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A Prospective Cohort Study of Cryptococcal Meningitis in Hiv Patients in a Tertiary Care Hospital

KEYWORDS: Cryptococcal meningitis, antifungal resistance, fluconazole

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ABSTRACT *Cryptococcal meningitis is an AIDS defining illness caused by an encapsulated fungal organism that has more predilections for the immune compromised. This hospital based prospective cohort study was done to analyze clinical and microbiological features of cryptococcal meningitis in patients with acquired immunodeficiency syndrome (AIDS). The study included demography, clinical presentation, CD4 counts, CSF analysis, microbiological diagnosis, biotype of the Cryptococcus, and the antifungal susceptibility testing of the isolates. 28 cases of Cryptococcal meningitis were confirmed during the three year study period. The CD4 count ranged from 60 to 198 cells /µl. All 28 patients were positive for culture, 71.4% were positive for India ink preparation and 89.2% were positive for Latex agglutination test. All the isolates were sensitive to Amphotericin B, Itraconazole and Voriconazole, and eight were resistant to Fluconazole. Combining all three tests can increase chances of early detection and help the clinician to initiate early antifungal therapy.*

Introduction:
Cryptococcal meningitis is caused by Cryptococcus neoformans, encapsulated yeast that can cause disease in both immunocompetent, as well as immunocompromized hosts [(1)]. The organism is inhaled into the host's lung where they establish colonies and cause disease, and then extra-pulmonary dissemination takes place including CNS and skin lesions. The incidence of C.neoformans infection has greatly increased with the emergence of AIDS and the increased occurrence of other immunosuppressed conditions [(2)]. The incidence is higher in the developing countries of Africa and Asia [(3)]. If untreated, cryptococcal meningoencephalitis is 100% fatal, and even when treated with the most effective antifungal drugs, cryptococcal infections can be fatal if the host does not have adequate T – cell dependent immune function [(4)]. The clinical manifestations of cryptococcosis are varied though the severity of the infection and the spread is more pronounced in the individuals with deficient cell mediated immunity as in AIDS [(5)]. Cryptococcal meningitis is usually treated with Amphotericin B with or without flucytocine, followed by maintenance therapy with fluconazole. We conducted this prospective cohort study to analyze cryptococcal meningitis in patients with AIDS. This included demography, clinical presentation, microbiological diagnosis, biotype of the Cryptococcus, and the antifungal susceptibility.

Materials and Methods:
The study was conducted in the Kasturba Medical College, Mangalore, India. 28 patients with cryptococcal meningitis in AIDS patients diagnosed based on clinical presentation, and further confirmed by laboratory tests were included in the study. All were newly diagnosed cases and no antibiotic or anti-fungal therapy was given at the time of CSF collection. HIV status of the individuals was confirmed by ELISA according to WHO guidelines. CD4 count of the patient was recorded by flow cytometry. All CSF samples were analyzed microscopically by gram staining and India ink preparation. Cryptococcal antigen detection was done using latex agglutination test (Murex). The sediment from the centrifuged sample was inoculated on two Sabourauds dextrose agar (SDA) with chloramphenicol and gentamicin slants and incubated at 37°C and other at room temperature respectively. All cultures were incubated for 3 weeks before a negative report was considered. Urease test (Christensen's urea agar) and phenol oxidase tests (Caffeic acid agar) were also performed. All the Cryptococcus neoformans isolates were biotyped on L Canavanine glycine bromothymol blue (CGB) agar [(6)]. The antifungal susceptibility testing was done for flucytosine, amphotericin B, fluconazole, voriconazole and itraconazole using ATB ™ Fungus 3, Biomerieux ® France.

Results:
Of the 28 cases of Cryptococcal meningitis, males accounted for 19 patients with male to female ratio of 2:1. Age predominance was in the range of 20 -40 years (median age; 32 years). Majority (25) presented with severe headache and fever. Other symptoms were nausea, disturbances in vision, neck stiffness, behavioural changes, convulsions and unconsciousness.

The cellular response of CSF in all cases was lymphocyte predominance. The CD4 count ranged from 60 to 198 cells /µl. Lymphocyte predominance persisted in repeat CSF samples on 5th day, 10th day, 14th day and 4 weeks.

All 28 were positive for culture. 20 patients (71.4%) were positive for India ink preparation. 25 (89.2%) patients were positive for Latex agglutination test. In three patients only culture was positive while India ink and antigen detection were negative. All isolates were identified as Cryptococcus neoformans var neoformans. All the isolates were sensitive to Flucytosine, Amphotericin B, Itraconazole and Voriconazole. Eight isolates exhibited resistance to Fluconazole with a MIC of 32 -64 mg/L.

All patients were treated with Amphotericin B and Fluconazole. Of the 28 patients, 20 patients survived, 5 succumbed to the illness and three left the hospital against medical advice.

Discussion:
Cryptococcal meningitis (CM) is the most common manifes-

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tation of systemic fungal infection in human immunodeficiency virus (HIV) infected patients and remains associated with significant morbidity and mortality. Untreated meningitis is fatal [7]. Cryptococcal meningitis is known for diverse clinical presentations ranging from mild head ache to overt signs of meningitis.

Cytology and biochemical findings of CSF are not specific. They may be similar to those of tuberculous meningitis, viral meningitis, and atypical purulent meningitis which may be cause of meningitis in the immunosuppressed individuals [8]. Hence the confirmation of diagnosis is solely relied on the microbiological diagnosis involving India ink preparation, antigen detection and culture.

India ink preparation and latex agglutination tests (LAT) of the CSF are simple and rapid tests that demonstrate the encapsulated yeast cell. The India ink preparation test has a relatively low sensitivity as compared to latex agglutination test [9]. Our results (India ink preparation 71.4% positive as compared to latex agglutination test 89.2% positive) are also in support of it. This low sensitivity is because the test is positive only when 10^3 -10^4 colony forming units (CFU/ml) are present in the CSF sample. Sensitivity can be improved by centrifuging the sample and using the pellet for the preparation[10]. In HIV infected patients capsule production may be reduced due to immuno-suppression, and the test may be negative. There is documented evidence of absence of capsule in the yeast which makes it difficult for diagnosis[11]. A quantitative LAT is both diagnostic and prognostic, as the titres decrease with the treatment [8,10].

Culture is considered as the gold standard in the diagnosis of cryptococcal infection[12]. In fact culture was positive in all cases in our study, and all the twenty eight isolates were Cryptococcus neoformans var neoformans. Culture however required 2-6 days for the results leading to a diagnostic delay in those cases where India ink preparation or the antigen detection tests were negative. We recommend that in suspected cases the cultures should be incubated for a minimum of 14 days before giving a negative report. Culture has an advantage of determining the species and also antifungal susceptibility testing.

Amphotericin B is used as the drug of choice for treatment during an episode of meningitis along with flucytocine or fluconazole, but fluconazole is used as a prophylactic antifungal drug against cryptococcosis. There is drug resistance developing against fluconazole. In our study we found eight isolates having a high MIC of 32 - 64 mg/L. Therefore antifungal susceptibility testing may be useful in all cases of cryptococcal meningitis.

It is obvious in our study that the LAT and India ink preparation are rapid but may give some false negative results. Culture if done in fresh untreated cases has a high sensitivity and is the most specific test. Combining all three tests can increase chances of early detection and helps the clinician to initiate early antifungal therapy. If promptly treated, majority show a good response to anti-fungal therapy. Emergence of fluconazole resistance is alarming since it is the most common drug used for the prophylaxis.

REFERENCE

1. 1. Thomas G Mitchell and John R Perfect. Cryptococcosis in the Era of AIDS – 100years after the Discovery of Cryptococcus neoformans. Clin Microbiol Rev., 1995; Vol.8, No.4: 515-548. | 2. Fran Fisher and Norma B Cook. Fundamentals of Diagnostic Mycology. W B Saunders Company. First Edition 1998. | 3. Uma Banerjee. Progress in diagnosis of opportunistic infections in HIV/AIDS. Indian J Med Res. 121, April 2005: 395-406. | 4. Subramanian S, Mathai D. Clinical manifestations and management of cryptococcal infection. JPGM. 51, 2005: 21-26. | 5. Reiko Ikeda, Takashi Sugita, Takako Shinoda. Serological relationships of Cryptococcus spp.: Distribution of antigenic factors in Cryptococcus and intraspecies diversity. JCM; Nov 2000; 4021-4025. | 6. Kyung J Wwon Chung, Itzhack polacheck and John E Bennet. Improved Diagnostic medium for separation of Cryptococcus neoformans var neoformans (Serotypes A and D) and Cryptococcus neoformans var gattii (Serotypes B and C). JCM; Mar 1982; 535 -537. | 7. Rajeshwari S, Prabha M R Adhikari, John T Ramapuram, Satish Rao, M RM S M Pai, Kiran. Amphotericin B for cryptococcal meningitis in HIV positive patients: Low dose versus high dose. Indian J Crit Care Med; 2007: 112-116. | 8. Hongzhou Lu, Yingjie Zhou, Youkuan Yin, Xiaozhang Pan, Xinhua Weng. Cryptococcal Antigen Test Revisited: Significance for Cryptococcal Meningitis Therapy Monitoring in a Tertiary Chinese Hospital. J.Clin.Microbiol; 2005: 43: 2989-2990. | 9. Saha DC, Xess I, Jain N. Evaluation of conventional & serological methods for rapid diagnosis of cryptococcosis. Indian J Med Res. 2008; 127(5):483-8. | 10. Satishchandra P, Mathews T, Gadre G, Nagarathna S, Chandramukhi A, Mahadevan A, Shankar S K. Cryptococcal meningitis: Clinical, diagnostic, and therapeutic overviews. Neurology India; 2007: 55: 226 -232. | 11. Shashikala, Kanungo R, Srinivasan S, Mathew R, Kannan M. Unusual morphological forms of Cryptococcus neoformans in Cerebrospinal fluid. IJMM; 2004: 22: 188 -190 | 12. Saldanha Dominic RM, Prashanth HV, Shalini Shenoy, Shrikala Baliga. Diagnostic value of latex agglutination in Cryptococcal meningitis. J Lab Physicians; 2009: 67-68 |