ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 01 | January - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY OF FUNGAL INFECTIONS IN HIV/AIDS PATIENTS IN TERTIARY CARE HOSPITAL: A CROSS SECTIONAL STUDY

Clinical Microbiology

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ABSTRACT

Introduction: Human Immunodeficiency Virus (HIV) is the most significant emerging infectious pathogen of the 20th century. The major causes of morbidity and mortality in HIV patients are the opportunistic infections. The present study was conducted to isolate and identify fungal pathogens in HIV/ AIDS patients, to study antifungal susceptibility pattern of *Candida* isolates and to study the co-relation of fungal infections with CD4 count. **Material and Methods**: A total of 250 HIV positive patients presenting with signs and symptoms suggestive of fungal infections were studied. Oral swabs, cerebrospinal fluid, sputum, broncho-alveolar lavage (BAL), Urine, Stool, Skin scrapping and Blood were processed for identification of yeast and moulds by standard conventional method of identification. Cryptococcal Antigen detection by Latex Agglutination Test (LAT) was done for CSF samples. Giemsa stain and Gomori'smethanamine Silver stain (GMS) was used for the demonstration of cyst of Pneumocystis jirovecii in sputum and BAL. Antifungal susceptibility for candida done by disc diffusion as per CLSI guidelines M44- A2. CD4 count estimate by flow cytometry. **Result:** The prevalence of fungal infection was (30.4%) with candidiasis as the commonest (26.0%) followed by aspergillosis (2.8%), cryptococcosis (1.2%) and dermatophytosis (0.4%).Oral candidiasis was the most common form of candidiasis (50.8%) being mainly caused by *C.albicans* (75.4%). The non albicans species isolated were *C.tropicalis* (12.3%), *C.glabrata* (9.2%) and *C.parapsilosis* (3.1%).Seven *Aspergillus* spp. were isolated from sputum specimens, of them five were identified as *Aspergillus niger* and two as *Aspergillus fumigatus*.The prevalence of cryptococcosis was 1.2%. 85% HIV positive patients of study population were with CD4 count < 200 cells/µl. **Conclusion** Opportunistic fungal infections,are prevalent in large number of HIV infected patients. Diagnosis and surveillance of these opportunistic fungal infections in AIDS will lead to early, accurate treatment and better management of these cases.

KEYWORDS

HIV, fungal infections,CD4 count

INTRODUCTION

Humankind has been besieged throughout its evolution by microorganisms that pose a continual challenge to the survival of the species.[1] Human Immunodeficiency Virus (HIV) is the most significant emerging infectious pathogen of the 20th century. Since the Acquired Immunodeficiency Syndrome was first recognized in 1981 from North America, the HIV/AIDS epidemic continues its expansion across the globe.[2,3]

Globally, there were an estimated 37.9 million (32.7 - 44million) people living with HIV in 2018. According to UNAIDS data, 5.9 million (5.1 – 7.1 million) people were living with HIV in Asia in 2018, and approximately 200,000 people died from AIDS-related illness in 2018. Young people aged 15 to 24 account for an estimated 32% of new HIV infections worldwide.[4]

The major causes of morbidity and mortality in HIV patients are the opportunistic infections. This could be attributed to decreased level of immunity in such patients due to destruction of CD4 cells. A weakened or impaired immune system provides favourable conditions for pathogenic and non-pathogenic micro-organisms. Thus, these patients become vulnerable to infections particularly those caused by fungi [5]

The clinical profile of AIDS in India is seen to be different from what it is seen in developed world, since the HIV infected individual in India lives in an environment with high prevalence of infectious diseases.[6] Tuberculosis is the most commonly reported OI among infections. Among the opportunistic fungal infections *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* often become life threatening. The most common and some of the uncommon mycoses seen in patients of AIDS are Candidiasis, Cryptococcosis, Histoplasmosis,Coccidioidomycosis, Blastomycosis, Penicilliosis, Sporotrichosis and *Pneumocystis jirovecii* (*Pneumocystis carinii*).[7-9]

The extensive use of antifungals for prophylaxis in these patients became the leading cause of colonization of Candida-non-albicans (CNA) species and increasing resistance to antifungal drugs. [10]

Opportunistic fungal infections are important challenges in the progress of modern medicine. Early diagnosis and prompt therapy are the important tools in effective management against opportunistic fungal infections.[11]

So the present study was conducted to isolate and identify fungal pathogens in HIV/ AIDS patients and to study antifungal susceptibility pattern of Candida isolates and to study the co-relation of fungal infections in HIV/ AIDS patients with CD4 count.

MATERIAL AND METHODS

The study was conducted in Department of Microbiology of Government Medical College attached to a tertiary care hospital from November 2018 to October 2020.

Patients visiting ART OPD and admitted in wards infected with HIV and presenting with clinical signs and symptoms suggestive of fungal infection were included in the study A detailed history and clinical findings were noted.

Various samples like Oral swabs, cerebrospinal fluid, sputum, broncho-alveolar lavage (BAL), Urine, Stool, Skin scrapping and Blood were processed in the mycology section for identification of yeast an moulds.

All the specimens were subjected to Gram stain and 10% potassium hydroxide (KOH) and examined for the presence of fungal hyphae and spores.

Two Sabouraud's Dextrose Agar (SDA) slopes with and without cycloheximide and chloramphenicol were used for culture of specimens and incubated at temperatures of 25^{0}C and 37^{0}C for 3 weeks for isolation of moulds and yeasts. Fungal growth was identified based on colony morphology, pigmentation, growth rate, microscopy using Lactophenol cotton blue mount (LPCB), slide culture on corn meal agar and growth on CHROMagar Candida medium. [12,13]

India ink preparation and Cryptococcal Antigen detection by Latex Agglutination Test (LAT) was done for CSF samples. [14]Giemsa stain and Gomori'smethanamine Silver stain (GMS) was used for the demonstration of cyst of Pneumocystis jirovecii in sputum and BAL. [13,15]

The strains of Candida species (n=65) were subjected to susceptibility testing against Fluconazole and Voriconazole by disc diffusion as per CLSI guidelines M44- A2.[16]

CD4 count estimated at department of Microbiology by SysmexPartec GmbH, CyFlow Counter using whole blood sample by flow cytometry. [17]

RESULTS AND OBSERVATION

A total of 250 HIV positive patients attending OPD and admitted in wards presenting with signs and symptoms suggestive of fungal infections were studied.

Table 1: Age and sex distribution of HIV positive patients (n = 250)

Age (years)	Male	Female	Total (%)
0-10	00	02	02 (0.8)
11-20	06	03	09 (3.6)
21-30	38	31	69 (27.6)
31-40	55	38	93 (37.2)
41-50	32	22	54 (21.6)
51-60	14	09	23 (9.2)
Total	145 (58%)	105 (42%)	250 (100)

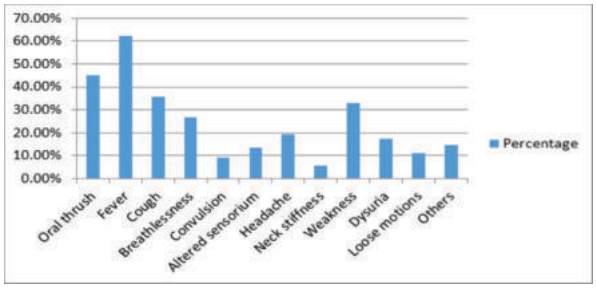

Chart 1: Clinical manifestations in study population(n = 250)

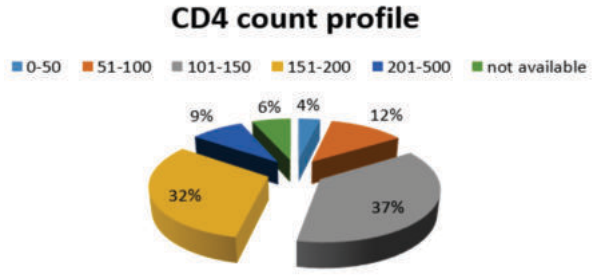

Chart 2: CD4 count profile of HIV positive patient (n = 250)

Table 2: Specimen-wise distribution of various fungal isolates (n = 250)

Specimen	Fungi	Total number
Oral swab (n = 86)	Candida spp.	33
CSF (n = 47)	Cryptococcus neoformans	03
Urine (n = 28)	Candida spp.	12
Sputum (n = 64)	Candida spp. Aspergillus spp.	17 07
Blood (n = 8)	Candida spp.	02
BAL (n = 6)	P.jirovecii	01
Skin scrapping (n =6)	T. mentagrophytes	01
Stool (n = 5)	Candida spp.	01
Total n=250		77(30.8%)

Table 3: Specimen-wise distribution of *Candida* species CSF (n = 65)

	C.albicans	C.tropicalis	C.glabrata	C.parapsilosis	Total (%)
Oral swab	25	04	03	01	33 (50.8%)
Sputum	12	03	02	00	17 (26.1%)
Urine	09	01	01	01	12 (18.5%)
Stool	01	00	00	00	01 (1.5%)
Blood	02	00	00	00	02 (3.1%)
Total	49	08	06	02	65

Table 4: Susceptibility pattern of *Candida* species for (n = 65)

Candida species	Fluconazole			Voriconazole		
	S	S-DD (%)	S (%)	S	S-DD (%)	S (%)
C.albicans (n =49)	38 (77.5)	06 (12.3)	05 (10.2)	42 (85.7)	03 (12.2)	04 (8.2)
C.tropicalis (n= 8)	05(62.5)	02 (25.0)	01 (12.5)	06 (75.0)	01 (12.5)	01 (12.5)
C.glabrata (n= 6)	03(50.0)	00	03 (50.0)	02 (33.3)	00	04 (66.7)
C.parapsilosis (n=2)	02(100.0)	00	00	01 (50.0)	00	01 (50.0)
Total (n = 65)	48 (73.9)	08 (12.3)	09 (13.8)	51 (78.5)	04 (6.1)	10 (15.4)

S – Susceptible, R – Resistant, S-DD – Susceptible dose dependent

Table 5: Correlation of fungi with CD4 count(n=74)

CD 4 count	Candida spp.	Aspergillus spp.	Cryptococcus neoformans	P. jirovecii	T. mentagrophyte	Total
0-50	02	-	-	-	-	02
51-100	18	02	01	-	-	21
101-150	29	04		01	01	35
151-200	10	01	01	-	-	12
201-500	04	-	-	-	-	04
Total	63*	07	02*	01	01	74

*There were total 250 cases, CD4 count of sixteen cases was not available out of them two isolated *Candida* species, one isolated *Cryptococcus neoformans* and thirteen cases were without fungal infections.

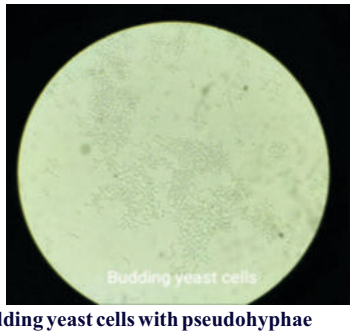

Image 1: Budding yeast cells with pseudohyphae

Image 2: *Cryptococcus* with capsule

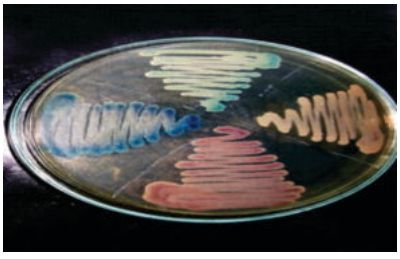

Image 3: *Candida species* on CHROMagar

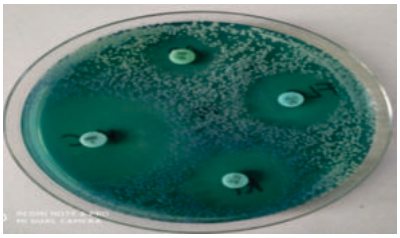

Image 4: Antifungal sensitivity test for *Candida species*

DISCUSSION

The major causes of morbidity and mortality in HIV infected patients are the opportunistic infections (OIs). Opportunistic infections range from viral, parasitic and bacterial to fungal. Among these, fungal agents are ubiquitously present becoming the most common cause of life threatening infections in immunocompromised individuals.[17]

The present study was conducted among 250 cases of HIV patients with signs and symptoms suggestive of fungal infections.

In the present study majority of the patients were in the age group 31-40 years (37.2%) . This result is in accordance with the findings of other workers. Kaur et al [18]and Ravikanti et al [19] showed maximum cases in age group 31-40 years 38.2% and 37.8% respectively. S Mallya et al [20] and A Wadhwa et al [21]showed maximum cases in age group 21-40 years 87.4% and 82% respectively.

Male: female ratio in the present study was 1.38: 1. Our findings are in consistent with Ravikanti et al[19] study which showed the M:F ratio 1.2: 1. However some other studies showed male predominance with higher ratio. Wadhwa et al[21] and R Kaur et al[18] showed male: female ratio as 4.8: 1 and 2.25: 1 respectively. Preponderance of males may be due to their migration to the metropolitan cities in search of work, staying away from their spouse for longer periods and the philandering habit of males being seen might have resulted in their acquiring HIV infection.

In this study fever was the commonest symptom (62.2%) followed by oral thrush (45.2%), cough (35.6%) and weakness (32.8%) . In study by Wadhwa et al[21] weight loss was the commonest finding (88.3%), followed by loss of appetite (75%) and fever (71.7%). In study by Ravinder Kaur et al[18] weight loss was commonest finding (78.2%) followed by oral ulcer (74.6%) and fever (67.1%). As published by NACO (National AIDS Control Organization), weight loss (89%), fever (88%), diarrhea (86%), weakness (72%) and cough (57%) are the common presenting symptoms in AIDS patients in India.[22]

The overall prevalence of opportunistic fungal infections in the present study was 30.8%. Various studies done by Ravikanti et al [19], S Agrawal et al [23]and R Kaur et al [18]32 showed overall prevalence of opportunistic fungal infections 13%, 32.43% and 41.1% respectively. Our prevalence corresponds with the study by S Agrawal et[22] but lower than that of study by R Kaur et al[18]. The low prevalence observed in the present study can be attributed to the antiretroviral treatment being received by patients in the study. It has been documented that ART reduces the risk for Ois[24].

Among the fungal infections in HIV patients, oral candidiasis has been reported most common opportunistic fungal infection[6,7]. .In the present study candidiasis was diagnosed in 65 cases (26%). These findings were consistent with studies by workers like Bhaumik et al[24], Shahapur et al[25],118 and A. Aggrawal et al[26], who reported candidiasis in 21.13%, 30.9% and 24.24% respectively in HIV infected patients.

Pulmonary candidiasis was observed in seventeen cases (26.5%), study by Ravinder Kaur et al[18] 32 showed 32.9% Candida spp. in sputum. R Sah et al[27] and ofoneme et al[28] showed 22.8% and 11.8% Candida spp. in sputum respectively.

In our study prevalence of Candida spp. in urine samples was 42.8% which is higher prevalence than other studies like study conducted by Ravinder Kaur et al [19]which showed Candida spp.in 15.1% in urine samples. However, it is mentioned that depending on the population examined, Candida is reported up to 44% in urine samples sent for culture[18].

In our study one out of five had Candida diarrhoea which denotes 20% prevalence of Candida spp. in patients with diarrhoea. Study conducted by Abhjit Sarkar et al[29] showed Candida spp. in 28.7% patients with diarrhea. Similar prevalence was found in two other studies by Uppal B. et al [30] in patients with diarrhoea.

The commonest Candida species was C.albicans 75.4% followed by C.tropicalis 12.3%, C.glabrata 9.2%,and C.parapsilosis (3.1%). These findings were almost similar to R Kaur et al[18] .

Resistance to fluconazole were seen in 10.2% among C.albicans, 12.5% in C.tropicalis and 50% in C.glabrata .Dewan et al[31] resistance 244 showed 20% resistance to fluconazole among Candida species and A Jaychandran et al[32] found fluconazole resistance 11.8% among C.albicans, 16.66% among C.tropicalis and 70% in C.glabrata.

In our study we found 15.4% resistance to voriconazole among Candida spp.Dewan et al[31]showed 13.4% resistance to voriconazole among Candida spp. A Chakroborti et al[33] showed 5.6% resistance to voriconazole among Candida spp.

Cryptococcosis was observed in 1.2% cases, all were clinically suspected as having cryptococcal meningitis. Similar findings were sown byVarious studies shown by Kaur et al[18] 3.3% and Ravikanti et al.[19]

In our study we found all cases of cryptococcosis in patients with CD4 count < 200 cells/µl. Similar findings were reported by A Wadhwa et al[21] and Ravikanti et al[19] with mean CD4 count in cryptococcosis as 138.8 cells/µl, and 55 cells/µl respectively.

In the present study seven 10.8% Aspergillus spp. were isolated from sputum which includes A.niger 71.4% and A.fumigatus 28.6%. R Kaur et al[18] found 13.2% Aspergillus spp. in sputum which includes A.niger (50%), A.fumigatus (35.7%) and A.flavus (14.3%).. In our study all seven Aspergillus spp. were isolated in patients with CD4 count < 200 cells/µl . This is in concordance with findings reported by Shah et al[27]with mean CD4 count in aspergillosis 142.3 ± 100.1 cells/µl and ≤ 200 cells/µl respectively.

One specimen of BAL showed cyst of P.jirovecii in Gomori's silver methanamine staining. One skin scrapping showed growth of T.mentagrophytes 0.4%.. R Parmar et al [34]and Shah et al[27] showed prevalence of dermatophytes 4% and 3% respectively.

CONCLUSION

Opportunistic fungal infections, due to severe suppression of the immune system, are prevalent in large number of HIV infected patients. 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.

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