ORIGINAL RESEARCH PAPER

Volume-7 | Issue-10 | October-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PREVALENCE OF FUNGAL MENINGITIS IN CLINICALLY SUSPECTED CASES.

Microbiology

Dr. Trupti V. Mathure* — Research Student, Department of Microbiology,T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai-40008 *Corresponding Author

Dr. Shashikant P. Vaidya — Assistant Director, Clinical Pathology Department, Haffkine Institute, Acharya Donde Marg, Parel (East) , Mumbai-4000 12

Dr. Sunita D. Deshpande — Professor, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai-40008

Dr Geeta V. Koppikar — Dean , T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai-40008

ABSTRACT

Fungal infections are an important cause of morbidity and mortality in immune-compromised patients. Keeping in mind the above facts, the study was conducted to understand the prevalence of fungal meningitis in clinically suspected cases in Nair Hospital, Mumbai,including 180 patients,who were clinically suspected for fungal meningitis. Collection and microbiological analysis of cerebrospinal fluid for identification of fungal isolates was carried out as per standard procedures. Total 35 fungal strains were isolated from total 180 cases and Cryptococcus neoformans var neoformans being the most prevalent strain followed by Candida species, causing fungal meningitis in HIV positive patients. There was significant association between fungal infections and HIV positivity.Amphotericin B was found to be the drug of choice for the fungal meningitis.

KEYWORDS

Fungal Meningitis, Hiv, *Cryptococcus*, Cerebrospinal Fluid

1. INTRODUCTION :

Fungal infections are an important cause of morbidity and mortality in immune-compromised patients. The use of more intensive regimens of immune-suppression, more potent antibiotics and the increasing incidence of acquired immune deficiency syndrome (AIDS) has led to a higher incidence of opportunistic fungal infections such as *Aspergillosis, Candidosis, Cryptococcosis, Zygomycosis* . The magnitude of the problem in India is comparable to that in the Western world. Early diagnosis is the cornerstone for the effective management. The clinical signs of fungal infections are non-specific and confident diagnosis relies on a combination of microbiological, histological and serological evidences. Frequently, Candidiasis is the first sign of impaired immune function. The severity of the fungal infections increases as the immune system becomes more dysfunctional. [1]

The neurological complications of HIV infection are both common and varied and contribute importantly to patient's morbidity and mortality. Nervous System opportunistic infections, fungal meningitis accounts for over 40 % of the AIDS patients with neurological manifestations. Serious infections are seen in severely immune-compromised patients usually with CD4 counts of less than or equal to 200 cells per ml Fungal diseases are becoming increasingly important due to their increased incidence. Currently adequate facilities for diagnosis of fungal diseases are not available with sufficient scientific back up even in the international scenario. Facilities for diagnosis, therapy and management of fungal diseases have become a necessity in the field of respiratory diseases and transplant cases all over [2]. Several fungal pathogens are known to cause meningitis such as *Cryptococcus, Candida, Rhodotorula, Aspergillus* and many other filamentous fungi. Cryptococcal infection which may cause meningitis, is the most prevalent and common invasive fungal infection[1].

Our observations in Nair Hospital, in year 1992 to 1998, had shown a gradual rise in the incidence of the fungal infections with meningitis in HIV positive patients, patients who receive immune-suppressive drug therapy and having some other predisposing factors. Hence the study was conducted in the Department of Microbiology to find out the prevalence of *Fungal meningitis* in clinically suspected cases.

2. MATERIALS 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.

2.2 Collection and microbiological analysis:

Cerebrospinal Fluid (CSF) was obtained from each patient from various clinical units by trained clinicians by the standard procedure [3] 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.

2.3 Identification of fungal isolates from CSF samples :

A) Initially Direct microscopy of CSF samples was done by Wet mount, Modified India Ink Preparation.[4] and Gram staining .

B) CSF sediment obtained after centrifugation was cultured on Blood Agar, Chocolate Agar , MacConkeys Agar, Soyabean Casein Digest Broth (SCD), Corn Meal Agar (CMA) along with Sabourauds Dextrose Agar (SDA) in duplicate and incubated at 37 ^{0}C and Room Temperature . Growth was observed everyday till 4th week before reporting sample as negative.

C) Biochemical Analysis of fungal isolates was carried out by Germ tube test , Urease test ,Nitrate reduction test, Sugar Assimilation test and Sugar fermentation test.

C) Blood Culture [5] was done in brain heart infusion broth to detect Cryptococcemia in patients showing *Cryptococci* on direct mount . Inoculated bloods cultures, were incubated for 48-72 hours at 37^{0}C and were subcultured on SDA without cycloheximide to check the colonies of *Cryptococcus isolates.*

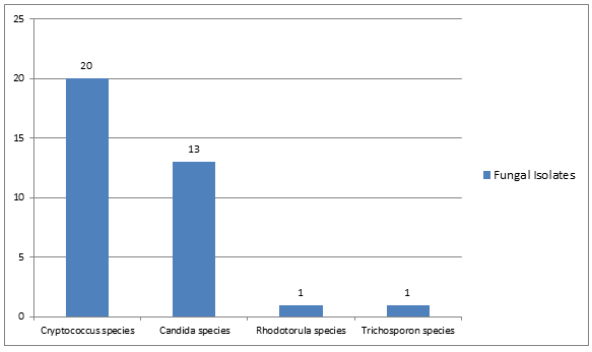
2.4 Determination of In vitro antifungal activity

All fungal isolates were tested in vitro against Amphotericin B (100 units/disc) and Fluconazole (10 microgm/disc) by Stokes method [6] using *Candida kefyr* as control strain.

3 RESULTS :

Graph 1: Number of Fungal isolates in CSF samples.

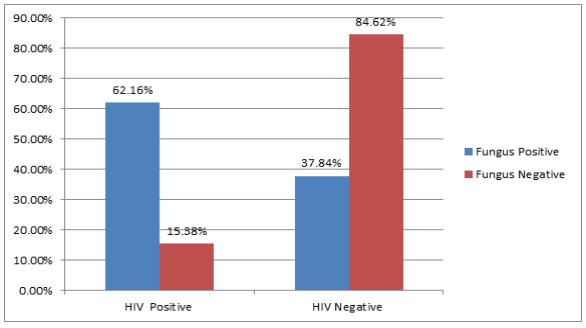
n=180



Study revealed *Cryptococcus* species in 20 cases, *Candida* species in 13 cases, *Rhodotorula* and *Trichsporon* species in one case each. Hence total 35 fungal strains were isolated from total 180 cases. A concomitant growth of *Cryptococcus* and *Candida* species was observed in one case.

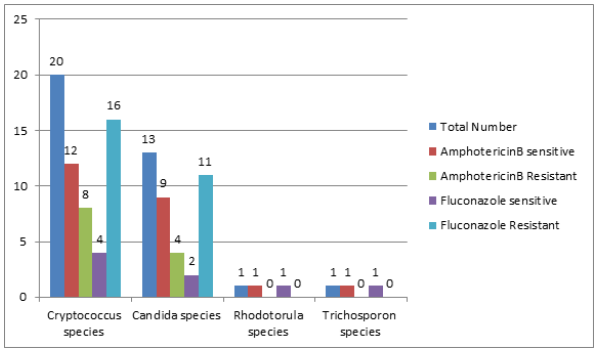
Graph 2: Fungus Positive and Negative Cases with respect to HIV Status

n=180



Out of 180 CSF samples studied, 25 % of cases were HIV positive and 75 % of HIV negative. In HIV positive cases 62.16% cases were found to be fungal positive, while in HIV negative cases37.84% cases were fungal positive . There was significant association between fungal infections and HIV positivity .(CI= **95% CI** 16.3, 33.7 (P< 0.001), Chisquare test with YaH's Correction = 31.95)

TABLE 7: Antifungal susceptibility test (Stokes method)



It was observed that maximum number of isolates was sensitive to Amphotericin B as compared to Fluconazole.

4. DISCUSSION :

Total 180 subjects, clinically suspected for fungal meningitis were studied and divided into two groups, HIV positive (25%) and negative (75%) cases. Out of total 180 subjects, 132 were males and 48 were females. In the present study fungal meningitis was diagnosed in 52.12% in HIV positive patients, out of which *Cryptococcal meningitis* accounts for 42.2%. Whereas in HIV negative patients, fungal meningitis was found to be 10.37%, out of which *Cryptococcal meningitis* accounts for 2.22%. Prevalence of *Cryptococcosis* was maximum in HIV positive group as compared to HIV negative group. *Cryptococcosis* was diagnosed maximum in males as compared to females. Male preponderance was observed in the present study. Over all prevalence of *Cryptococcal meningitis* in the study was found to be 12.2% which is in accordance with the WHO incidence rate of 5-33% worldwide. [7]

Among other 15 cases, 13 were confirmed as *Candidal meningitis*, one as *Rhodotorula* meningitis and another one as *Trichosporon meningitis* cases. *Candidal meningitis* is still a rare clinical situation, although it is becoming more frequent. Candia species were isolated in all 13 cases, of which 5 were *C. tropicalis*, 3 were *C. pseudotropicalis* ,3 were *C. albicans* ,1 was *C.guillermondi* and 1 was *C.geotrichum* which were confirmed by doing biochemical tests such as urease test, germ tube test, sugar assimilation test, Sugar fermentation tests and growth on Corn meal agar . Speciation was done by correlating the results of different biochemical tests. Huttova et al had reported various neonatal fungemia caused due to *C albicans* and *C. parapsilosis* and had concluded that fluconazole was effective antifungal therapy [8]. Rodrigues et al had reported three cases of *Candidal meningitis* in HIV positive drug abusers [9] Jarlov et al had reported *C. albicans* meningitis in a 27 weeks neonate who was treated successfully with Liposomal amphotericin-13 from Sweden. [10] Porter et al had reported a single strain of *C. albicans* associated with separate episodes of fungemia and meningitis [11],Sarma et al had reported a case of *C. lusitania* causing fatal meningitis from India[12]. Meningitis caused due to *Rhodotorula rubra wa*s reported by Gyaurgieva et al from England in HIV positive patient and the fungus was isolated from CSF of the patient [13], Pore RS et al had also reported meningitis caused by Rhodotorula species.[14] and Marinova et al had reported *Rhodotorula* fungemia in a 13 year old boy after neurosurgery from Switzerland[15].

In the present study 86.09% of the Cryptococcal meningitis cases were HIV positive which correlates with a retrospective study of Pedrol et al from Spain in 1985-90 which shows that 76.9% of the *Cryptococcal meningitis* cases were HIV positive [16].

According to Aquinas et al from Bangalore in 1996 *Cryptococcal meningitis* was the most common opportunistic fungal infection in patients with AIDS contributing to the increased morbidity and mortality.[17] 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 [18] Hence a high index of clinical suspicion and routine mycological surveillance is essential to identify this infection [17].

Significant advances in antifungal therapy have occurred in the last decade. Most of these advances had been tried to the introduction of the Itraconazoles and Fluconazoles.[19] In the present study in vitro antifungal susceptibility test had been carried out using Amphotericin- B and Fluconazole against all fungal isolates by Stoke's method using *Candida kefyr* as a control strain [20]. It was observed that maximum number of isolates were sensitive to Amphotericin- B as compared to Fluconazole. 60% of Cryptococcus species were sensitive to Amphotericin- B, hence it could be still considered as a drug of choice. Kauffman et al, White et al, Treseler et al and 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 [21].It was the preferred as initial treatment for many fungal infections. 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 *Cryptococcal meningitis*, and once the diagnosis was established, treatment with Amphotericin- B was mandatory and a response should be expected[22].

5. CONCLUSION :

With the advent of AIDS pandemic, the incidence of fungal meningitis was seen to be increasing mainly due to yeast like fungi. The etiological agents for fungal meningitis found mainly in the present study were *Cryptococcus* species, *Candida* species, *Rhodotorula* species and *Trichosporon* species , out of which *Cryptococcal meningitis* was the predominant one. Our study concluded that *Cryptococcus neoformans var neoformans* was the most prevalent strain causing fungal meningitis in HIV positive patients and Amphotericin B was found to be the drug of choice for fungal meningitis.

6.ACKNOWLEDGEMENT

The authors are extremely thankful to Microbiology Department of T.N. Medical College and B.Y.L. Nair Charitable Hospital, India for

providing research facilities and moral support to conduct this work.

REFERENCES

1. Powderly WG. `Fungal infections' Diagnosis and Management in patients with HIV disease. HIV Clinical Management, 1999, Vol. 6.
2. Sharma PU. An over-view of the symposium on Mycoses. Recent trends in Mycoses, 1998; 1-8.
3. CSFcollection procedure: https:// www. labce. com/ spg 546367_ csf_ specimen_ collection_ process.aspx
4. Zerpa R, Huicho L and Guillén A. Modified India ink preparation for Cryptococcus neoformans in cerebrospinal fluid specimens. J Clin Microbiol. 1996;34(9): 2290–2291.
5. Fuglsang-Damgaard D, Pedersen G and Schønheyder HC. Positive blood cultures and diagnosis of bacterial meningitis in cases with negative culture of cerebrospinal fluid. Scand J Infect Dis. 2008, 28; 40(3): 229 – 3.
6 Gosden PE, Andrews JM, Bowker KE, Holt HA, MacGowan AP, Reeves DS, Sunderland J and Wise R. Comparison of the modified Stokes' method of susceptibility testing with results obtained using MIC methods and British Society of Antimicrobial Chemotherapy breakpoints. J Antimicrob Chemother. 1998 ;42(2):161-9.
7 Baum RR , Jose A and Gonclaves R .Clinical Epidemiological study of 171 cases of Cryptococcosis. Clinical infectious diseases, 1994; 18: 369-380.
8 Huttova M, Hartman OI, Kralinsky K, Fillca J, Uher J, Kurak J, Krisan S and Krcmery V. Candida fungemia in neonates treated with fluconazole report of 46 cases including eight with meningitis. Pediat. Infect. Dis. J.1998; 17 (11): 1012-5
9 Rodriquez AF, Bengoca AK, Arce AD, Arrizabalaga J, Iribarren JA, Wichnaana MA, Goenaga MA. Candidal meningitis in HIV infected patients: treatment with fluconazole. Scand J. infect. Disease (Sweden), 1998; 30 (4): 417-8.
10 Jarlov JO, Born P, Bruun B. Candida albicans meningitis in a 27 weeks' premature infant treated with liposomal amphotericin B (Ambisome).Scand J Infect Dis. (Sweden), 1995; 25 (4): Pusa-20.
11 Porter SD, Nobel MA, Rennie R.'A single strain of Candida albicans associated with separate episodes of fungemia and meningitis' J. din. Microbiology (USA), 1996; 34 (7): 1813-4.
12 Sarma PS, Dunatray P, Padhye AA:Candida lusitaniae causing fatal meningitis'. Posgrad. Med J (Eng), 1993; 69 (817); 878-80.
13 Gyaurgieva OH, Bogomolova TS, GorshKova GI. Meningitis caused by Rhodotorula rubra in an HIV infected patient ,J. Med. Vet. Mycology (Eng), 1996; 34 (5): 357-9
14 Pore RS and Chen J.`Meningitis caused by Rhodotorula. Sabouroudia.1976; 14: 331-335
15 Marinova I. , Szabadosona V, Brandeburnova O, Kromery V, Jr.Rhodotorula Sp. fungemia in an immunocompromised boy after neurosurgery successfully treated with miconazole and 5-flucytosine : Case report and review of the literature. Republic Chemotherapy (Switzerland),1994;40 (4): 287-9.
16. Pedrol EE, Mallolas J, Gonzalez CJM. Cryptococcosis: Presentation of 26 cases (Spanish). Medicine Clinica, 1992; 98 (10): 361-5.
17. Aquinas SR, Tasey SD, Ravindran GD, Nagamani D. Ross C. Cryptococal meningitis in AIDS-Need for doagmpsos. L f J. Assoc. Physicians, India, 1996; 44(3): 178-80.
18. Petty RK, Kennedy PG. The neurological features of HIV positive patients in Glasgow-A retrospective study of 90 cases. Quarterly Journal of Medicine. 1992; 82 (299): 223-34.
19. Musial CE, Franklin R, Cockerill Ill and Rosters GD. Fungal infections of the Immunocompromised Host. Clinical and Laboratory aspects. Clin. Microbiological Review, 1988; 349-364.
20. National Work shop on-Anti-microbial susceptibility testing and control of antibiotic therapy. Dept. of microbiology, Aimed Forces Medical College, Pune, 27, 253-256.
21. Treseler CB and Sugar AM. 'Fungal Meningitis' Infect. Dis Clin North. Am (USA), 1994; (4): 789-808.
22. Grant IH, Armstrong D. Fungal infections in AIDS. Cryptococcosis. Infect Dis.Clin. North Am (USA),1988; 2 (2): 457-64.