Original Research Paper

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Opportunistic Fungal Infections in HIV/ AIDS

Medical Science

KEYWORDS : - Candida, Cryptococcus, P.jirovecii

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ABSTRACT

Aim: The prevalence of opportunistic fungal infections in HIV/AIDS was studied. Material and Methods: The study group comprised of 207 HIV seropositive patients receiving care at KMC Hospital, Mangalore. Various samples e.g. Sputum, Oral Swab, Blood, CSF, Nail clippings and Skin scrapings were collected and analysed for identification of fungus using wet mount, India Ink, Gram stain, Modified Toluidine Blue O Stain, Giemsa Stain, Methylene Blue Stain , and cultured on SDA and Chocolate Agar media. Statistics: It is an open, observational, descriptive, and prospective study. Result: Of the 207 HIV positive cases 81(39.1%) were positive for Candida spp. of which 67 (82.7%) were male and 14 (17.3%) were female. A total 15 (7.2%) cases were positive for Cryptococcus neoformans , 14(6.8%) patients were found to be positive for cyst of P.jirovecii and two (0.97%) were found to be positive for Penicillium marneffei infection. There was no significant difference in the prevalence rate between male and female. Conclusion: The highest incidence of opportunistic fungal infection was observed in the age group of 20-40years. Most prevalent fungal infection was identified as Candida spp. followed by Cryptococcus neoformans, Pneumocystis jirovecii and Penicillium marneffei..

INTRODUCTION

Microorganisms the tiny living creatures, such as bacteria, viruses, fungi and parasites are present everywhere. Despite their overwhelming abundance, relatively few of the thousands of species of microorganisms invade, multiply and cause disease in people. Host and parasite variables contribute to determine the consequences of infection. Host immunosuppression caused by the Human Immunodeficiency Virus (HIV), culminates into fatal syndrome called Acquired Immuno Deficiency Syndrome (AIDS).1

In India early cases of HIV/AIDS concentrated primarily to Chennai and Mumbai then spread to other parts of the country, often following major highways and labour migrants. By mid-2003 Tamilnadu had nearly 50% of reported AIDS. Maharashtra shares about 19.74% of cases. Out of all states Karnataka which has 29 districts and all are included into high prevalence rate of HIV i.e Category A. Among them Hassan and Belgaum district with prevalence of more than 3%. And in Dakhsina Kannada prevalence (in 2006) among patients attending STD clinics 3.6% , of women attending antenatal clinics 0.25%, antenatal clinic rural 1.25% and female sex workers 6.64%.2,3

Among fungal pathogens Candida spp (especially Candida albicans) is the most common infections of HIV infection. In one prospective study, 84% of HIV infected patients had oropharyngeal colonization by Candida spp on at least one occasion and 55% developed clinical thrush. Although oropharyngeal candidiasis is frequent in men, recurrent vaginal candidiasis is common early manifestation of HIV infection in women and among others cryptococcosis. In some parts of the world like in Thailand the third most common opportunistic infection among HIV infected person is Penicillium marneffei. Unlike candidiasis and cryptococcosis, infections by Histoplasma capsulatum and Blastomyces dermatitides are prevalent in specific geographic regions.4

Diagnosis and surveillance of these opportunistic fungal infections in AIDS will lead to early, accurate treatment and better management of these cases. HIV/AIDS may not be curable but most opportunistic infections can be prevented. Prophylaxis against some of these infections will not only prolong life of an HIV infected patients but also improve the quality of life. So an early and accurate diagnosis of the etiological agent is important.

METHODS

The study was conducted at the Department of Microbiology, Kasturba Medical College and Hospital, Mangalore over a period of 1 and a half years from August 2009 to March 2011. The study group comprised of 207 HIV seropositive patients. The appropriate samples were collected based on the presenting symptoms and clinical features. And various methods were used to study the prevalence of opportunistic fungal infections in this part of India and subsequent identification. Results were analyzed using descriptive statistics. The study protocol was approved by Institutional Ethics Committee.

COLLECTION AND PROCESSING OF THE SAMPLE

Various samples e.g. sputum, oral swab, blood, cerebrospinal fluid (CSF), nail clippings and skin scrapings were collected under universal aseptic precautions in suitable sterile universal containers. And transported to the laboratory immediately and processed without delay in a class II biological safety cabinets. Repeated sampling from the same site was done for authentic diagnosis as repeated demonstration/isolation of same organism from same site is essential.

SPUTUM

Early morning induced sample was collected in a sterile universal container and in the case of intensive care unit (ICU) patients tracheal aspirate was collected in aspirate containers.

- Wet Mount:Thick and mucoid sputa should be digest ed and concentrated with mucolytic agents such as aqueous solution of enzyme N-acetyl-L-cystein (With out added sodium hydroxide). After the sediment was resuspended in sterile water, a drop was examined in a wet preparation.
- Staining: Purulent part of the sputum smeared and heat fixed. Stained with Gram Stain, Modified Tolui dine Blue O Stain, Giemsa Stain. Later smear was ob served under oil immersion objective for identification of oval cyst with violet cytoplasm and thick cyst wall against a clear background. It was used to find out Pneumocystis jirovecii5,6
- Culture,The sputum samples were streaked on to Chocolate Agar plates and on to Sabourauds plain and with Cycloheximide Agar slopes. Chocolate Agar was incubated at 370C for 18-24 hrs under 5-10% of CO2 and Sabourauds Agar slope was incubated at room

temperature (250 to 300C); isolated colonies were identified by different methods. Pasty colonies on Sab ourauds Dextrose Agar(SDA) were subjected to Gram Stain and Germ Tube Test for identification of Can dida spp.5,6

BLOOD

Blood was collected and inoculated into Brain Heart Infusion (BHI) broth or Soya Trypticase Broth and incubated at 370C for 18 hrs. Positive culture was sub cultured on to Blood Agar or Chocolate Agar and SDA. If negative further subcultures were done on 4th and 7th day. SDA tubes were incubated at room temperature (250 to 300C). The plates or tubes were checked for growth over 2weeks.If no growth observed after 2wks then reincubated for another two weeks, before discarded as negative. Any isolated of Candida spp. obtained identified by direct microscopy, Gram stain of colonies, colony morphology and Germ Tube test.5,6

CEREBROSPINAL FLUID

The CSF specimen was processed immediately. The sample centrifuged and deposits were used for staining and culture.Microscopic examination was done with Gram Stain & Indian Ink for Negative Staining. It was used to observe Cryptococcus neoformans.The centrifuged deposits were cultured on Chocolate Agar, SDA. Chocolate agar is incubated at 350C in the presence of 5-10 % CO2. SDA was incubated at 25-370C. Both the culture plates were checked for growth for 4 days before discarded as negative. 5,6

MUCOCUTANEOUS SCRAPINGS

Two swabs were collected from the oral thrush . One swab was used to make a Gram Stain smear and the other was streaked on to SDA slope.

10% potassium hydroxide (KOH) wet mount was used for the skin scrapings and examined under the high power objective for fungal elements.

The above samples were cultured on SDA at room temperature (25-30)0C and checked for 7days before discarded as negative. All the above samples were dried out to prevent the growth of saprophytic bacteria and fungi. Creamy colonies of Candida spp. were looked for and Gram stain and germ tube test was done to confirm Candida albicans. Any fungal hyphal colony grown were analysed by Lactophenol Cotton Blue(LPCB) wet mount. 5,6

NAL CLIPPINGS

Wet mount with 20% KOH and cultured on SDA at room temperature (25-30)0C and checked for 7days before discarded as negative and colonies identified both macroscopically and microscopically. 5,6

RESULTS

The age groups, sex and caste distribution of 207 HIV seropositive patients were studied (Fig-I, II, III).

Of the 207 HIV positive cases 81(39.1%) of them had oral thrush of which 67 (82.7%) were male and 14 (17.3%) were female. Oral thrush was commonly seen in 21-30 yrs age groups with 38(46.9%) cases followed by 31-39yrs 29 (35.8%), ≥40yrs 12(14.8%) and ≤20yrs 2(2.5%) . Among the Candida spp., 58 strains (71.6%) were Candida albicans. (Fig-IV)

A total of 14 (6.8%) patients were found to be positive for cyst of P.jirovecii from induced sputum samples stained by Modified Toluidine Blue O method. 11cases (78.6%) were male when compared to female 3(21.4%). The prevalence of PCP was 8 (57.1%) in age group of 21-30yrs followed by 05 (35.7%) in 31-39yrs and 01 (7.1%) in ≥40yrs (Fig-V)

Total of 2 cases (0.97%) were found to be positive for Penicillium marneffei in age group of 31-39yrs.

A total of 15cases (7.2%) were positive for Cryptococcus. Prevalence in the age group 21-30 yrs was 5(33.3%) followed by 31-39yrs 09(60%) and ≥40 yrs 01(6.7%). There was not much difference in the prevalence rate between male and female (53.3% and 46.7% respectively(Fig-VI)

DISCUSSION

HIV gradually reduces immune system functions which lead to several opportunistic infections including fungal infections. In the present study, the patients (Total 207 number, of which 169 male and 38 female) present with more than one symptom, the common being dyspnoea, fatigue, malaise, loss of weight, fever, chronic cough, loss of appetite and chronic diarrhoea. Preponderance of cases was seen among the age group of 21-40yrs (181 cases, 87.4%), the sexually active age group.

Out of the 207 HIV positive cases 81(39.1%) had oral candidiasis among which 67(82.7%) were male and 14(17.3%) were female. Preponderance in both males and female was seen in the age group of 21-30yr. Among the Candida sp., 58 strains (71.6%) were Candida albicans. Study by V.P Baradkar et al 7 showed prevalence of oral candidiasis 32.12% with most common species Candida albicans (76.92%). Oral candidiasis as most common (34.5%) opportunistic fungal infection was seen in Kaur et al 8 study. P G Shivananda et al 9 reported oral candidiasis as the most common (59.0%) infection in AIDS. The current study shows similar findings with Candida as most common fungal infection (39.1%)

Study by PG Shivananda et al9 reported prevalence rate of Pneumocystosis jirovecii infection in HIV patients 7%. Our finding of prevalence of 6.8 % with preponderance in males of the age group (21-30)yrs is similar to this finding. Pneumocystic pneumonia is the commonest opportunistic infection in North America.10 In the developing part of the world the incidence is low.11Among all cases there was no clinical evidence of extrapulmonary pneumocystosis and respond well to trimethoprime-sulfamethoxazole. The rarity of pneumocystis pneumonia among patients with AIDS may be due to the fact that they have many other endemic infections prior to reaching a severely immunocompromised state and consequent relative early mortality

In northern Thailand penicilliosis is found as third most common opportunistic infection (after oral thrush and cryptococcal meningitis) in HIV/AIDS.12 According to Supparatpinyo et al 12 and P N Singh et al 13 the penicilliosis is endemic in South Asia, Manipur of North East India. Similar prevalence of 0.97%(prepordance in males) was found in our study

Cryptococcal meningitis varies from place to place. A study in Tamilnadu by G. Manoharan et al14 revealed that cryptococcosis to be 34.8% which is much higher than other reports.(PG Shivananda et al 9 7% , Sara chako et al 15 7%). Prospective studies have suggested that 10–20% of all deaths in HIV-infected patients in Africa are attributable to cryptococcal infection.16 .Our study showed prevalence of cryptococcal infection 7.2% with prepordence in age group of 31-39yrs. 5%-10% of HIV-1-infected patients will develop CM as an AIDS-defining illness (Fessler et al, 1998) 17*Cryp-*

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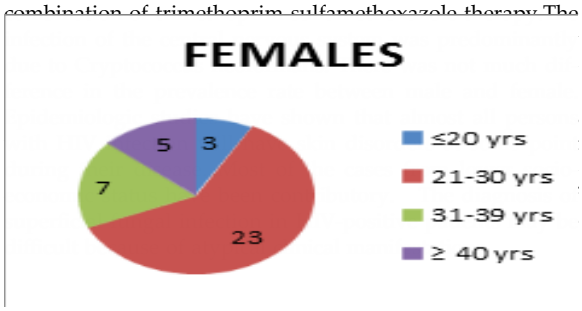
tococcus is the third most common cause of CNS infection in AIDS patients, ranking behind HIV and *Toxoplasma*. Before HAART, cryptococcal infection of the CNS occurred in up to 10% of patients, usually when the CD4 count dropped below 100 cells/μL. 18A retrospective study at National Institute of Mental Health and Neurological Science (NIMHANS) revealed HIV as the predominant predisposing factor for cryptococal infection 19

Epidemiologic studies have shown that almost all persons with HIV infection will have skin manifestation at some point during their illness 20.Superficial mycotic infections such as seborrheic dermatitis, tinea pedis, tinea corporis, and onychomycosis are common in patients infected with HIV. The diagnosis of superficial mycosis in HIV-positive patients may be difficult because of atypical clinical manifestations. Dermatophytic infections are common in HIV-infected patients; however, these skin diseases may not occur any more frequently than in comparable groups. Studies have been few and their results contrary.21

CONCLUSION

HIV / AIDS in the developing countries is emerging as one of the most serious public health problem. The disastrous effect of HIV on the host immune system leads to overwhelming opportunistic infections raising the morbidity and mortality. The highest incidence of HIV infection in AIDS was observed in the age group of 20-40yrs. Most prevalent fungal agent causing opportunistic infection in HIV was identified as Candida spp. followed by Cryptococcus neoformans , Pneumocystis jirovecii and Penicillium marneffei . The percentage of Pneumocystis jirovecii infection was quite low probably on account of preexposure prophylaxis. The sensitivity also could have been enhanced by using BAL specimens and all patients responded well to combination of trimethoprim sulfamethoxazole therapy The



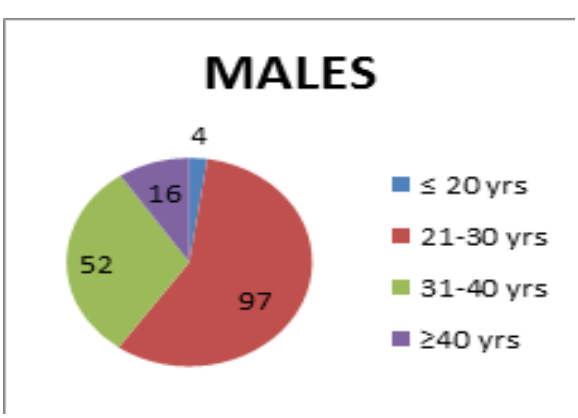


Fig-I,II- Age wise distribution of HIV positive cases in male and female

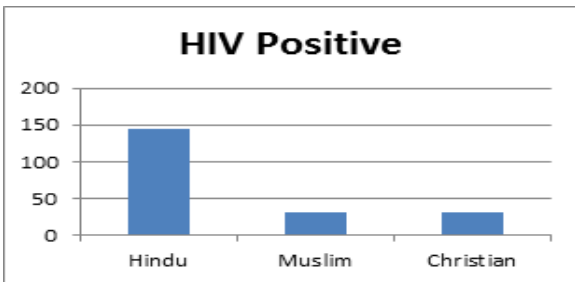


Fig-III Caste wise Distribution Among HIV Positive Cases

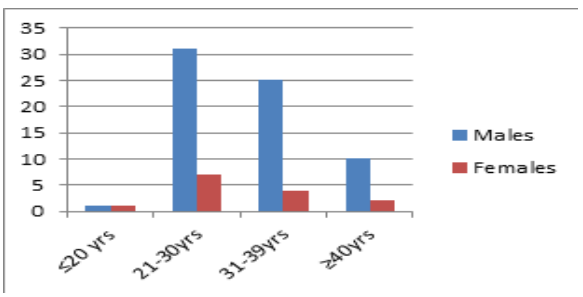


Fig-IV Oral Thrush in different age groups of HIV Infected Individuals

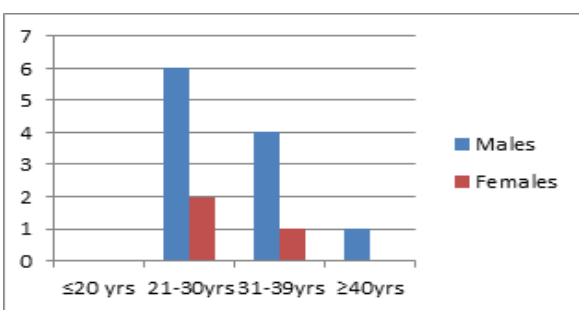


FIG-V- Pneumocystosis in different age groups of HIV Infected Individuals

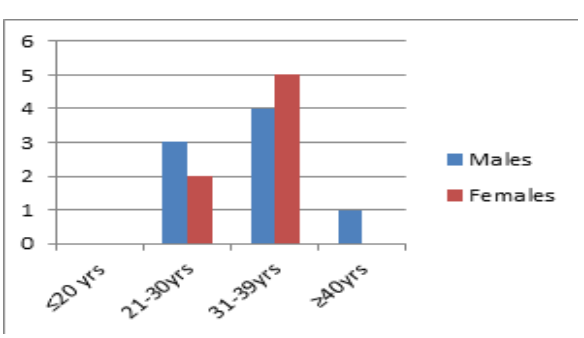


FIG-VI- Cryptococcal Meningitis in different age groups of HIV Infected Individuals

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