World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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The spectrum of opportunistic fungal infections in relation with ART in HIV patients

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World Journal of Biology Pharmacy and Health Sciences, 2022, 11(01), 062–068

Publication history: Received on 04 June 2022; revised on 23 July 2022; accepted on 25 July 2022

Article DOI: https://doi.org/10.30574/wjbphs.2022.11.1.0099

Abstract

Recent years have witnessed a phenomenal increase in prevalence as well as severity of opportunistic fungal infection (OFIs) despite of availability of different therapeutic options. This could be attributed to various reasons including increased prevalence of immunocomprised patients. Awareness of spectrum of fungal infections in immunocompromised conditions and their epidemiological changes is critical for early diagnosis and proper management. Method: A total of 277 clinical samples were collected from 216 HIV patients suspected of OFI. Sample processing and identification of fungi carried out by standard conventional methods. Result: Out of 216 patients, 30% (65) showed positive fungal etiology with single or multiple system involvement. Oral candidiasis ranked highest (46%) followed by Cryptococcal meningitis (35%), Candidial pneumonia (10%), Oesophageal candidiasis (7%), Dermatophytoses (6*%)*, Pulmonary aspergillosis (4%), Systemic candidiasis (4%), Pneumocystis pneumonia (3%) etc. Discussion: As most of opportunistic fungi (OF) are part of the normal human microbial flora, clinical significance of fungal isolates must be confirmed by standard criteria laid down such as repeated isolation, supportive clinical findings and radiographical evidences. Although Antiretroviral Therapy for minimum 6 months duration provides protection against OFI by maintaining CD4 count, compliance to treatment important.

Keywords: Fungal infection; Antiretroviral Therapy; Immunocompromised; Opportunistic fungi; Candida

1. Introduction

Recent years have witnessed a phenomenal increase in prevalence as well as severity of OFIs mainly attributed to a spread of AIDS pandemic and increasing use of immunosuppressive therapies. A *Candida* and *Aspergillus Spp.* are isolated most commonly from immunocompromised patients. Thus considering change in spectra of fungal pathogens involved in causation of OI and the specific therapy needed for each of them, constant monitoring of these infections is essential for better management [1].

The isolation of fungi in clinical samples must be interpreted carefully as most of them are normal colonizers, consequently its pathogenic significance in samples needs to be documented by repeated isolation or isolation from the sterile site such as blood, cerebrospinal fluid or tissues. Microbiological findings supported by clinical and radiological evidence indicates infection. Histopathological examination is useful for confirmation in cases with suspected fungal tissue involvement

The widespread emergence of antifungal drug resistance in particular for Candida isolates is important due to associated morbidity and mortality. The widespread emergence of antifungal drug resistance is the result of irrational use of drugs. Realizing this, antifungal agents should be prescribed preferably after confirmation of diagnosis. In vitro susceptibility tests should be performed for Candida isolates to detect resistant strains [2,3].

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In economically sound countries new, advance and automated tests like Mass spectrometry, various molecular assays have revolutionized mycology diagnostic field, their availability and cost limits its use in other countries with high prevalence of HIV. Thus even though conventional methods are notoriously time consuming, continues to be gold standard particularly in resource constrained areas of India. Rapid, affordable and accurate testing for diagnosis of fungal infection remain the need of the hour.

A change in the spectrum of *Candida* spp. has been noticed in major hospitals in India. Non-*Candida albicans species* have been isolated from 30-90% cases of disseminated candidiasis. Amongst these the most common isolate reported is *C. tropicalis* followed by *C. glabrata*, *C. parapsilosis*, *C. guilliermondii* and *C. krusei*.

This study was undertaken to determine the spectrum of various OFI in immunocompromised patients so that they can be detected and treated earlier for better outcome.

2. Methods

A prospective study was planned and carried out at tertiary care teaching hospital in western India over a period of 2 years (2012 to 2014). A total of 216 HIV-diagnosed OPD/IPD patients with clinical evidence of fungal OI (opportunistic infection) were recruited in a study. Diagnosis of HIV infection was done at the ICTC center as per NACO guidelines. A total of 277 clinical samples (Oral swabs, Sputum, BAL, CSF, Blood, Skin scraping, hair, nail, esophageal Brushing) were collected. The study was approved by the local ethical committee.

Sample processing for identification of *Candida* was done by direct microscopy, colony morphology on sabourauds dextrose agar and on cornmeal agar (Hi Media), colony color on HiCrome Candida agar (Hi Media), physiological studies including germ tube test, Carbohydrate assimilation tests or species identification by Modified Wickerham method using yeast nitrogen base agar (Hi Media) as per standard recommended procedures.

Identification of cryptococci was done by India ink test, Cryptococcal Antigen Latex Agglutination Test (CALAS ®, Meridian Bioscience), colony morphology on SDA, colony color on Birdseed agar (Hi Media), Urease production on Christensens urea agar (Hi Media) as per standard recommended procedure. Inositol assimilation and nitrate assimilation tests were carried out to differentiate *Rhodotorula Spp*. from *Cryptococcus Spp*.

Identification of moulds was done by direct microscopy using KOH mounts, colony morphology on SDA, Lactophenol cotton blue mount and growth on slide culture. For diagnosis of *Pneumocystis jirovecii,* Gomori methamine silver (Hi Media) and Giemsa staining (Hi Media) of induced sputum and BAL samples were done [4,5,6].

The statistical analysis was done by using statistical software SPSS (version 16.0) and graphs prepared by Microsoft excel.

3. Results

We analyzed 216 HIV patients regardless of their ART status. Sixty patients presented with multisystem involvement, hence more than one sample was collected.

A total number of fungal infections diagnosed in study group were 30%. Of these, oral candidiasis ranked highest 13.89% followed by cryptococcal meningitis (10.65%), *Candida* pneumonia (3.24%), Oesophageal candidiasis (2.31%), Dermatophytoses (1.85%), Systemic candidiasis (1.39%), Aspergillus pneumonia (1.39%), *Pneumocystis* pneumonia (0.93%), *Candida* meningitis (0.93%), Cryptococal pneumonia (0.93%) and *Rhodotorula* meningitis (1.39%)

Among the *Candida* species isolated, *C. albicans* (11.19%) was the most common followed by *C. tropicalis* (3.61%) and *C. parapsilosis* (1.08%) while *C. glabrata*, *C. krusei* and *C. guilliermondii* were isolated from one patient each.

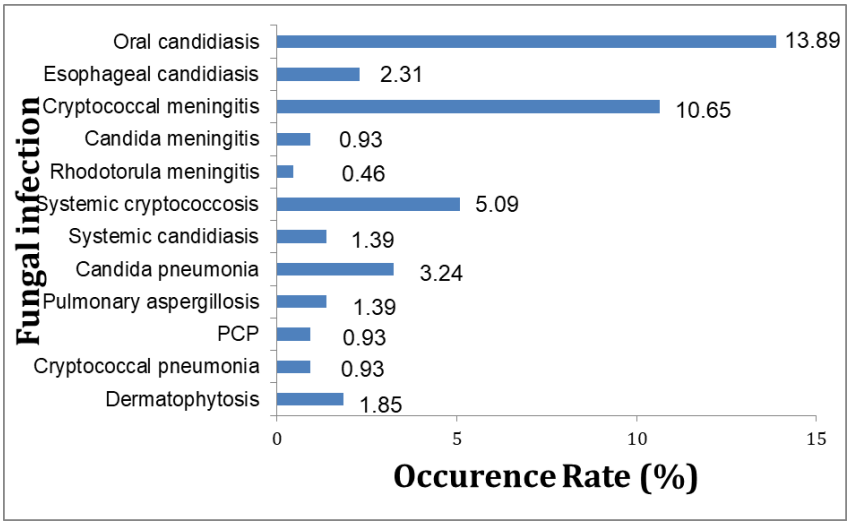

Figure 1 Occurrence rate of fungal infections in HIV seropositive patients

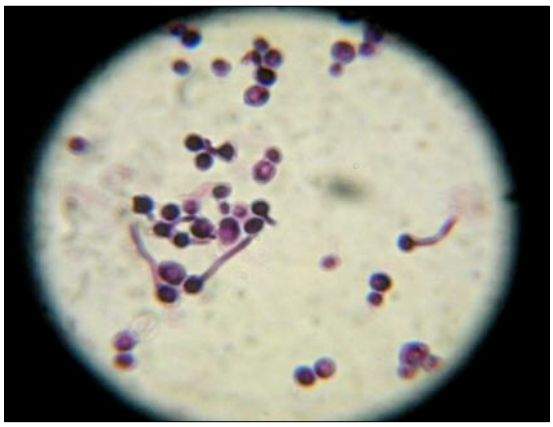

Figure 2 Germ Tube Test showing yeast cells with germ tube (Gram stain)

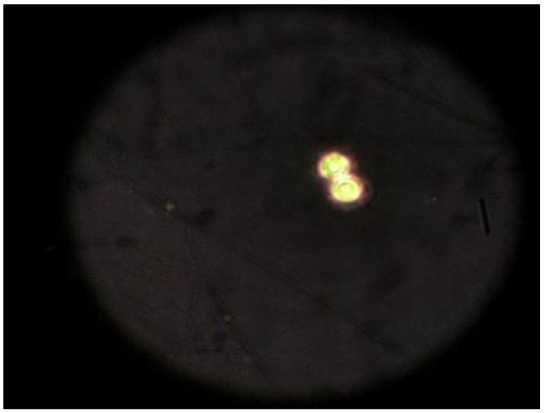

Figure 3 India ink of CSF showing encapsulated yeast cells of *C. neoformans*

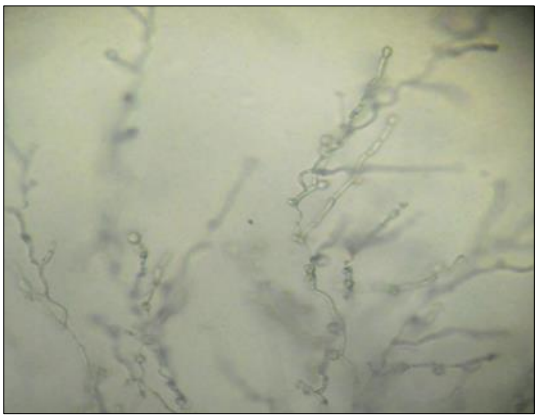

Figure 4 *C. albicans* with pseudohyphae & large, terminal chlamydospores on Corn Meal Agar

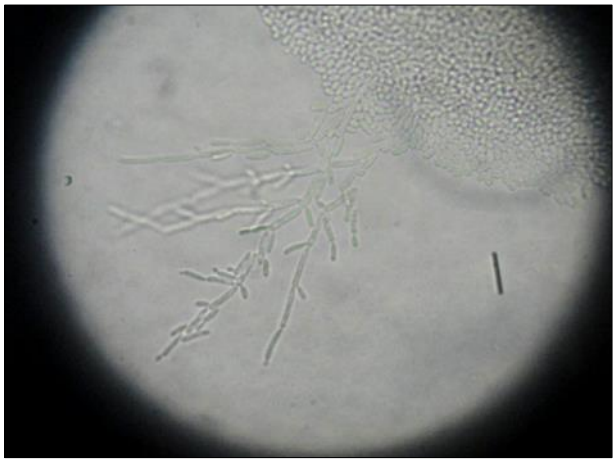

Figure 5 *C. parapsilosis* showing short, pencil like pseudohyphae with blastoconidia

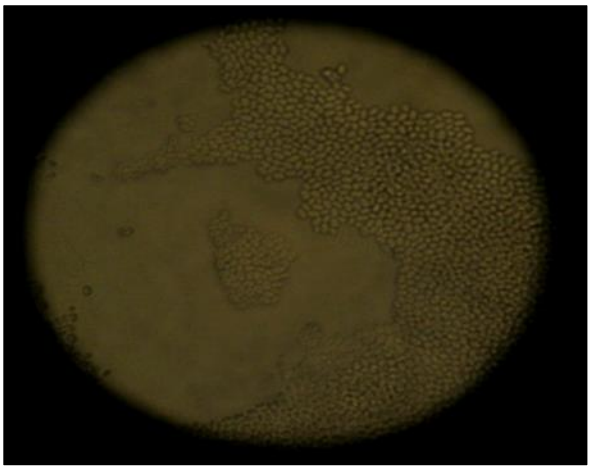

Figure 6 *C. glabrata* with yeast cells on Corn Meal Agar

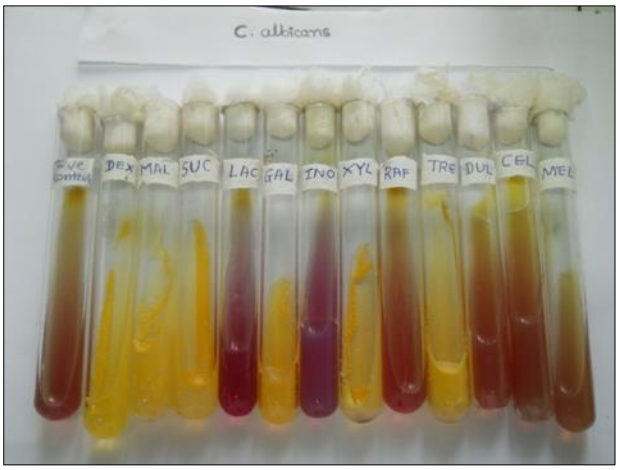

Figure 7 Carbohydrate assimilation reactions of *Candida albicans*

4. Discussion

Given the immunocompromised nature of patients and wide variety of fungal pathogens involved, OFI pose significant diagnostic and therapeutic challenge. Considering their lethal potential, early clinical suspicion and establishing clinical significance is of utmost importance.

In the current study, pathogenicity of fungal isolate was confirmed by repeated isolation or isolation from the sterile body site and supported by clinical findings, radiological investigations or fulfillment of other standard criteria laid down to rule out fungal contaminants. Clinical diagnosis of bronchopulmonary candidiasis in this study was made as per Chakravarthy and Sandhu's Criteria as- sputum positive consecutively at least four times for same *candida* spp., heavy growth on culture, and/or bronchial aspirate positive for same *Candida* spp. and improvement in patients condition after treatment with specific antifungal drugs [7].

In our study group, we observed an occurrence rate of 30% for OFIs among those suspected and fulfilling the diagnostic criteria, which corroborates the findings of other studies. Out of 216 patients suspected for OFI, 110 patients were on antiretroviral treatment. However they contributed to only 11% (7 patients) with 6 of them on ART treatment started within last 6 months duration (out of 32) which is statistically significant to those not on ART and with those on ART for more than 6 months duration. One Indian study showed risk of developing OI in patients on ART to be 5.56% with drastic reduction in risk after completion of 6 months of treatment [8]. This implies early initiation of ART and good patient compliance to treatment are necessary.

We observed predominance of various candida and cryptococcus infections including oro-esophageal candidiasis, cryptococcus meningitis, Candida pneumonia among OFIs in HIV infected persons. Oral candidiasis was the most common infection, which is also reported in literature as an indirect marker of HIV progression in the absence of CD4 count and HIV viral load [9,10]. A relatively very low number of patients showed other fungal infections including aspergillus pneumonitis and PCP.

The blood stream infections by emerging non-Candida albicans species particularly *C. tropicalis, C. parasilosis* and *C. glabrata* are being increasingly reported in immunocompromised patients. However BSI occurrence remains surprisingly low in HIV patients. The present study observed very low occurrence of candidemia in AIDS patient (1.39%) which is consistent with most of studies reported incidence of around 1% [11,12], and out three isolates detected, two were found to be non-Candida albicans- *C. parasilosis* and *C. tropicalis*. Though blood is sterile clinical specimen, its sensitivity for isolation of fungal etiological agent is low. Thus repeated blood cultures are to be performed for isolation of *Candida* and *Cryptococcus Spp*. Isolation of filamentous fungi is rare from blood specimen.

Fungal infections in a total of 32 patients (36% of diagnosed fungal patients) found to have systemic infection and/ or dissemination to deeper body organs with Candida being most common associated pathogen. These infections were further defined into proven, probable, possible invasive fungal infections based on criteria laid by European organization on research and treatment in cancer and the mycosis study group (EORTC MSG) [13]. Ten patients were of proven IFIs (positive histopathology for esophageal, pulmonary or CNS Infection) while remaining 22 patients had probable IFIs (Microbiological, clinical and radiological evidence)

Out of 23 cryptococcal meningitis patients, 11 showed presence of Cryptococcus in blood sample which gives total prevalence of 5.09% for cryptococcemia. Cryptococcal meningitis patients showed serious disease and common findings were fever, neck stiffness, vision disturbances, shock etc.

Though the global incidence of cryptococcosis has decreased due to antiretroviral therapy, the proportion of HIV positive patients with cryptococcosis is still on rise in India [14]. Occurrence of Cryptococcemia in Cryptococcal meningitis patients indicates poor prognosis [15]. The primary focus for cryptococcus infection is usually in the lung but cryptococcal pneumonia remains under diagnosed and not recognized until dissemination. This may be due to extensive prevalence of tuberculosis in India which masks a diagnosis of pulmonary cryptococcal infection [16]. Two (0.93%) patients were diagnosed with pulmonary cryptococcosis based on a positive respiratory specimen with clinical and radiographic evidence of active pulmonary infection.

We observed a case of meningitis caused by *Rhodotorula glutinis*. Patient responded to intravenous amphotericin B treatment. The same growth was obtained in two separate CSF collections of the patient [17,18].

Patients with respiratory involvement presented with long term fever, non-productive cough, chest pain, shortness of breath and dysphagia. In two (0.93%) patients with pneumonia, *Pneumocystis jiroveci* was demonstrated while in another two (0.93%) *Cryptococcus spp.* was isolated from sputum sample. Even though pulmonary candidiasis documented to be a very rare, our study shows higher prevalence (3.24%). Pneumocystis pneumonia has lower incidence in India which could be due lack of awareness and more sensitive diagnostic modalities like fluorescent staining, PCP NAT testing [19].

Dermatophyte infections occurs at frequencies more or less same in HIV positive and negative persons [20]. Even though incidence of dermatophytosis was not high in our study, rapid and extensive spread infection was observed in 3 out of four culture positive patients. These patients showed Tinea cruris rash extending beyond the groin into the trunk. Fungal culture of all three cases grew *Trichophyton Spp* and

The CD4 cell count was analyzed for 190 out of 216 patients (90%). Value closest to incident OFI was taken into consideration. A two third of OFI occurred at low CD4 cell count of below 300 cells/mm3.

In our study, out of 30 patients with oral candidiasis, 22 (73.34%) patients had CD4 count less than 200/µl, 5 (16.67%) patients were in range of 200-500 cells/µl while count was unavailable for remaining 3 patients. The mean CD4 cell count was observed to be 142 and 93 cells/mm3 for candidiasis and Cryptococcosis respectively while for most of other fungal infections it was below 100 cells/ mm^3 including aspergillus pneumonitis and PCP. Correlation of OFI with CD4 count observed was in agreement with other studies [21,22].

Our study carries a limitation of limited sample size. We also didn't analyze occurrence of OFI attributed to type of ART regimen of as corresponding medical record was not available.

5. Conclusion

A *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* contributes most to the OFI while emergence of non-Candida albicans over C albicans increasingly recorded. A rising trend of disseminated infection in immunocompromised patients is a point of concern and therefore, early initiation of ART, high clinical suspicion of OFI and early treatment are of utmost importance for better outcome.

Compliance with ethical standards

Disclosure of conflict of interest

Author states that there is no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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