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Prognosis of human immunodeficiency virus infected patients with *Cryptococcal meningitis*

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Abstract
The incidence of and mortality related to *Cryptococcal meningitis (C. m.*) remain exceedingly high worldwide. With advent of HIV, *C. m.* cases are on rise along with prognosis of patients. Hence observations were made of prognosis of patients with HIV positive and negative status suffering from *C. m.* at Nair Hospital, Mumbai. The study included 180 patients, clinically suspected for fungal meningitis. Screening for HIV of these patients was done by rapid diagnostic kits and interpreted according to NACO guidelines. Also identification of Cryptococcus isolates from CSF samples was carried out by standard procedures. 25% and 75% of cases were HIV positive and negative respectively. *C. m.* was diagnosed significantly higher in HIV positive cases as compared to HIV negative cases. Male preponderance was observed in present study. Maximum number of isolates were sensitive to Amphotericin B as compared to Fluconazole. Out of total 22 patients with *C. m.* 5 cases showed mortality. Out of which 4 were HIV positive and one was HIV negative. While 17 cases with *C. m.* were discharged which were then failed to have follow up. Out of which, 15 were HIV positive and two were HIV negative. Our study concludes that *Cryptococcus neoformans var neoformans* was the most prevalent strain causing fungal meningitis in HIV positive patients. New point-of-care testing and follow up modules have potential to improve early diagnosis and treatment of *C. m.* in resource-limited settings markedly, however, substantial barriers exist in adhering to the accepted guidelines.

Keywords: *Cryptococcal meningitis*, prognosis, human immunodeficiency virus, amphotericin b, fluconazole

1. Introduction
Fungal meningitis tends to be a sub-acute or chronic process; however, it may be just lethal as bacterial meningitis if untreated. Most central nervous system (CNS) fungal infections can be divided into those that occur in normal hosts and those that occur in the immunosuppressed host. Cryptococcal infection is common in both groups. Cryptococcal disease is the most common life threatening fungal infection in patients with AIDS [1].

Cryptococcal meningitis (*C. m.*) is an invasive fungal infection of the CNS caused by the encapsulated yeast, *Cryptococcus neoformans*. The major predisposing factor in the development of cryptococcosis is profound cell-mediated immune deficiency, seen most commonly with advanced human immunodeficiency virus (HIV) infection. Though recognized a century ago, Cryptococcus became a major human pathogen with the emergence of the acquired immune deficiency syndrome (AIDS) epidemic [2]. Cryptococcosis, the fourth most common opportunistic infection in AIDS patients has a wide spectrum of clinical presentation. Therefore testing for this pathogen has become routine for patients infected with HIV. The situation has become more alarming with the onslaught of the AIDS pandemic. Cryptococcosis is now being regarded as one of the AIDS defining infection by WHO and incidence of it has been reported as 5-33% worldwide [1].

The incidence of and mortality related to *C. m.* remain exceedingly high worldwide, particularly in settings where access to antiretroviral therapy is still limited. The global burden of HIV-associated Cryptococcal disease is estimated at nearly one million cases annually [3]. While long-term survival has improved with widespread use of antiretroviral therapy in high-income countries, early mortality remains high [4]. Furthermore, expanding access to antiretroviral therapy in resource-limited settings has not yet led to compelling

Improvements in mortality, with 10-week mortality rates between 24% and 37%, even under optimal research conditions [5]. Early mortality rates are often 50% in routine practice where access to diagnostics or medications is limited or unavailable, intracranial pressure is uncontrolled, or in settings where other barriers to the management of *C. m*. exist [6]. Abraham M. *et al*. had reported first time a case of meningitis caused by *Cryptococcus neoformans var gatti* serotype B in AIDS patients from India which was reported very rarely [7].

Our observations in B.Y.L Nair Charitable Hospital, in 1992 to 1998 years had shown a gradual rise in the incidence of the Cryptococcosis infection in HIV positive patients and those patients who receive immunosuppressive drug therapy and having some other predisposing factors. The number of diagnosed cases of Cryptococcosis had increased from 6.6% to 13.75% from the year 1992 to 1998. The data regarding the *C.m*. cases and the prognosis is still not very clear in India. Also with advent of HIV, *C.m.* cases are on rise along with the prognosis of the patients. Hence the study and observations were made of prognosis of patients with HIV positive or HIV negative status suffering from *C.m.*

2. Material and methods

This prospective longitudinal study was carried out over a period of three years, from January 1997 to December 1999 at the Department of Microbiology, after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai.

2.1 Participants

The study included 180 patients, who were clinically suspected for fungal meningitis. These subjects were showing sign and symptoms of fever with chronic headache, body ache, Nausea, vomiting, staggering gait, altered sensorium and neck stiffness. Also patients on long-term immunosuppressive drug therapy and malignancy and organ transplant were included. Each patient's demographic data is collected in specially structured format.

2.2 HIV testing

Blood was collected from the subjects and serum was separated. Serum samples were stored at -20ºc and thawed when the tests were put up. Screening for HIV was done by rapid diagnostic kits and interpreted according to NACO guidelines. The report for HIV testing was collected from ICTC with consent and strict confidentiality. Antibodies to HIV 1(subgroups O and C) and HIV 2 was checked by Microlisa kit manufactured by J. Mitra and company. All the serological tests were done as per the instructions supplied by the kit provider with appropriate quality check. Also all the serogical work was carried out taking care of bio-safety measures and standard discard procedures.

2.3 Collection of CSF and microbiological analysis for detection of Cryptococcus.

Cerebrospinal Fluid (CSF) was obtained from each patient from various clinical units by trained clinicians by the standard procedure [8] and sent to Microbiology department for the further analysis. CSF samples were centrifuged at 3000 rpm for 10 minutes immediately and sediment was studied by microscopy and culture methods. Identification of Cryptococcus isolates from CSF samples was carried out by direct microscopy, culturing on various differential media, biochemical tests and blood culture [9].

3. Results

Table 1: Fungal Positivity of the patients with respect to HIV Status n=180

HIV Positive cases		HIV Negative cases	
45 (25%)		135(75%)	
Fungal positive cases	Fungal negative cases	Fungal positive cases	Fungal negative cases
23 (51.11%)	22(48.89%)	14 (10.37%)	121 (86.63%)

Out of 180 CSF samples studied, 25 % of cases were HIV positive and 75% cases were HIV negative. In HIV positive cases, 51.11% cases were fungus positive and in HIV negative cases 10.37% cases were fungus positive. Total Fungal positive cases and negative cases were found to be 37 (20.56%) and 143 (79.44%) respectively. There was significant association between fungal and HIV infections (95% CI: 16.3, 33.7 (P<0.001) Chisquare test with YaH's Correction = 31.95)

Table 2: HIV Status of patients suffering from *Cryptococcal meningitis* n=180

Groups	Suspected Cases	Diagnosed Cases
HIV positive cases	45 (25%)	19 (42.22%)
HIV negative cases	135 (75%)	03(02.22%)
Total	180	22

Chisquare =46.7(P, 0.001)

C. m. was diagnosed significantly higher in HIV positive cases as compared to HIV negative cases. Cryptococcosis was diagnosed more in adult males than in adult females. Out of total 180 subjects, 132 were males and 48 were females. Cryptococcosis was diagnosed maximum in males as compared to females. Male preponderance was observed in the present study.The ratio of M: F was 2.6: 1

Table 3: Antifungal susceptibility test (Stokes method)

Cryptococcus species Isolates	Amphotericin B		Fluconazole	
	sensitive	resistant	sensitive	resistant
20	12 (60%)	08(40%)	04(20%)	16(80%)

In vitro antifungal test was carried out by stokes method using *Candida kefyr* as a standard control strain. It was observed that maximum number of isolates were sensitive to Amphotericin B as compared to Fluconazole.

Table 3: Death and recovery of patients with HIV and *Cryptococcal meningitis* n=22

No of Patients diagnosed with *C. m.*	Deaths		Recovery	
	HIV positive cases	HIV negative cases	HIV positive cases	HIV negative cases
22	04 (18.18%)	01 (4.54%)	15 (68.18%)	02 (9.09%)

Out of total 22 patients with *C. m.*, 5 cases showed mortality. Out of which 4 were HIV positive and one was HIV negative. While 17 cases with *C. m.* were discharged which were then failed to have follow up. Out of which, 15 were HIV positive and two were HIV negative.

4. Discussion

Total 20 Cryptococcus species were isolated and confirmed by various biochemical tests. Niger seed agar which confirmed it as *Cryptococcus neoformans*. Further separation of these isolates upto their varietal status was carried out by inoculating on L- Canavanine Glycine Bromothymol blue medium. Out of these 20 isolates, one was speciated as *C. neoformans var. gatti* and other 19 were separated as *C. neoformans var. neoformans*. Antifungal susceptibility test results revealed maximum sensitivity towards Amphotericin B as compared to Fluconazole, hence Amphoterin B could be still considered as a drug of choice.

Pathogenesis of Cryptococcosis is determined by the status of host defences and because of the ubiquity of *C. neoformans* in nature and the relatively low prevalence of symptomatic infection; the incidence of exposure far exceeds the incidence of disease. Therefore most people are probably, resistant and only individuals with compromised host defences are likely to develop Cryptococcosis. Bulmer and Tacker had suggested that disease can be initiated in person with normal defences by exposure to a usually large inoculum and hence the size of the inoculum matters. The strains of *C. neoformans* differ in virulence, and the establishment of symptomatic infection, whether in healthy or compromised subjects, depends upon inhalation of more virulent yeast cells [10].

Clinical manifestation tends to overlap between patients with AIDS and severely immune-compromised patients without AIDS. Certain findings are generally more common in AIDS than non- AIDS patients[10] HIV-associated *C. m.* has a slowly progressive sub-acute onset, typically presenting as a meningo-encephalitis in severely immune-compromised persons. *C. m.* generally presents (about 95%) in persons with a CD4 count, 100 cells/μL, and *C. m.* is the first AIDS-defining illness in the majority. In general, *C. m.* should be considered in any patient with advanced HIV infection and symptoms referable to the central nervous system [11].

The symptoms and signs of meningitis in patients with or without AIDS are similar. The duration of symptoms and signs may be severe in AIDS patients because of the high burden of organisms and poor inflammatory response in these patients whereas apparently non-immunocompromised hosts with *C. m.* may have symptoms that persist for months. Such patients may present with true chronic meningitis. Khanna N *et al*. had analysed predisposing factors, laboratory findings and outcome in patients with Cryptococcal infection of the CNS from South India with special reference to HIV infection [12]. According to Aquinas SR *et al*, presence of Cryptococcemia in *C. m.* was an indicator of poor prognosis, hence a high index of clinical suspicion and routine mycological surveillance is essential to identify this infection [13].

Cryptococcosis is difficult to prevent because of its sporadic nature, occurrence during severe immune-suppression, and the meagre epidemiological data on prevalence of infection in specific areas. The first trial to evaluate prophylaxis with fluconazole to prevent Cryptococcosis among patients with AIDS suggested a positive benefit. Anti-fungal prophylaxis in certain high risk groups may be useful but it needs to be carefully considered. Active immunisation in the form of a vaccine is an ideal strategy for prevention among high-risk patients [10]. Over all prevalence of *C. m.* in the study was found to be 12.2% which is in accordance with the WHO incidence rate of 5-33% worldwide [1].

In the era of AIDS pandemic, Khan *et al*, from Lucknow, had reported 10% positivity of *C. m.* from 1991 to 1994**[14]** which showed a rise in the prevalence rate as compared to only 9 cases of Cryptococcosis in 12 years period reported by Talwar *et al*. from Chandigarh [15].

In the present study 86.09% of the *C. m.* cases were HIV positive which correlated with a retrospective study of Pedrol *et al* from Spain in 1985-90 which showed that 76.9% of the *C. m.* cases were HIV positive [16]. According to Aquinas *et al* from Bangalore in 1996 *C. m.* was the most common opportunistic fungal infection in patients with AIDS contributing to the increased morbidity and mortality [13].

Hence our present study correlated with this findings that Cryptococcosis was maximally diagnosed in HIV positive patients than HIV negative patients. However, Petty *et al*. had noted the prevalence rate as low as 3.3% from HIV positive patients [17]. Hence a high index of clinical suspicion and routine mycological surveillance was essential to identify this infection [13].

Present study revealed a male preponderance, the proportion of males to females being 2.6:1. Similarly the male to female ratio, 3:1 was reported by Rozenbaum *et al*. [18] A similar study by Kirti *et al* in 1996-97 from Mumbai, stated the ratio of male to female being 9:1 [19] The Mean age of patients in our study was 31 years among *C. m.* cases. Mean age reported by Aquina *et al*. from Bangalore was 40 years [13]. Powderly WG *et al*. had reported average age as 42-46 years [20]. From above reports it appeared that *C. m.* occurs chiefly in the young adult males. An overall preponderance of Cryptococcal infection in young adults was known and could be related to an active outdoor life style [18]. However Moosa *et al*. from South Africa in 1995 had reported females out-numbering males in HIV associated *C.m.* cases [21]. This was in contrast with the studies carried out in the West, where males were affected predominantly [22].

Kauffman *et al*, White *et al*, Treseler *et al*. & Lyons *et al*. stated that Amphotericin- B with or without flucytosine remains the drug of choice for many fungal infection especially those that were life threatening. It was the preferred as initial treatment for many fungal infections [23, 24].

Study carried out by Shindo *et al*. in 1990, had proved that Amphotericin- B was essential to administer to the patient as the initial treatment for *C. m.*, when antigen titre and India ink was positive. Once the diagnosis was established, treatment with Amphotericin- B was mandatory and a response should be expected [25].

Death occurred in five of our patients with *C. m.* (22.5%) others 17 patients were discharged after the treatment and the follow up of this patients was not possible. Kedare K. had reported mortality rate of 36.3% from Mumbai [18] and SR Aquina *et al*. had reported mortality rate of 42.9%. of these 28.6% survived and an equal number was lost to follow up [13].

Possible prognostic factors which characterise the outcome of patients with AIDS include antigen titres and positive culture from extra meningeal source. A high titre and positive culture from extra-meningeal source is associated with worse prognosis [26]. Even in the present study mortality rate was 100% in fungemic cases. Fungal infection are being increasingly recognised. In addition to generally recognised

pathogens, numerous other fungi are seen to cause infections especially under condition of abnormal patient susceptibility. With the advent of new and expanding population of immune-compromised hosts, the list of fungi is expected to increase. Furthermore, expanding access to antiretroviral therapy in resource-limited settings has not yet led to compelling improvements in mortality, with 10-week mortality rates between 24% and 37%, even under optimal research conditions [27]. Early mortality rates are often 50% in routine practice where access to diagnostics or medications is limited or unavailable, intracranial pressure is uncontrolled, or in settings where other barriers to the management of *C. m* exist. [24] Follow up modules have potential to improve early diagnosis and treatment.

5. Conclusion

Our study concludes that *Cryptococcus neoformans var neoformans* was the most prevalent strain causing fungal meningitis in HIV positive patients. Symptoms typically begin indolently, and can include fever, malaise, and headache. Altered mental status is a marker for advanced disease and is predictor of poor outcome. Analysis of CSF during primary disease is notable for a lack of inflammation in AIDS patients. The presence of cryptococcal antigen in cerebrospinal fluid or serum is central in the diagnosis of *C. m.* New point-of-care testing has the potential to improve early diagnosis of *C. m.* in resource-limited settings markedly, and has been recommended by the World Health Organization. However, substantial barriers exist in adhering to the accepted guidelines in resource-constrained areas of the world where *C. m.* is most prevalent. Fluconazole is widely available worldwide, and is an essential component through all phases of treatment, though is not preferred for induction monotherapy. Innovative trials utilizing different combinations of antifungal therapy are ongoing, and are leading the way in efforts to improve the care of HIV patients with *C. m.* The optimal time to initiate antiretroviral therapy in patients with *C. m.* remains unclear, and trials are underway to answer this question.

In future, we are optimistic that Cryptococcosis will be under control perhaps as a result of the development of a novel vaccine, effective immunotherapy, improvement in the delivery of current antifungal drugs, or methods of intervention based on knowledge of the virulence factors [10].

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