Summary

Over the past two decades, the incidence of the fungal infection has increased dramatically. The morbidity and mortality associated with these infections are substantial, and it is clear that fungal diseases have emerged as important public health problems. The array of opportunistic fungal pathogens is almost unlimited (Gupta *et al*, 2004; Lattif *et* al, 2004; Schell, 1995).

Clinical research on fungal infections is largely a neglected area of health care in India. The present study carried out a detailed analysis of *Candida* infections in i) HIV/AIDS and ii) diabetic patients with candidiasis admitted to All India Institute for Medical Sciences, New Delhi, India.

Oropharyngeal candidiasis was at the top of the list of the opportunistic infections in HIV/AIDS patients before the era of HAART. Although the incidences of the opportunistic infections have been reduced around the globe by HAART the situation remains the same in most of the developing countries, including India where patients can hardly afford this treatment. 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.

Diabetes mellitus has been considered a predisposing factor for vulvovaginal candidiasis. Diabetes mellitus is increasing by an alarming rate indeed and by the year of 2025 the total number of people with diabetes mellitus is projected to reach 300 million worldwide (Amos *et al*, 1997).

The problem is alarming in developing countries, particularly 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 (Bjork e*t al*, 2003).

Vulvovaginal candidiasis is a common complication of diabetic women.

In order to get insights into the present scenario of candidiasis setting in an Indian hospital, the present 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, and (2) vulvovaginal candidiasis in diabetic women.
The clinical samples were collected as follows:
1. HIV/AIDS patient suffering from suspected oropharyngeal candidiasis 125 HIV/AIDS patients have been studied.

2. Diabetic women with suspected vulvovaginal candidiasis, 200 high vaginal swabs collected during this study.

3. Non-diabetic women with suspected vulvovaginal candidiasis, 150 vaginal swabs from 75 non-diabetic patients. Diabetes mellitus was excluded form the non-diabetic by performing an oral glucose tolerance test as per the criteria of the American Diabetes Association (Gabir *et al,* 2000).
4. Wound swabs from burn patients.

1. Oropharyngeal candidiasis in HIV/AIDS patients.

* Out of the 100 HIV/AIDS patients studied, 75 were found to be positive for oropharyngeal candidiasis. This study affirmed that about 75 per cent of the HIV/AIDS patients studied, had oropharyngeal candidiasis.

* Species identification revealed that *C. albicans* was the most predominant species isolated from HIV/AIDS patients.

* Antifungal susceptibility testing by NCCLS M27A-2 method for all the *Candida* isolates revealed significant level of azole resistance was not found among the *Candida* isolates from this group of HIV/AIDS patients.

* DNA fingerprinting of all *C. albicans* isolates with CARE-2 probe was also done. Visual analysis of DNA fingerprint patterns showed that although isolates collected from different patients over a period of time were different. This indicates that the source of infection is not common to all patients, suggesting that commensal or endogenous isolates might be become pathogens.

* Using the DENDRONE software did cluster analysis of these fingerprint patterns. The dendrogram, generated, showed that the isolates were unrelated groups. Similar to visual analysis, quantitative analysis also revealed that most of the isolates collected from different HIV/AIDS patients were unrelated.

* Correlation between $CD4^+$-T-cell counts and onset of oropharyngeal candidiasis in HIV/AIDS patients was also studied. The present study suggested that severity of oropharyngeal candidiasis detected clinically can provide a rough estimate of $CD4^+$-T-cell counts depending on which anti retroviral therapy can be instituted if needed.

2. Vulvovaginal candidiasis in diabetic women.

Diabetes mellitus has been considered a predisposing factor for vulvovaginal candidiasis. Diabetes mellitus is increasing by an alarming rate indeed and by the year of 2025 the total number of people with diabetes mellitus is projected to reach 300 million worldwide (Amos *et al*, 1997). The problem is alarming in developing countries, particularly 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 (Bjork e*t al*, 2003).

* Vulvovaginal candidiasis is a common complication of diabetic women. 350 vaginal swabs were collected from 175 patients. Two swabs have been collected from each patient of the hundred diabetic and seventy-five non-diabetic women.

* The present study showed that the percentage rate of the vulvovaginal candidiasis was about 45% prevalence in diabetic and 25% in non-diabetic women.

* The species identification revealed that *C. albicans* was the most predominant species (47%) followed by *C. glabrata* (40%), *C. krusei* (7%), *C. parapsilosis* (4%) and *C. tropicalis* (2%).

* The in-vitro susceptibility patterns were checked in all the isolates against fluconazole NCCLS m27-A2 standard protocol. In totality 22% *Candida* species from diabetic patients were resistant to fluconazole.

* DNA fingerprinting of all *C. albicans* isolates form diabetic and non-diabetic patients with CARE-2 probe was also done. Visual analysis of

DNA fingerprint patterns showed that although isolates collected from different patients over a period of time were different. By using the cluster analysis, it was further confirmed that all the *C. albicans* isolates were genetically unrelated.

* In the present study an attempt was also made to evaluate the genetic nature of the predominant non-*C. albicans Candida* isolates of *C. glabrata* isolated from diabetic and non-diabetic patients with vulvovaginal candidiasis. Arbitrarily primed PCR (AP-PCR) technique was employed. Most of the *C. glabrata* isolates obtained from diabetic patients and non-diabetic to be belonging to genotype B.

* The present study is probably the first ever study survey of oropharyngeal candidiasis in HIV/AIDS patients and vulvovaginal candidiasis in diabetic patients in India. In which comprehensive laboratory data were generated; this will help not only in patient management but also in future clinical research.

3. The Glyoxylate cycle enzyme activities in the pathogenic isolates of *C. albicans* obtained from HIV/AIDS, diabetic and burn patients.

Recently it has been found that *C. albicans* harbors enzymes involved in the glyoxylate cycle, which helps its survival inside the macrophages during infection, especially the two key specific enzymes namely, isocitrate lyase and malate synthase [Lorenz & Fink, 2001].

* The data from the present study showed and confirmed the presence of all the glyoxylate cycle enzymes as a virulence factor of the *C. albicans*.

* The present study is the first report of the presence of the glyoxylate cycle enzyme activities in a group of clinical *C. albicans* strains, isolated from clinically different patients.

4. Identification of *Candida* species by Randomly Amplified Polymorphic DNA (RAPD).

In a clinical mycology laboratory, yeasts are always identified by Conventional methods which includes combination of morphological and biochemical criteria. Conventional means of identification of pure include lengthy and time-consuming morphological, fermentation and assimilation tests that can take several days to identify the isolates in a culture (Hui *et al*, 2000; Fujita & Hashimoto, 1992). Early and accurate diagnosis of these infections is important for several reasons (i) including timely institution of antifungal therapy and (ii) to decrease the unnecessary use of toxic antifungal agents.

* By employing the Randomly Amplified Polymorphic DNA (RAPD) assay, originally referred to as the arbitrarily primed PCR (AP-PCR). In the present research a method has been characterized that produces simple diagnostic fingerprints that are unique to different isolates of *Candida* species.

* **The** results of the RAPD study showed that a promising random primer **(ALI-8)** can be used as a diagnostic tool for the diagnosis of different *Candida* species as this probe demonstrated a species-specific band pattern.