

INTRODUCTION
&
AIMS AND OBJECTIVES

1. Introduction.

Fungal infections may be divided into two broad categories: (i) nosocomial and (ii) community acquired. Virtually all nosocomial fungal infections may be considered opportunistic mycoses, because fungi, which are ordinarily non-pathogenic, harmless saprobes, may cause life-threatening infection in seriously ill and/or immunocompromised patients. In contrast, community-acquired fungal infections encompass not only opportunistic mycoses but also the endemic mycoses, in which susceptibility to the infection is acquired by living in geographical area constituting the natural habitat of a pathogenic fungus. Fungi frequently cause disease in patients with human immunodeficiency virus (HIV) infections. The spectrum of illness ranges from asymptomatic mucosal candidiasis to overwhelming disseminated infection and life-threatening meningitis. The importance of fungal diseases among patients with HIV infection was recognized in the early days of the acquired immunodeficiency syndrome (AIDS) epidemics. More than 100 species of *Candida* exist in nature; only few species are recognized as causing disease in humans.

Candida albicans accounts for approximately 50–60% or more causes of candidiasis in humans. *C. glabrata* recently has become important because of its increasing incidence worldwide, and it is intrinsically less susceptible to azoles and amphotericin B. Some *Candida* species, *C. tropicalis* and *C. dubliniensis*, are increasingly isolated from clinical samples. Another important *Candida* is *C. krusei*, it is of clinical significance because of its intrinsic resistance to azoles and its less susceptibility to all other antifungals, including amphotericin B.

Clinical research in fungal infections is largely a neglected area of health care setting in India. It is an important emerging infection, especially in immunocompromised patients. Incidence of fungal infection is increasing

in India but due to lack of awareness and specialised expertise, the investigation on the subject is still limited.

There are a number of the new developments in fungal infection briefly they include:

- (1) Changes in fungal infection epidemiology.
- (2) There is a new understanding of pathogenesis.
- (3) There are new methods of diagnosis.
- (4) New approaches for treatment (New formulations and the combination therapy).

The present problems of fungal infection are:

- (1) The incidence of fungal infections is increasing.
- (2) The spectrum of fungal disease is changing.
- (3) Selection of fungi is a reality.
- (4) Drug resistance of fungi is on the rise.
- (5) Cross resistance is increasingly recognized
- (6) The unit epidemiology is underscored.

There is a paucity of planned studies on this subject in India. In spite of the fact that the incidences of fungal infections are rapidly increasing mostly due to rise in number of immunocompromised patients and also due to lack of awareness and limited surveillance, this aspect largely remains neglected area of health care in most developing countries including India.

Oropharyngeal candidiasis tops the list of opportunistic infections in HIV/AIDS patients before the era of highly active antiretroviral therapy (HAART). Oropharyngeal candidiasis represents the most common clinical presentations of mucosal candidiasis associated with HIV/AIDS. The pseudomembranous and erythematous variants of oropharyngeal candidiasis represent the most common clinical presentations of mucosal candidiasis associated with HIV-infection. Though the incidences of

opportunistic infections have been reduced around the globe after HAART, the situation is different in most of the developing countries including India where patients can barely afford this treatment. World Health Organisation (WHO) predictions put India as one of the biggest repository of HIV/AIDS patients in coming decades. Thus opportunistic infections are expectedly also to increase alarmingly specially the candidiasis. Therefore an early specific diagnosis and subsequent treatment to combat this infection is not only the concern of western hospitals but is also equally relevant to developing countries like India.

Vulvovaginal candidiasis occurs more frequently and more severely in the women with weakened immune systems. There are some other conditions that may put a woman at risk for genital candidiasis. The predisposing factors of vulvovaginal candidiasis are disturbed glucose tolerance e.g. diabetes mellitus, pregnancy, use of high-oestrogen oral contraceptives, taking of wide spectrum antibiotics and corticosteroids, and use of tight-fitting synthetic underwear.

C. albicans is the most common species and it is usually isolated (77–90%) from the vaginal mycoses. Vulvovaginal candidiasis caused by non-*C. albicans Candida* species is also on the rise. It may be because of wide use of antifungal drugs, which has resulted in the suppression of the more susceptible *C. albicans* and selection of other more resistant species. It has been found that these non-*C. albicans Candida* species are often less susceptible to antifungal agents, especially to the widely used azoles. Diabetes mellitus is increasing by an alarming rate in India, where the number of people with diabetes figure is expected to rise to more than 80.9 million by the year of 2030 from 31 million, as reported in the year 2000 from India (Wild *et al*, 2004; Bjork *et al*, 2003).

The majority of the cases of candidiasis in humans are caused by *C. albicans*. *C. albicans* is an opportunistic pathogen and causes infections in different anatomical locations. The frequency of this infection has risen drastically in the recent decades due to the increasing number of the immunocompromised patients. This organism expresses several virulence factors that contribute to the pathogenesis.

Recently the enzymes involved in the glyoxylate cycle have been found in *C. albicans*, which have a role in its virulence, especially the two key regulatory enzymes, namely isocitrate lyase and malate synthase. Glyoxylate cycle enzymes are therefore the prime targets for antibacterial and antifungal agents. There are however, very few studies on the glyoxylate cycle enzyme activities in the clinical isolates.

Rapid identification of *Candida* species has become more important because of an increase in infections caused by species other than *C. albicans*, including species innately resistant to azole antifungal drugs, the most frequently used drugs for treating patients with candidiasis. The invasive candidiasis is now considered the fourth rank as the causative agent of nosocomial invasive infection with high mortality and morbidity. In a clinical mycology laboratory, yeasts are always identified by conventional methods which include a combination of morphological and biochemical criteria. These conventional tests are lengthy and time-consuming. Morphological, fermentation and assimilation tests can take several days to identify the isolates in a culture. Molecular diagnostic techniques have initiated a revolution in the diagnostic and monitoring of infectious diseases.

1.1 Aims and objectives.

In order to get insights into the present scenario of candidiasis setting in an Indian hospital, this study was mainly designed and the main aims were to do a) phenotypic characterization and b) genotypic characterization of the *Candida* species isolated from clinical samples.

In the present study the opportunistic candidal infection were monitored; (1) oropharyngeal candidiasis in HIV/AIDS, (2) vulvovaginal candidiasis in diabetic women, and (3) Wound candidal infection in burn patients.

(1) Oropharyngeal candidiasis in HIV/AIDS patients.

In order to get an insight into the present scenario of oropharyngeal candidiasis in an Indian hospital, in the present prospective study, an attempt was made to characterize the *Candida* species isolated from oropharyngeal candidiasis in HIV/AIDS patients, mainly to know: **a)** The spectrum of isolates **b)** its in vitro anti-fungal susceptibility pattern against common antifungal fluconazole used for prophylaxis and treatment of oropharyngeal candidiasis. In addition, genetic relatedness of the prevalent isolate was also recorded to generate basic data to evaluate the primary infection or infections due to resistant strains. In selected number of patients it was also tried to correlate the CD4⁺-T-cell counts with the severity of infection.

(2) Vulvovaginal candidiasis in diabetic women.

Patients with diabetes mellitus are at risk for vulvovaginal candidiasis. There are very few reports regarding Vulvovaginal candidiasis from India and this research was the first study on vulvovaginal candidiasis in diabetic patients from which has been carried out in India with extensive clinical and laboratory aims. The main aims of the present study on vulvovaginal candidiasis in diabetic women were: **(i)** whether vulvovaginal candidiasis is a significant problem in diabetic women who attended the

tertiary medical care hospital for their medical advice **(ii)** the prevalence percentage of various *Candida* species in these patients and the change of spectrum of isolates if any comparing with the earlier clinical report from same hospital **(iii)** its in-vitro fluconazole susceptibility pattern and **(iv)** the genetic relatedness of predominant strains of *C. albicans* and *C. glabrata* were also recorded to generate basic data about genetic nature, in order to estimate the primary source of infection.

In this study, an attempt was also made to **(i)** characterize and study the four glyoxylate cycle enzymes by measurement of the enzyme activities in the human clinical isolates of *C. albicans* isolated from three different groups of patients these are HIV/AIDS, diabetic and burn suffering from candidiasis and **(ii)** to elucidate if there is any correlation of the status of antifungal susceptibility and the glyoxylate cycle enzymes.

The conventional methods may take several days for the final diagnosis of *Candida* up to species level. The early diagnosis and subsequent treatment to combat this infection of immunocompromised patient is not only the concern of western hospitals but is also equally relevant to developing countries like India. One of the main aim of this study was therefore to develop a PCR based approach in order to develop a DNA probe to identify different *Candida* species based on common amplicon present on arbitrary primed PCR (AP-PCR) profiles.