The patient with increased counts of cells, the main cells was lymphocytes. For two patients there was no detection of cells in the CSF. Zerpaet al., 1996 reported the absence of cells in50% of CSF patients. It was known that CM can be vague and sometimes the reduced glucose levels caused the higher level of protein.

Between the patients of primary episodes of CM, the preparation of Indian ink was positive in 10 cases (68.75%), 100% was positive for LAT in 13 cases and 12 cases had positive culture (75 %). Among patients presenting with relapse, India ink preparation was positive in 2 (66.67%), LAT was positive in 3 (100%) and culture and gram positive was positive in 1 (33.33%). Growthof Cryptococcus on SDA was observed within 72 hours in all except two CSF sampleswhich grew on the 5th and 7th day respectively (Fig-1). The research work by Ralston et al., 2017 reported the Indian ink positivity, LAT and Cryptococcus culture were found to be 91%, 100% and 100%. The positivity rate of Indian ink was 70%, LAT was 100% and culture was 85% showed the similarity to the result of this study.

Fig-1: Detection of Cryptococcus by various tests

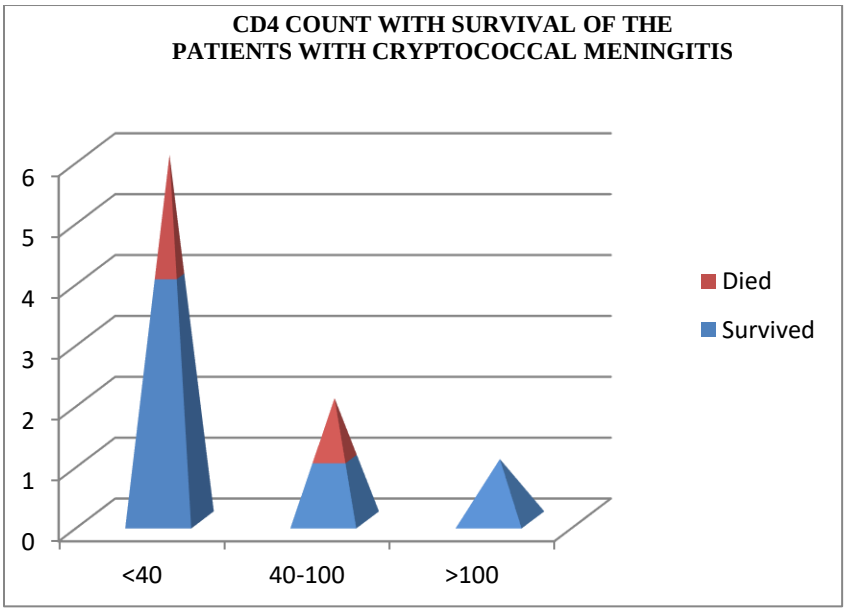


The 13 isolates of cryptococcal hydrolyzed urea and produced the brown colonies in the medium of CFA.However, only one isolate was growing on medium of CGB and integrate proline. The isolates of

C.neoformansintegrates the inositol, dextrose, maltose and sucrose and two of them does not integrate with cellobiose and no one integrate with lactose The isolate of C.gattiiintegrate with inositol, dextrose, maltose and sucrose but not with lactose and cellobiose. There were 15.79% of 3 relapse cases were admitted with presence of CM. A report by Lin and Heitman, 2006, 23% of relapse CM admitted to the hospital. Between the 3 cases having relapse episode, India ink, LAT & culture was positive in 2 (66.67%), 3 (100%) and 1 (33.33%). In this study, among the 3 patients had a relapse episodes had quiet secondary prophylaxis and continue with four months later with a relapse.

Most of the patients died had CD4 count was < 40cells/µl during the time of presentation with CM. One patient had CD4 count <100cells/µl with CM of primary episode and who live with infection was 71.63cells/µl and the patients who yield of infection was 34.71cells/µl. The correlation among the patients died with CD4 count <40cells/µl to counts >40cells/µl was statistically significant (p = 0.018) (Fig-2). Two patients had increased CD4 counts with relapse because of IRIS compared to the count of primary episodes.

Fig-2: Correlation of CD4 count with survival of the patients with cryptococcal meningitis



Conclusion

The study concludes that the most prevalent laboratory confirmed isolate is the Cryptococcal meningitis among the patients of HIV infected. There is a reduction in the cryptococcal meningitis patients because of good facilities for diagnosis at earlier stage of HIV infection. So it is difficult to diagnose because of the diagnostic tool challenges and higher prevalence. Our work creates awareness to the need for developing diagnosis and management for meningitis patients in resource limited settings.

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