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PREVALENCE OF *CANDIDA* SPECIES IN THE ORAL CAVITY OF DIABETIC AND NON-DIABETIC SUBJECTS IN GULBARGA DISTRICT, KARNATAKA, INDIA.

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ABSTRACT

Prevalence of Species of *Candida* in the oral cavity of one hundred and three diabetic subjects and one hundred non-diabetic subjects were studied. Species of *Candida* were isolated was *C.albicans* (54.36%), *C.tropicalis* (14.56%), *C.krusei* (4.85%), *C.parapsilosis*(1.94%) was isolated from oral cavity of diabetic subjects where as *C.albicans* (27%) was isolated from non diabetic subjects. Isolation and identification of species of *Candida* in diabetes mellitus will helps in antifungal therapy.

KEY WORDS : **Candida, Oral Cavity, Diabetic patients**

INTRODUCTION

Candida is notorious opportunistic pathogen.[1] The sites where *Candida* species are commonly present in human oral cavity, gastrointestinal tract and vagina, followed by the skin, whilst isolation from the eye and urine is much less common.[2] Oral candidiasis in the form of thrush has been well known since the time of Hippocrates.[3] Prevalence of *Candida* species infections has been increased during past decade.[4] Diabetes mellitus is predisposes to superficial and systemic infections and oral candidiasis in particular is thought to be more prevalent among these individuals. This is classically an acute infection but in diabetes mellitus thrush may, if untreated, persist for several months and the infection could be considered chronic rather than acute.[5] There have been reports of out breaks of *Candida* infections in hospitals and in general community. In addition there is considerable interest in the epidemiology of recurrent oral candidiasis[6]. Further more the majority of studies have been done on 'normal individuals'. The epidemiological investigations of species *Candida* to date have been hampered. Accurate figures for oral *Candida* species in the community would not appear[2]. Arunloke Chakrabarthi(1999)[7] noted that the exact prevalence rate of species of *Candida* is not known. Therefore present study was undertaken to antibiotic or steroid therapy or had been using antiseptic mouthwashes or were wearing denture wearers.

check the prevalence of *Candida* species in the oral cavity of diabetic and non-diabetic subjects.

Materials and methods

One hundred and three Diabetic subjects and one hundred age and sex matched Non-diabetic subjects participated in the present study with prior informed consent. Non-diabetic subjects had fasting and 2 hour-post-prandial blood sugar estimation. After detailed clinical history if the subjects gave symptoms suggestive of any chronic complications of Diabetes like neuropathy, cardiovascular complications or nephropathy and associated hypertension, they were further subjected to a systematic physical examination of cardiovascular, neurological and respiratory systems and for the presence of severe anaemia, oedema, jaundice, lymphadenopathy and any abdominal masses. Routine investigations like urine analysis, complete haemogram, chest X ray, ECG (in relevant cases) apart from fasting, 2 hour-post-prandial blood sugar, Glycosylated haemoglobin and in a few cases lipid profile were done. Besides this, where it was felt necessary abdominal ultrasound and blood test for HIV were also done. Individuals were excluded from the study in either group if they had received any

Each individual was supplied with a universal container containing 10-ml. of sterile

phosphate buffer saline solution (PBS 0.1 M pH 7.2), and was asked to rinse mouth under the observation of clinician[8]. After rinsing the mouth for 60 sec. thoroughly, subjects were asked to expel the mouth rinses in to a sterile container. Oral rinse was centrifuged and made to 1 ml. Oral rinse was vertex mixed prior to plating.

The sample was taken with 3.26 mm. internal diameter inoculating loop, which holds a drop of sample, inoculated on sabourauds dextrose agar with chloramphenicol. The plates were then incubated at 37^0C for 48 h. The growth of *Candida* was identified by smooth, white or creamy coloured buttery colonies. Confirmation of *Candida* colonies was done by grams stain to observe cells as large gram-positive round or oval cells with budding. All positive cultures for *Candida* were preserved in sabourauds dextrose agar with chloramphenicol, for further identification of the species[9].

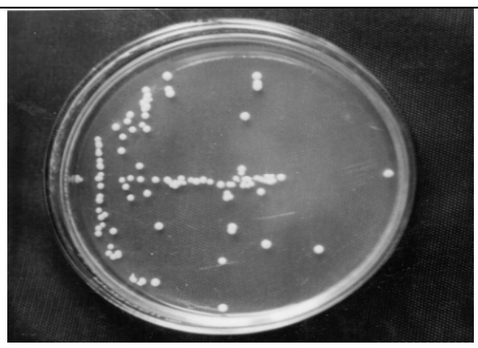


Fig.1.Growth of *Candida* species on sabourauds dextrose agar with chloramphenicol

Colony characters : The candidal isolates grown on sabourauds dextrose agar with chloramphenicol (**Fig.1**) were studied for their morphological characters such as: colony appearance, colony colour, colony shape, colony texture, and production of hyphae and /pseudo hyphae.

Germ tube production: All *Candida* isolates were inoculated in to 0.5 ml. of pooled human sera and incubated at 37^0C for 2 h. At the end of 2 h. of incubation, one or two drops of suspension were placed on a clean glass slide, mounted with a cover slip and examined for the presence of germ tube.(**Fig.3**)

Chlamydospore production; Each *Candida* isolates were picked up with a straight wire and was streaked through deep cutting on corn meal agar plate at an angle and was then covered with a sterile cover slip to produce a relative anaerobic condition. The plates were incubated at 25^0C for 3-5 days. At every 24 hour intervals the plates were examined under microscope for the presence of chlamydospores (large double walled round bodies).

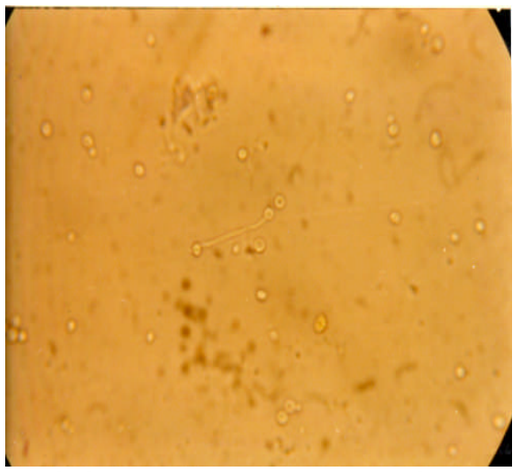


Fig. 2. Candida albicans – producing Germ tube in human serum.

Surface growth;A drop of *Candida* isolate suspension was inoculated in to a test tube containing half filled malt broth. The tubes were incubated at 30^0C for 48 h. and observed for surface growth as pellicle or climbing on the slides of the glass.

Fermentation of sugars;The test candidal isolates was transferred to sugar free nutrient agar slants. After three successive transfers on to the sugar free nutrient slants, growth of *Candida* cultures were then inoculated in to different test tubes containing Andrade's indicator with 2% each sugar (dextrose, lactose, sucrose and maltose) and were incubated at 37^0C. Periodical observation was done to check for the growth, acid production and gas production.

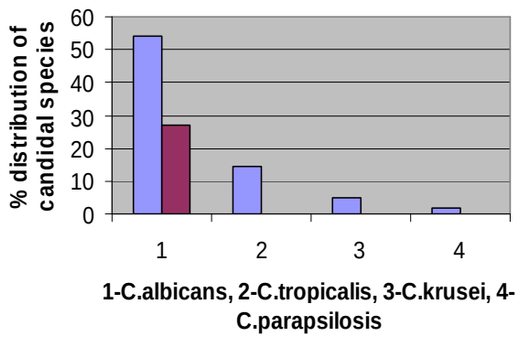
Sugar assimilation (Auxonogram): Basal sugar free medium was prepared, autoclaved and flooded on the plates. Suspension of each different *Candida* isolates (after three successive transfers on to the sugar free nutrient agar) was prepared in sterile distilled water. The turbidity of suspension was standardized (Mc Farland I) and poured on above plates. The excess suspension was removed and the plates were allowed to dry. Different carbohydrates were offered in the form of discs, which have been impregnated with 10% carbohydrate solution. Five discs of each sugar were placed in each plate at five different locations on the plate. The plates were then incubated at 37^0C. for 24 hour. Assimilation of sugars was indicated by a relatively dense growth around each disc.

Results

Twenty-seven (27%) species of *C.albicans* was isolated from 100 non-diabetic subjects whereas 56 (54.36%) species of *C.albicans*, 15 (14.56%) species of *C.tropicalis*, 5 (4.85%) species of *C.krusei* and 2 (1.94%) species of *C.parapsilosis* was isolated from 103 diabetic subjects (**Table. 1 , Fig. 2 and Graph 1**).

Discussion

Candida species are normal inhabitants of the oral flora of many individuals. The asymptomatic carrier state is affected by a number of known factors, including the immune status of host, the strain of *Candida*, the local environment, smoking, prior use of antibiotics, and the general health of the host[1]. Because *Candida* species are normal oral inhabitants, thrush and other forms of oral candidiasis may be classified as specific endogenous infections[10]. A variety of species of *Candida* have been isolated from oral candidal carriers. Candidal species are responsible for most frequently encountered opportunistic oral fungal infections. [2]



Graph 1. Distribution of Candida species in Diabetic and Non Diabetic patients.

Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility. Different species of *Candida* have been shown to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet differences in disease severity and susceptibility to different antifungal agents have been reported[11]. Evidence suggests that some species of *Candida* have a great propensity to cause systemic, nosocomial, and superficial infections than do other species.[12]

Isolation of *Candida* from oral cavity does not imply disease, since its asymptomatic prevalence in healthy persons ranges from 3 to 48%[13]. Belazi maria (2005)[14] studied 128 diabetic patients and noted *C.albicans* was the most frequently isolated species where as Fisher (1987)[15] isolated Candidal species from oral cavity of 210 patients out of 412 diabetic patients. Species of *Candida* isolated was *C.albicans* (89%), *C.tropicalis* (6.2%), *C.krusei* (2.8%), *C.glabrata* (2.8%), *C. stellatoida* (2.8%), *C.parapsilosis* (0.5%). In the present study (Table 1) *C.albicans* (54.36%), *C.tropicalis* (14.56%), *C.krusei* (4.85%) and *C.parapsilosis* (1.94%) were isolated from diabetic subjects whereas only *C.albicans* (27%) was isolated from non-diabetic subjects. Safia (2010)[16] isolated *C.albicans* (68.9%), *C.glabrata* (11.1%), *C.parapsilosis* (6.7%), *C.krusei* (4.4%), *C.tropicalis* (2.2%) and other yeast species (6.7%) from diabetic subjects where as *C.albicans* (40%) was isolated from healthy control subjects. In many epidemiological studies of oral candidiasis the most commonly isolated *Candida* species is *C.albicans*. Odds (1988)[17] reported that other pathogenic members of the genus *Candida* often isolated from the oral environment are (in descending order of virulence) *C. glabrata, C. tropicalis, C. parapsilosis, C. pseudotropicalis, C. krusei* and *C. guilliermondi.* Arunaloke Chakrabarti[7] reported that the apparent emergence of non-albicans of *Candida* species is certainly of interest. Despite such diversity among non-albicans species, it is the general belief that they are of low virulence and the disease manifestations is determined by health of the host. Regina Helena (2006)[18] studied oral colonization of insulin treated diabetes mellitus patients and reported 56% of C.albicans, 39.8% non albicans and other yeast 4.3%. *C.albicans* was prevalent, followed by *C.parapsilosis*, *C.tropicalis*, *C.glabrata*. Antifungal susceptibility of non albicans *Candida* shown resistance in 21.9%, mainly in *C.rugosa* (100%), *C.glabrata* (57%) and *C.krusei* (50%). Different species of Candida isolated from diabetic subjects also showed resistant to antifungal drugs[16]. Earlier reports indicated that *C.tropicalis*, *C.krusei* and *C.parapsilosis* and few other *Candida* species also causes potentially fatal blood stream infection[19]. Hence, it should be noted that any species of *Candida* isolates from diabetic subjects be treated cautiously, because it might act as potential pathogen.

CONCLUSION

C.albicans, *C.tropicali*, *C.krusei* and *C.parapsilosis* were isolated from the oral cavity of diabetic subjects whereas only *C.albicans* was isolated from non-diabetic subjects. Candidal species such as; *C.tropicalis*, *C.krusei* and *C.parapsilosis* may also

Table1: Prevalence of species of *Candida* in diabetic and Non-diabetic subjects.

S.No.	Case Type	Total number of cases	Species of *Candida* isolated	Number of isolates	Percentage of prevalence
1.	**Diabetic subjects**	103	C.albicans	56	54.36
			C.tropicalis	15	14.56
			C.krusei	5	4.85
			C.parapsilosis	2	1.94
2	**Non-diabetic subjects**	100	C.albicans	27	27.00

be responsible to cause oral candidal infection in diabetes mellitus apart from routinely isolated *C.albicans.* Complete identification of species of *Candida* isolates from diabetes mellitus helps in antifungal therapy rather than traditional 'swab and smear' technique.

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