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Research Article

A infecting fungus was discovered from HIV-positive people's mucus.

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Abstract

In the two months between June and July 2010, fungal pathogens were isolated and identified from the sputa of one hundred HIV-positive patients who were hospitalised to the ART centre at K.G.H. in Visakhapatnam. Sputum samples from HIV-positive people were gathered in sterile containers and processed according to conventional procedures. Three days separated the collection of the two successive samples. Only two samples were deemed positive for fungal infections if they produced the same growth. The age range of the patients, in both genders, was 6 to 55 years. Thirty-three females and 67 guys provided samples. Of the 100 samples, 54 had single fungal isolates, 20 had mixed fungal species, and 26 had no fungal isolates at all. Four samples each of Cryptococcus neoformans, Penicillium spp., Aspergillus fumigatus, and Aspergillus niger, two samples of each of Scedosporium apiospermum, Cunninghamella bertholletiae, Sporothrix schenckii, and Geotrichum candidicum were isolated. The majority of the isolates, forty-two, were Candida species. Two samples of Penicillium marneffei isolates were from patients whose CD4 levels were less than 100.

Keywords : *Opportunistic fungal infections, immunocompromised individuals, CD4 counts, penicillium marneffei.*

INTRODUCTION

Opportunistic infection by pathogens that seldom infect immunocompetent individuals is a major cause of morbidity and mortality among HIV positive individuals in late stages of HIV infection with low CD 4 count ≤ 500/cumm. Infections by opportunistic fungi have become more common in the past few years. Recent reports indicate that 26% of patients who are continuously and severely immunosuppressed have invasive fungal infections.

Candida albicans infections can be the first sign of immunodeficiency and manifest when the CD4 count is 500–200/cumm. A cryptococcal infection manifests as a CD4 count < 150/cumm. In patients with a CD4 count of less than 100/cumm, penicillosis is seen. It is thought that the phagocytic cells and lymphocytes (both T&B) work in tandem to defend the host from fungi.

However, it is yet unknown to what extent each is implicated specifically. Research has demonstrated that neutrophils consume and destroy the vegetative hyphal structures of Aspergillus and candida (Jagdish, 2009). Primary defence against infections involves the skin and mucosal surfaces. The primary method of mucus membrane clearance against inhaled fungal spores is the mucociliary action. The mucosal barrier may be compromised in HIV positive persons, making them more susceptible to fungal respiratory infections due to their propensity for recurring respiratory infections. The host's overall resistance and exposure to an adequate inoculum size of the organism are prerequisites for fungal infections. Immune deficiencies make infections caused by well-established organisms more likely to develop. Bernadette Martinelli Furthermore, Histoplasma capsulatum. Mould species that are frequently isolated in HIV-positive people include Aspergillus spp., Rhizomucor, Absidia spp., Cunninghamella spp., and Apophysomyces spp. (Harrison's, 2008). After tuberculosis and cryptococcosis, P. marneffei infection is the third most common infection among HIV-positive individuals in Thailand (Anantanarayan and Paniker's, 2009). As of right now, the CDC has identified Manipur State, India, as a new P. marneffei endemic location (Guidelines for prevention and treatment of opportunistic infections in adults and adolescents infected with HIV, 2009). after C. glabrata and C. krusei become resistant to fluconazole, the source of candida infections has switched from Candida albicans to C. glabrata and other non-Albicans species after the advent of antifungal drugs. In immunocompromised people, fungaemia can spread and result in a serious illness known as fungaemia.

MATERIALS AND METHODS

The Andhra Medical College's Ethics Committee gave us permission to carry out the study. Annexure 1 contained a copy of the Ethics Committee's approval. HIV patients provided two consecutive sputum samples, separated by three days, and their CD4 levels were displayed in Table 1. Every patient reported having a fever and cough for longer than a week. Samples of sputum were gathered in a sterile container with a wide aperture. To prevent commensal flora from the oral cavity from contaminating the sputum, patients were instructed to wash their mouth with distilled water before collecting phlegm (Koneman colour Atlas and Text book of Diagnostic Microbiology, 1997). Specimens were prepared using KOH mount and Gram's staining for direct smears. Gram's discoloured streaks were checked for the presence of fungi and inflammatory cells (pus) using an oil immersion microscope objective. Grains staining smears were used to evaluate the sputum's quality. When the quantity of squamous epithelial cells in the material is fewer than 10/LPF, it is deemed acceptable (Bailey and Scott's, 2002; Koneman colour Atlas and Text book of Diagnostic Microbiology, 1997). Further processing was applied to the samples that revealed pus cells and fungal elements in the direct Gram's staining (Koneman colour Atlas and Text book of Diagnostic Microbiology, 1997; Topley and Wilson's, 2005).

Two sets of Sabouraud's dextrose agar were inoculated with sputum (one set with gentamicin alone and the other with gentamicin and cycloheximide), and the inoculations were then cultured for four weeks at 25°C in BOD. During the first week, SDA bottles were checked for growth once every two days, and then twice a week for the next four weeks. Standard procedures were used to process the SDA medium with growth (Mackie and McCartney, 2008). Mucoid, yeast-like growth was examined by urease testing, capsular staining, germ tube staining, inoculation onto maize meal agar, and Gramme staining. All of the isolates exhibiting mucoid and yeast-like development were stained with Gram's solution, and gram-positive budding yeast cells were detected. Capsular staining with 10% Nigrosin: To identify Cryptococcus neoformans, all isolates exhibiting mucoid and yeast-like development were examined for capsulated budding yeast cells.

Test for germ tube formation : Every candida isolate underwent this procedure. After being injected with human serum, a colony was cultured at 37°C. Wet mount was produced and checked for germ tubes after two to four hours. Chlamydospore formation: In maize meal agar, the ability of each candida isolate to produce chlamydospores was examined. The plates were inoculated, allowed to incubate at 25°C, and then looked at under a microscope to check for chlamydospores.

Urease test : For Cryptococcus, Christensens' Urease test was conducted. Urease positivity was observed in Cryptococcus neoformans. For filamentous growth, lactophenol cotton blue (LPCB) mounting was used to observe conidia's organisation.

Slide cultures : In mycology, slide cultures are used to examine the undisturbed morphological aspects of fungi, specifically the relationships between reproductive structures like as hyphae, conidia, and conidiophores.If the same fungal isolate was obtained from two samples, only that fungal isolate was identified as the causal agent.

P. marneffei identification : P. marneffei is a dimorphic fungus. It is a mycelial fungus that develops quickly on SDA at 25°C. It produces greenish yellow sporulating colonies with a red centre and dark green margins that are diffusible to brick red colour. It develops smooth, glabrous, off-white yeast-like growth with little pigment at 37°C on SDA. Microscopically, P. marneffei is known for the Corda's phenomenon, in which the fruiting heads occasionally exhibit terminal conidia that are larger than the ones beneath them.

RESULTS

54 of the 100 sputum samples produced isolates of a single fungus species. Twenty of the 26 samples showed mixed fungal isolates, whereas the remaining samples showed no signs of fungal growth. Candida spp. were the most common isolates among the 54 single isolates. Forty-two samples included those yeasts. Candida non albicans in 24 and Candida albicans in 18. As indicated in Table 2, other fungi that were isolated included several species of Penicillium spp., Aspergillus fumigatus, and Cryptococcus neoformans (4 samples), as well as Cunninghamella bertholletiae (Figure 2), Geotrichum candidicum, and Sporothrix schenckii (Figure 3), all of which were found in two samples each. Thirteen of the mixed isolates contained the isolation of Candida spp., and seven contained the isolation of several moulds. P. marneffei was isolated from samples that included A. fumigatus and C. albicans in one sample each. Both of P.

marneffei's CD4 levels were below 100.

Candida spp. constituted the largest group of the 96 isolates, numbering 55 (57%). 26 samples (27%) had C. albicans, while 29 samples (30%) had non-Albicans spp. Aspergillus spp. in 13 samples, or 13.5%, was the next most prevalent isolate. Penicillium spp. in 6 samples, or 6.25%, included two isolates of P. marneffei (Figure 4). A. fumigatus and A. niger each occurred in 6 samples, and A. flavus in 1 sample. Five samples (5.2%) each had C. neoformans and S.schenckii, whereas four samples (4.16%) had Geotrichum candidicum.

Isolated microorganisms included Wangiella dermatitidis, Scedosporium apiospermum, Absidia corymbifera (Figure 6), Cunninghamella berthollatiae, Nigrospora spp. (Figure 7), and Prototheca spp. in two samples each.

DISCUSSION

In HIV positive patients, the incidence of fungal infections has increased along with the number of opportunistic fungal infections in general. The recent HIV-related pandemic of AIDS has contributed to an increase in the prevalence of two prevalent fungal illnesses, namely esophageal candidiasis and cryptococcosis. Candida is an opportunistic infection that thrives in the upper respiratory tract's regular flora and becomes more prevalent when immunity is poor. In most cases, people who test positive for HIV also have additional risk factors such as lymphopenia, neutropenia, abnormalities in selective T cells, changed function of monocyte macrophages, frequent use of antibiotics for the prevention and treatment of different bacterial infections, and decreased immunity as a result of low CD4 count. Aspergillosis, zygomycosis, cryptococcosis, and pulmonary candidiasis are examples of opportunistic fungal infections that are facilitated by all of these variables.

While infections with S. schenckii (Figure 3) and P. marneffei are emerging in HIV positive patients, invasive fungal infections such as canidiasis, aspergillosis, mucormycosis, cryptococcosis, histoplasmosis, and coccidiodomycosis are well-known (Carol, 1998). According to Topley and Wilson (2005), additional fungi that cause lung infections in HIV/AIDS patients include Absidia corymbifera (Figure 6), Scedosporium apiospermum, and Wangiella dermatitidis (Figure 5).

The development of fluconazole-resistant Candida albicans in AIDS patients experiencing repeated bouts of oral thrush and decreased fluconazole sensitivity in Candida krusei and Candida glabrata is the current cause for concern regarding candidiasis. Among Candida spp., C. albicans is typically regarded as the main pathogen (Topley and Wilson's, 2005). 96 isolates were found in 74 sputum samples used in this investigation. Of the 96 isolates of Candida spp., 55 (26 samples including C. albicans and 29 samples containing non-Albicans) demonstrated a shift in the trend of candidial infections in favour of non-Albicans spp. Isolating additional non-Albican species is becoming more and more important as non-Albican species like Candida krusei and Candida glabrata become resistant to fluconazole, a major antimycotic medication used for prophylaxis. In Hyderabad, India, Sailaja et al. (2004) also isolated more non-Albican spp. in 18 cases compared to C. albicans in 6 cases. Twenty (62.5%) of the 32 isolates that Aruna Agarwal et al. (2005) from Punjab had C. albicans identified (Usha et al., 2005).

Twenty Candida albicans and ten non-Albicans were isolated from 462 samples of lower respiratory tract infections by Jha et al. (2006) of Khatmandu. 350 C. albicans that were isolated from sputum samples underwent resistotyping, as reported by Prasobh et al. (2009) in Tamil Nadu. Geographically, every study mentioned above came from the same area. In each of these investigations, C. albicans was isolated from sputum samples. In Rakhamanova's (1998) study on opportunistic fungal infections in HIV/AIDS patients, 4% of lung infections were found to have positive cultures for Candida albicans and Candida neoformans. The present investigation and the study of Sailaja et al. (2004) found that Candida albicans spp. dominated. Yongabi et al. (2009) recovered 12 strains of C. albicans from 98 sputum samples. The predominant species in the Jha study was albicans. Other investigations mentioned only Candida albicans. However, all of these investigations noted the significance of Candida albicans as the causal agent in lung infection. In 13 samples (13.5%), Aspergillus spp. were the next most often isolated fungi in the current investigation. While A. niger was recovered in one instance by Sailaja et al., A. fumigatus and A. niger were isolated from six samples each, and A. flavus from one sample. At autopsy, Nash et al. (1997) identified 17 cases of pulmonary aspergillosis associated to AIDS. After examining 342 AIDS cases, Mylonakis et al. (1998) came to the conclusion that individuals with fewer than 50 CD4 cells/cumm are typically at risk for invasive lung aspergillosis.

Five samples (5.2%) from the current investigation and two samples from a study by Sailaja et al. (2004) included C. neoformans. Rakhamanova (1998) reported 4% isolation, while Yongabi et al. (2009) reported 2.04%. A retrospective analysis

conducted by Mirvium L Cameroon from 1981 to 1989 found 12 occurrences of cryptococcal pneumonia, of which 10 had positive cultures. All of these studies identified C. neoformans as the causal agent of lung fungal infection. Further organisms identified were: Cunnighamella bertolletiae, A. corymbifera, Nigrospora spp., Prototheca spp., Penicillum spp. (including two P. marneffei) in six, Geotrichum candidicum in four, Wangiella dermatitidis and Scedosporium apiospermum in two, and so on. In our geographic region, the aforementioned fungus were isolated for the first time. In two samples used in this investigation, the developing fungus P. marneffei was identified from sputum. The same was documented by Singh et al. (1999) from Manipur, India, in four autochthonous cases. Fifty cases from the same hospital had widespread P. marneffei infection, according to Ranjana et al. (2002). CDC noted that penicillosis was observed in HIV positive patients who have been to Manipur, India, a new endemic location for this fungus.

Less than 100/cumm for CD4 counts (Guidelines for prevention and treatment of opportunistic infections in adults and adolescents infected with HIV, 2009). P. marneffei was isolated from lymph nodes and blood cultures of HIV-positive patients with multifocal lymphadenopathy and extensive hepatosplenomegaly by Gupta et al. (2007) of New Delhi. Bhagyabathi.

Conclusions

It is determined that the most often isolated species in the current investigation were Candida species (C. albicans in 26 samples and non albicans in 29 samples). Aspergillus species and Cryptococcus neoformans were the next frequent species. The isolation of Geotrichum candidicum, Wangiella dermatitidis, Scedosporium apiospermum, Absidia corymbifera, Cunnighamella bertholletiae, Nigrospora spp., and Prototheca spp., albeit in fewer cases, highlights the need for additional research on fungal pathogens in HIV positive patients. In the current study, P. marneffei, an emerging fungal pathogen, was isolated from two individuals with CD4 levels below 100. At Andhra Medical College in Visakhapatnam, we attempted to isolate fungal infections from the lower respiratory tract for the first time. It still requires a thorough investigation.

In addition to bacterial pathogens, all HIV-positive patients with cough must be screened for fungal pathogens. Early diagnosis and treatment thus prevent more consequences.

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