Case Report

Disseminated cryptococcosis and fluconazole resistant oral candidiasis in a patient with acquired immunodeficiency syndrome (AIDS)

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Abstract

Disseminated cryptococcosis and recurrent oral candidiasis was presented in a-heterosexual AIDS patient. *Candida tropicalis* (*C.tropicalis*) was isolated from the oral pseudomembranous plaques and *Cryptococcus neoformans* (*C. neoformans*) was isolated from maculopapular lesions on body parts (face, hands and chest) and body fluids (urine, expectorated sputum, and cerebrospinal fluid). *In vitro* drug susceptibility testing on the yeast isolates demonstrated resistance to fluconazole acquired by *C. tropicalis* which was a suggestive possible root cause of recurrent oral candidiasis in this patient.

Key words: HIV; AIDS; cryptococcosis; candidiasis; immunocompromised; fluconazole

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Case Report

A 34-year-old heterosexual HIV-I positive male developed mucopurulent productive cough, recurrent oral plaques, occasional syncope, and neurological symptoms that included headache and dizziness. Physical examination revealed discrete erythematous maculopapular lesions on his face (Figure 1), neck, chest, and both hands. There were not any significant enlargement of the cervical lymph nodes, and oral examination revealed pseudo-membranous plaques (Figure 1). He was previously treated with anti-fungal drugs (fluconazole and amphotericin-B), primary anti-tuberculous drugs (isoniazid, rifampin, ethambutol, and streptomycin), and an antigiardial drug (tinidazole) for giardiasis, caused by, *Giardia lamblia*. The CD4+ lymphocyte count for this patient was 40 cells/µl with a CD4+/CD8+ ratio of 1:72. Despite a history of multiple unprotected sexual exposures, the patient tested negative for venereal disease.

The skin biopsies and body fluids, such as, cerebrospinal fluid (CSF), urine, and mucopurulent expectorated sputum, showed encapsulated yeasts in India-ink-wet-mount preparation. The cultures for acid-fast bacilli (AFB) on Lowenstein-Jenson and non-selective Middle-brook 7H12 agar media were negative. Periodic-acid-Schiff and Grocott-Gomori-methylamine-silver-stained smears were negative for *Pneumocystis carinii*. Serum and CSF tested positive for capsular Cryptococcal polysaccharide antigen using the latex agglutination test with a titer of 1:1015. Skin sections revealed gelatinous troma (Figure 2) filled with numerous encapsulated yeast cells (Figure 3). Biopsied specimens of skin and other body fluids (CSF, urine, and sputum) yielded the growth of *C. neoformans* on Sabouraud's dextrose agar (SDA) medium. The resultant mucoid-cream-colored colonies were negative for germ tube and positive for urease test. Colonies failed to grow on Cyclohexamide-supplemented SDA. Colonic growth at 37°C on plain SDA was weakly positive. Microscopic examination of the Gram-stain preparation from a portion of scraped oral lesions showed Gram-positive yeasts and pseudo-hyphal forms. The remaining portion the scraped oral lesions were inoculated on SDA which then showed a typical growth of *C. tropicals*. Identification of *C. tropicalis* was further confirmed by the germ tube test; morphological characteristics were determined on cornmeal tween-80-agar and Vitek-32 and API 20C

AUX identification systems (bioMérieux, Craponne, France). Drug susceptibility testing to fluconazole, ketoconazole, clotrimazole, posaconazole, and amphotericin-B was performed using the CLSI micro-dilution method [1]. The *in-vitro* drug susceptibility study revealed fluconazole resistance to *C. tropicalis* only; whereas *C. neoformans* were found to be susceptible to all antifungal agents. Lab investigations for further clinical management could not be followed as the patient died on the third day of his hospitalization.

Discussion

The two species of genus *Cryptococcus* (*C. neoformans* and *C. gatti*) are pathogenic and responsible for the life-threatening infections in immunocompromised patients, particularly those that are diagnosed with HIV/AIDS [2-3]. *C. albicans* and other species of *Candida*, such as, *C. tropicalis*, are responsible for causing oral and systemic candidiasis in immunocompromised patients. Succeeding infection occurs due to high to moderately virulent strains of *Candida* against the intensity of the immune response generated by the host cells. Nonpathogenic *Candida* may colonize in immunocompromised hosts, such as, neutropenic and HIV-AIDS patients. The colonization of avirulent *Candida* species does occur due to poor cell mediated immune response, mounted by the compromised hosts [4].

Figure 1: Erythematous maculopapular lesions on the face and curdy coating on the tongue (oral candidiasis)

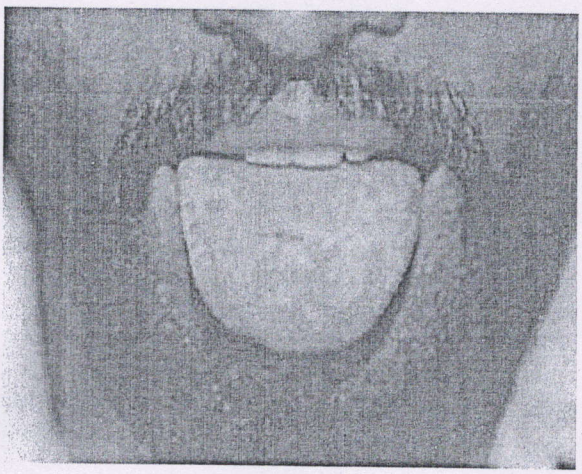
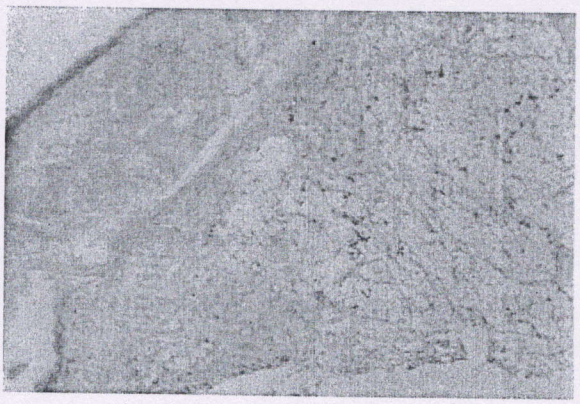


Figure 2: Histopathology of a skin biopsy from erythematous maculopapular lesions (tissue sections stained with Hematoxyllin and Eosin) shows (100x) a gelatinous stroma - (no inflammatory or granulomatous reactions).

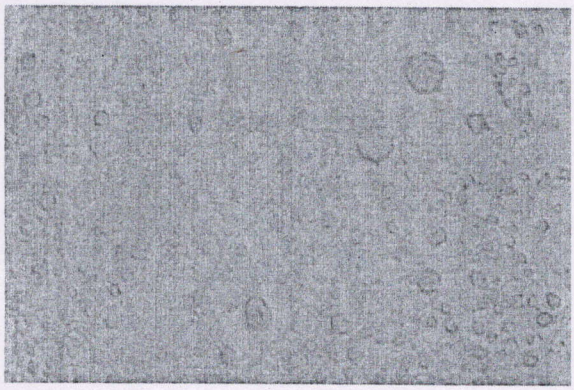


Development of Cryptococcal infection is determined by a cell-mediated immune response against an encapsulated yeast population and its virulence. Immunocompetent patients with cryptococcosis usually demonstrate granulomatous inflammatory reactions (infected sites) to encapsulated yeasts or antigens [5]. However, in this patient, there were no granulomatous reactions or inflammatory responses seen in any tissue sections; instead, there were extensive disseminated gelatinous-appearing lesions and large number of encapsulated yeasts. Comparable findings are seen in other full-blown AIDS patients as well [6,7,8].

In this case study, the CD4+ cell count was an ideal marker to assess the immunological status of the patient because from the T lymphocyte population CD4+ and CD8+ cells are the key elements in mounting immune response against pathogenic organisms [9]. However, it has been observed from the *in-vitro* studies on human T cells that CD4+ plays a significant role in mediating CD8+ proliferation against *C. neoformans* [10]. The HIV infected patients are more prone to Cryptococcal infection when their CD4+ counts drop below 50-100 cell/mm [11,12]. *In vitro* studies on human T cells, particularly CD4+ and CD8+ cells against *C. neoformans*, have demonstrated interleukin and transferrin receptor expression with subsequent proliferative response [10]. The production of IFN-gamma from CD8+ cells have been found in inactivating encapsulated yeasts of *C. neoformans* within macrophages [13]. Hence CD8+ cells in immune response may be functioning independent of

CD4+ cells. Some experimental studies suggest, that appropriate CD4+ signaling could allow CD8+ cells to respond against *C. neoforamans.* Further studies are required in human T cells to explore the potential role of interleukin-2 and IFN-gamma in resolving the ambiguity of CD4+-CD8+ linked response against *C. neoformans* infection. Inadequate T cell (CD4+/CD8+) response against *C. neoformans* infection leads to the death of patients suffering from AIDS [14,10]. *C. neoformans* is the most common fatal mycosis in AIDS patients, but is less common in non-cell-mediated immunodeficiency [15,16], which suggest that CMI is an important immune mechanism in the clearance of *C. neoformans.*

Figure 3: Histopathology of Skin biopsy (Erythematous maculopapular lesions): tissue sections stained with Periodic acid-schiff's stain under 400x shows intensely pinked muco-polysaccharide capsule of *C. neoformans* in gelatinous stroma.



In the present case report, it appears that the low CD4+ lymphocyte count supports the opportunistic condition to establish mixed fungal infections caused by *C. neoformans* and *C. tropicalis*. Unlike *C. albicans*, which can be occasionally found as a commensal, *C. tropicalis* has almost always been associated with the development of fungal infections in immunocompromised patients [17]. Some reports from India suggests that *C. tropicalis* is the most prevalent yeast species responsible for causing infection in HIV/AIDS patients [18]. However, in the present case study, fluconazole-resistant *C. tropicalis* was isolated from the oral-pseudomembranous lesions, which was an uncommon finding in the Indian subcontinent. Such observations might have been overlooked due to a lack of drug susceptibility studies or tracking of recurrent *Candida* infections in immunocompromised or immunocompetent patients in India.

The extensive use of fluconazole in the clinical management of AIDS and in other high-risk patients could be the basic cause of increasing prevalence of *Candida* species other than *C. albicans.* Among these species *C. tropicalis* is predominant in some parts of the world [19,20,21,22]. As a result, *C. tropicalis* has been emerging in both immunocompromised and immuno-competent patients; hence, it has been suggested that the use of fluconazole in a high-risk group of patients should be avoided [19]. Irrespective of host immune response and other predisposing factors, a pathogenicity of *C. tropicalis,* might be another contributing factor for its mucosal colonization in the buccal cavity. Similarly, colonization in the gastrointestinal tract can lead to the penetration of sub-mucosal layers. Some clinical studies have shown that *C. tropicalis* is more virulent than *C. albicans* in neutropenic and non-neutropenic patients [23,24]. The purified tropiase from *C. tropicalis* (a novel acid proteinase) demonstrated hemorrhagic activity and its ability to increase capillary permeability [25]. In-spite of the recurrent infections due to *C. tropicalis* in AIDS patients, there was no evidence of its dissemination. However, dissemination did occur due to encapsulated yeasts of *C. neoformans.* The dominant mechanism of *C. neoformans* over *C. tropicalis* in immunocompromised patients is still not clearly understood.

Antifungal drug intolerance is certainly a major challenge in the clinical management of AIDS and other neutropenic and non-neutropenic patients (with several underlying subclinical conditions) [24,26]. An interesting clinico-mycological scenario in this report is that *C. neoformans* isolates were susceptible to fluconazole; however, *C. tropicalis* was found to be resistant. Such clinico-mycological aspects must be carefully considered before switching to any antifungal therapeutic options. In addition, *C. tropicalis* has often been reported as resistant to fluconazole [27]. The plausible mechanisms of fluconazole resistance have been extensively studied in the strains of *C. albicans* isolated from patients with HIV-AIDS-related oropharyngeal candidiasis [4]. Based on the literature survey, we believe that this could be the first case report from India presented with disseminated cryptococcosis and recurrent oral candidiasis due to fluconazole-resistant *C. tropicalis*.

Development of fluconazole resistance in *C. tropicalis* could be due to several mechanisms. Nevertheless, some plausible mechanisms [28] could

include, escalation of emanation (efflux) of the azole compounds due to (i) over-expression of efflux pumps, such as the adenine tri-phosphate binding cassette (ABC) transporter and major facilitator superfamily (MFS) membrane transporters; (ii) up-regulation of the *ERG11* gene, coding for the azole target lanosterol 14 (α)-demethylase; (iii) *ERG11* sequence related point mutation and (iv) decreased affinity of azoles for their target-induced azole resistance. The acquired azole resistance could be the result of either one of these mechanisms or coupled with other steps in a harmonized manner [29]. In general, azole resistance in *C. tropicalis* suggests that over-expression of *CtERg11,* associated with missense mutation in this gene, and seemed to be responsible for acquired azole resistance.

The intracranial pressure due to the heavy load of encapsulated yeasts and extensive colonization of fluconazole-resistant *C. tropicalis* (or intolerance to antifungal agents) could be the major cause of the death of this patient. Epidemiological studies suggest that, increasing prevalence of *C. tropicalis* related to candidiasis in immunocompromised patients is due to the underlying subclinical conditions and subsequent antifungal agents associated toxicity; in addition to the genetic relatedness of the *Candida* species [30,31,32]. Although, morbidity and mortality rates due to infections caused by *C. tropicalis* have been reported more often, than those attributed to *C. albicans* [33]; it would be worth comparing this scenario in the cases of AIDS with disseminated cryptococcosis.

Comparing the susceptibility patterns of oral isolates during the highly active anti-retroviral therapy (HAART) era with those from 1994, suggest that: most cases of HIV-associated oropharyngeal candidiasis observed during the HAART era were caused by azole-susceptible strains. However, the reversion of isolates from azole-resistant to azole-susceptible was related to strain substitution [34]. Ever since the introduction of HAART, incidences of opportunistic infections such as, cerebral toxoplasmosis, *Pneumocystis carinii* pneumonia and others have been declining. However, the prevalence of Candidial esophegitis remained unchanged [34]. These findings suggests that HAART does not play a significant role in minimizing any fluconazole drug resistance in *Candida* species and still remains a challenging problem for its long-term therapeutic application in HIV-infected or immunocompromised patients.

Conclusion

The major gap in the pathogenicity of *C. neoformans* is the limited understanding of its dominant mechanism over *C. tropicalis* in AIDS patients. Secondly, the interlinked cellular mechanisms between CD4+ and CD8+ cells against *C. neoformans* infection remain unclear. Hence, some additional studies need to be performed on the expression of IL-2 receptors and IFN-gamma production by human T cells (T cells - derived from the various clinical stages of HIV infection; may be from sero-rectaive to full blown AIDS stage).

Although, fluconazole is still the first line of treatment in a number of cases of invasive candidiasis, its therapeutic application should be restricted to selected high-risk patients in order to minimize the risk of emergence of azole-resistant strains of Candida. Consistent use of standard, rapid and reliable methods for species identification in conjunction with drug susceptibility testing is advocated; particularly, for the cases of recurrent candidiasis. This approach would allow systematic tracking of emerging *Candida* species, other than *C. albicans* in various clinical conditions. Additionally, its application in monitoring drug-resistant strains or changing patterns of *Candida* species, other than *C. albicans* in clinical settings may support clinicians in determining appropriate therapeutic agents for effective clinical management. The therapeutic use of HAART, in combination with granulocyte colony-stimulating factor (G-CSF) cytokine in HIV-infected patients may be useful in the management of concomitant infections caused by fluconazole-resistant strains of *C. tropicalis*.

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