INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Official publication of Indian Association of Medical Microbiologists,
Published quarterly in January, April, July and October)
Indexed in Index Medicus/MEDLINE/PubMed, 'Elsevier Science - EMBASE', 'IndMED'

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The journal is printed on acid free paper.

INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Publication of Indian Association of Medical Microbiologists)

ISSN 0255-0857 **Volume 25** **Number 4** **October-December, 2007**

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Indian Journal of Medical Microbiology, (2007) 25(4): 401-4

Brief Communication

CLINICAL AND MYCOLOGICAL PROFILE OF CRYPTOCOCCOSIS IN A TERTIARY CARE HOSPITAL

***MR Capoor, D Nair, M Deb, B Gupta, P Aggarwal**

Abstract

This study examined the extent of cryptococcosis in clinically diagnosed cases of meningitis in HIV-1 seropositive and apparently immunocompetent patients. One hundred and forty-six samples, obtained from 126 chronic meningitis patients comprised of cerebrospinal fluid (CSF), blood, sputum and urine. The samples were processed by standard microbiological procedures. Cryptococcal isolates were identified by microscopy, cultural characteristics, melanin production on niger seed agar and hydrolysis of urea. The isolates were further speciated on cannavanine glycine bromothymol blue (CGB) media. Cryptococcal antigen detection of CSF samples was performed by latex agglutination test (LAT). Minimum inhibitory concentration (MIC) of amphotericin B for the isolates was also tested. Cryptococcosis was diagnosed in 13 patients (eight HIV-1 seropositive and five apparently immunocompetent). *Cryptococcus neoformans* var. *neoformans* was the predominant isolate. Cryptococcal antigen was detected in all, whereas microscopy could detect yeast cells in nine patients. The isolates were sensitive to amphotericin B. CD4 cell counts ranged from 8 to 96/cu mm. The study concludes that all CSF samples with clinical diagnosis of subacute and chronic meningitis should be subjected to tests for detection of *Cryptococcus* in clinical laboratory irrespective of the immune status.

Key words: *Cryptococcosis, meningitis, HIV, C. neoformans, C. gattii*

The incidence of cryptococcosis, caused by encapsulated yeast *Cryptococcus*, has risen dramatically over the past 20 years. The human immunodeficiency virus (HIV) epidemic and other forms of immunosuppression are common factors explaining this rise.[1] Cryptococcosis is also seen in apparently immunocompetent individuals. The mortality and morbidity in developed countries are declining due to better access to highly active antiretroviral therapy (HAART) and prophylactic treatment regimens designed to prevent opportunistic infections (OIs).[2]

Cryptococcal meningitis (CM) is an acquired immunodeficiency syndrome (AIDS) defining illness in patients with CD4 cell counts below 100/cu mm. *Cryptococcus neoformans var. neoformans* (*C. neoformans*) is the species predominantly reported from immunocompromised patients, while *Cryptococcus neoformans* var. *gattii* (*C. gattii*) infection has often been associated with immunocompetent individuals. The environmental association of *Cryptococcus* with bird excreta and commonly grown trees are well documented from India.[3] In sub-Saharan Africa, CM occurs in 30% of AIDS patients and is likely to remain a substantial cause of death unless HAART becomes available. Next to sub-Saharan Africa, India has the second largest burden of HIV-related pathology.[4] However, the reported incidence of associated CM does not appear to be commensurate. Hence, there is a suspicion of missed diagnosis of CM.

Since the clinical presentation and radiological evidence of cryptococcosis are usually non-specific, diagnosis in early stage is difficult to arrive at. Means of early laboratory diagnosis include conventional (microscopic, culture) methods and latex agglutination test (LAT). Molecular methods involving polymerase chain reaction (PCR) are also important diagnostic and research tools.[1] Not much published Indian data are available on whether the pattern of HIV-related opportunistic infections (OIs), especially cryptococcosis, has changed with institution of HAART or on its coexistence with other OIs.[3-6] Routine testing for antifungal susceptibility of clinical isolates is also necessary to obtain baseline data and to observe any shift in sensitivity pattern in the population.[7]

This study was undertaken to determine the pattern and course of cryptococcosis. It also tries to examine the extent to which cryptococcal infections are missed in chronic meningitis cases, by subjecting the samples submitted for tuberculosis, to test for cryptococcosis also.

Materials and Methods

The study duration was one year and seven months (September 2003 to March 2005). One hundred and twenty-six patients admitted at medicine, neurology and paediatric wards with diagnosis of meningitis were included. The details of the demographic profile of the patients (age, gender, urban/rural habitat), their clinical features, predisposing

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Received: 08-02-07
Accepted: 04-03-07

factors, radiological findings, relevant laboratory findings, other OIs, course of illness, treatment and outcome were noted from hospital records.

From 126 patients, physicians had sent 146 samples [cerebrospinal fluid (CSF) - 104, blood - 16, sputum - 13, urine - 13] for microbiological examination. The samples were processed by standard microbiological (mycological and bacteriological) procedures for microscopy and culture.[1] The bacteriological media were incubated at 37°C and Sabouraud dextrose agar was additionally incubated at 30 °C. They were inspected after 24 h and, if negative, were further incubated for up to 21 days with inspections for growth at regular intervals. Lowenstein-Jensen media and Bac T/Alert MB (bioMérieux, France) bottles were used for culture of mycobacteria and were incubated upto eight weeks. Cryptococcal isolates were identified based on the following findings: characteristic appearance under microscope of narrow-based budding yeast with capsule, cultural characteristics, melanin production on niger seed agar, hydrolysis of urea, and carbon and nitrogen assimilation by agar disk method. The isolates were speciated to *C. neoformans* and *C. gattii* on cannavanine glycine bromothymol blue (CGB) media.[1]

Cryptococcal antigen detection of CSF samples was performed by LAT (Pastorex, CRYPTO Plus; Bio-Rad, France). Cryptococcosis was diagnosed when one or more types of tests employed gave positive results. Disseminated cryptococcosis was diagnosed when more than one site gave positive culture results.[3] Minimum inhibitory concentration (MIC) of amphotericin B for the isolates was tested by broth dilution method using RPMI medium and MOPS buffer, as per NCCLS (M 27-A2) guidelines.[8]

HIV status of the patients was treated as the indicator of immunocompetence. Testing for HIV statuses were conducted on those patients whose status was not already known. This was done with their prior consent and after pre-test counselling. Three enzyme immunoassays (EIA) using different antigens were undertaken, namely, Comb Aids (Span Diagnostics, India), Retroquic HIV (Qualprodiagnostics, India) and HIV EIA (Labsystems, Finland), as per national policy for strategy of HIV testing. CD4 cell counts were determined by FACS counter (Becton Dickinson, USA). Appropriate diagnostic tests were performed to rule out other opportunistic infections. Serological tests for *Toxoplasma gondii*, *Cytomegalovirus*, *Herpes simplex* (IgG, IgM antibodies) were carried out using ELISA (SPA Italiana laboratories, Italy).

Results

A total of 126 patients from whom the 146 samples were obtained, had been identified as relevant for the purpose of our study. Of these, 13 patients emerged with the diagnosis of cryptococcosis, as depicted in Table. These 13 were taken up for further detailed study.

The age of the patients ranged from 7 to 48 years with a median value of 40 years. Twelve were male and all were from urban areas. Clinical symptoms reported were headache (10), fever (9), altered sensorium (3) and neurological deficits (4). Eight patients, all male, were found to be HIV-1 seropositive and the remaining five were apparently immunocompetent.

CSF cytology showed low lymphocyte count in five patients, lymphocytosis in five patients and cell count was normal in three patients. Cryptococcocal antigen was detected in 13 CSF samples, whereas nine of these samples were also positive for encapsulated yeast on microscopy. *Cryptococcus* grew on culture from 10 CSF samples. Of the 10 isolates of *Cryptococcus*, seven were *C. neoformans* and three were *C. gattii*. All isolates were sensitive to amphotericin B and had MIC ranging from 0.063 μg/mL to 0.5 μg/mL.

Eight patients (six HIV-1 seropositive and two seronegative) received full induction course of amphotericin B (1 mg/kg bodyweight per day) for two weeks followed by maintenance therapy of fluconazole (400 mg/day) for 8 weeks. Out of three patients, two were on HAART for one year prior to admission. Other infections were treated with appropriate antibiotics.

Cryptococcosis in HIV positive patients

All eight HIV positive patients were of high-risk category and heterosexual and included four truck drivers, three migrant labourers and one puppeteer. Seven cases presented as first episode of CM and one (patient H) was a relapse case earlier diagnosed elsewhere. Two patients had developed meningitis in spite of being on HAART for one year. CSF microscopy was positive in all samples and the yeast load was high (1-2 yeast cells/HPF). LAT was positive in all CSF samples in dilution of 1:1024. Of the eight isolates from six patients, six were *C. neoformans* and two were *C. gattii*. Two patients suffered from disseminated cryptococcosis (patients A and B). The CD4 cell counts were low in all and ranged from 8 to 96/cu mm. Findings of contrast enhanced computed tomography (CECT) head were consistent with meningitis. The other OIs recorded in this group were tuberculosis oropharyngeal candidiasis, staphylococcal septicaemia and toxoplasmosis. Two patients died before amphotericin B therapy was initiated. Six received antifungal treatment for 10 weeks. However, they were all lost to subsequent follow-up.

Cryptococcosis in HIV negative patients

In five apparently immunocompetent patients, CSF showed lymphocytosis and the antigen was detected in all. One CSF sample was positive on microscopy for encapsulated yeasts. While one CSF was sterile, *C. neoformans* was isolated from three and *C. gattii* from the remaining one. The relevant findings in CECT (head) were presence of granuloma, hydrocephalus and meningitis. Two patients received amphotericin B for 10 weeks and three left

Table: Summary of clinical data of thirteen patients with cryptococcosis

Patient/ age, sex	Predisposing, Factor	Clinical / Radiology	Sample	Microscopy	LAT (titre)	Culture	Other OIs	Treatment	Outcome
A/40y, M	HIV	meningitis	CSF, Urine	+	1:1024	*C. neo*	PTB	ATT,AFT	Survived
B/35y, M	HIV	meningitis, pneumonia	CSF, Sputum	+	1:1024	*C. neo*	TB (Diss) OPC	ATT,AFT HAART	Survived
C/34y, M	HIV	meningitis	CSF	+	1:1.024	-	TBM OPC	ATT,AFT HAART	Survived
D/48y, M	HIV	meningitis	CSF	+	1:1024	-	TBM	ATT	Expired
E/30y, M	HIV	meningitis	CSF	+	1:1024	*C. gat*	Toxo	-	Expired
F/45y, M	-	meningitis	CSF	-	1:4	*C. neo*	-	-	LAMA
G/15y, M	-	multiple granuloma	CSF	-	1:8	-	-	-	LAMA
H/45y, M	HIV	meningitis	CSF	+	1:1024	*C. neo*	*Septicemia S. aureus*	AFT	Survived
I/40y, F	-	multiple granuloma	CSF	+	1:8	*C. neo*	-	AFT	Survived
J/24y, M	HIV	meningitis	CSF	+	1:1024	*C. gat*	-	AFT, HAART	Survived
K/7y, M	-	meningitis, hydrocephalus	CSF	-	1:4	*C. gat*	-	-	LAMA
L/45y, M	-	meningitis, hydrocephalus	CSF	-	1:8	*C. neo*	-	AFT	Survived
M/44y, M	HIV	meningitis	CSF	+	1:1024	*C. neo*	TBM	AFT,ATT	Survived

Y - Year, M - Male, F - Female, LAT - Latex agglutination test, OIs - opportunistic infections, HIV - Human immunodeficiency virus, CSF - Cerebrospinal fluid, PTB - Pulmonary tuberculosis, diss - Disseminated, TBM - Tubercular meningitis, OPC - Oropharyngeal candidiasis, Toxo - Toxoplasmosis, ATT - Antitubercular treatment, AFT - Antifungal treatment, HAART - Highly active antiretroviral therapy, LAMA - Left against medical advice, *C. neo* - *Cryptococcus neoformans*, *C. gat* - *Cryptococcus gattii*

against medical advice before laboratory diagnosis could be communicated. Two patients had neurological sequelae after treatment.

Discussion

The systemic cryptococcal infection can masquerade clinically and radiologically as tuberculosis, which is endemic in India. CM is usually not considered as first differential diagnosis. Other conditions, which may mimic CM, are cerebral stroke, brain tumour and enteric fever.[3] Therefore, awareness of the disease and a high index of suspicion are crucial to arrive at both clinical and aetiological diagnosis of CM.

In the current study, the overall positivity of microscopy, culture and LAT in CSF, were comparable to reports in the literature (70-90% for microscopy, 80-92% for culture, 95-100% for latex agglutination).[9] The CSF examination (Gram stain, India ink) employs simple and easy techniques and can be performed in any clinical microbiology laboratory. In one sample (patient I), presence of budding yeast cells in Gram stain raised suspicion which led to inclusion of a fungal culture. India ink was equivocal, perhaps due to presence of microcapsule. Repeated attempts to demonstrate yeast cells in microscopy and culture on lumbar puncture are an important requirement in diagnosis. LAT was positive in all samples. Pronase-based LA kits are highly sensitive and specific, but are expensive.[3] The quantitative analysis of antigen has prognostic value and helps in guiding chemotherapy and period of hospitalization. The isolates were speciated to *C. neoformans* and *C. gattii* on CGB media, which is a simple and easy test to perform.[1] All the strains were sensitive to amphotericin B. The susceptibility pattern of a primary and relapse isolate was similar. *In vitro* resistance of *Cryptococcus* to antifungal agents is reported to be rare.[5,7]

Out of a total of 13 patients, eight (six HIV positive and two HIV negative) received antifungal treatment as per standard regimen. Seven of them presented with first episode of CM and one was a relapse case (patient H), due to discontinuation of maintenance therapy. Patients C and J developed cryptococcosis in spite of being on HAART for 1 year. For patient B, HAART was started on admission. HAART helps in better survival of AIDS patients by immune reconstitution and decreases fungal OIs for which specific prophylaxis is not given.[2] Disseminated cryptococcosis was seen in patients A and B. The morbidity and mortality in CM is 10-30% in developed countries and 50-100% in developing countries, where medical facilities are less accessible.[2] We recorded concomitant infections with other

bacteria. The association of CM with tuberculosis and other OIs is well documented.[3,4,10] The spectrum of OIs in AIDS is governed by host immune status and by endemicity of microorganisms prevalent in the environment.[4] Isolation of C. *gattii* in two HIV positive patients reflects the changing pattern of cryptococcosis. It is well documented that it rarely infects AIDS patients, as its natural reservoir is rare in urban areas where the AIDS epidemic is centred.[11,12]

The five HIV negative patients who were apparently immunocompetent, had few yeast cells and low antigen titre compared to HIV positive cohort. There was no mortality or dissemination or associated OIs. Neurological sequelae were seen in two patients. These observations and isolation of *C. gattii* in this group were comparable to previous studies.[9,13]

The increase in reporting of cryptococcosis in both immunosuppressed and immunocompetent individuals in recent years reflects in some measure an enhanced clinical awareness and improved diagnostic capability. In view of protean clinical manifestations and increased incidence of cryptococcosis in immunosuppressed and apparently immunocompetent patients, genetic characterization of these strains is crucial to know about strain variation and virulence. We believe that an awareness among clinicians and microbiologists will go a long way in arriving at a definite clinical and aetiological diagnosis of cryptococcosis, which would have been otherwise missed or undergone treatment of tuberculosis.

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Source of Support: Nil, **Conflict of Interest:** None declared.