

A COMPARATIVE STUDY OF CARRIER STATE OF CANDIDA AND ITS SPECIATION IN ORAL FLORA – AMONG HEALTHY INDIVIDUALS, PERSONS WITH DM AND HIV SERO POSITIVE INDIVIDUALS

BADANIE PORÓWNAWCZE NOSICIELI CANDIDA – OSÓB ZDROWYCH, CUKRZYKÓW ORAZ HIV-POZYTYWNYCH POD KĄTEM DALSZEGO ROZWOJU ICH FLORY JAMY USTNEJ

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Our Dermatol Online. 2012; 3(2): 102-106

Date of submission: 08.01.2012 / acceptance: 07.02.2012

Conflicts of interest: None

Abstract

The aim was to determine colonization rate of candida in oral cavity of healthy individuals, diabetics and HIV seropositive individuals.

Material and methods: Samples were collected from oral cavity of 50 HIV sero positive individuals, 50 diabetics and 50 healthy individuals by swabbing palatal mucosa, dorsum of tongue and buccal mucosa with a sterile swab. Samples were processed by inoculating on Hi Chrome Agar and speciation was done by growth on Hi Chrome agar, germ tube test, chlamydospore formation on CMA, pellicle formation in SDA broth and growth at 45°C.

Results: 27 HIV sero positive individuals (54%) carried candida in their oral cavities (single strain in 44% and combination of strains in 10%). Whereas it was 44% in diabetics (single species in 38% and a combination of species in 6%) and 24% in healthy individuals (only single species). Candida albicans accounts for 41.66% in healthy individuals, 68% in diabetics and 42.42% in HIV seropositive persons. Other species isolated were Candida tropicalis, Candida parapsilosis, Candida glabrata in all 3 groups in addition to Candida dubliniensis in healthy and HIV seropositive individuals and C. krusei in HIV seropositive persons only. P value- less than 0.05 between healthy persons & diabetics and between healthy persons & HIV seropositives (significant).

Conclusion: candidal carriage was higher in diabetics and HIV seropositive individuals. Candida albicans was the commonest species in all three groups. Candida krusei was seen only in HIV positive persons.

Streszczenie

Celem badania było określenie stopnia kolonizacji grzybem Candida jamy ustnej u osób zdrowych, cukrzyków oraz HIV- pozytywnych.

Materiały i metody: Próbkę z jamy ustnej pobrano za pomocą sterylnej wymazówki z błony śluzowej: podniebienia, grzbietu języka oraz policzka u 50 HIV- seropozytywnych osób, 50 – cukrzyków oraz 50 zdrowych osób. Próbkę były hodowane na agarze Hi Chrome i dalsza specjacja odbywała się na tymże agarze, w teście filamentacji oraz za pomocą kształtowania się chlamydosporów na CMA, tworzenia się błony w obrębie bulionu i wzrostu w temperaturze 45°C.

Wyniki: 27 HIV- pozytywnych osobników (54%) nosiło w sobie Candida (44% pojedynczy szczep, a kombinację kilku 10%). Podczas gdy w populacji cukrzyków odsetki wynosiły odpowiednio 44% (38% pojedynczy szczep i 6% kombinację kilku) a u zdrowych osób 24% (tylko pojedyncze odmiany). Candida albicans jest odpowiedzialna za 41,66% kolonizacji u zdrowych osób, 68% u osób chorych na cukrzycę i 42,42% u HIV-pozytywnych. Innymi wyizolowanymi szczepami były Candida tropicalis, Candida parapsilosis, Candida glabrata obecne we wszystkich 3 badanych grupach, a dodatkowo w grupach zdrowych i HIV-pozytywnych Candida dubliniensis podczas gdy Candida krusei tylko w grupie osób HIV-pozytywnych. Współczynnik $P < 0,05$ pomiędzy grupą osób zdrowych a cukrzyków oraz osób zdrowych i HIV-pozytywnych wskazuje na istotność korelacji.

Wnioski: Nosicielstwo Candida było wyższe u cukrzyków i osób HIV-pozytywnych. Candida albicans była najpowszechniej występującą odmianą we wszystkich przebadanych grupach. Candida krusei występowała jedynie u osób HIV-pozytywnych.

Key words: Candida albicans; colonization; opportunistic infections; Type 2 DM; HIV infection

Słowa kluczowe: Candida albicans; kolonizacja; infekcja oportunistyczna; Typ 2 DM; infekcja HIV

Introduction

Candida species and *Candida albicans* in particular are ubiquitous dimorphic fungal organisms that are part of normal microflora of healthy individuals [1]. They are commonly found on the skin, throughout GIT and female genital tract [2]. However, they are also opportunistic pathogens that can quickly transform from harmless mucosal commensals to a highly pathogenic organism of the same tissue with significant mortality and morbidity under appropriate conditions [1]. Variations regarding the presence of *Candida spp.* in healthy individuals may be a function of various factors such as climate, age and diet of surveyed population [1]. The factors predispose people to candidiasis include AIDS, burns, pregnancy, high fruit diet, steroids, antibiotic treatment, immunosuppressants, cancer treatment, heart surgery, diabetes mellitus and use of catheter [2]. The frequent occurrence of candida infections in patients with DM has been recognized for many years and oral candidiasis in particular is thought to be more prevalent among these individuals [3-11]. With introduction of antifungal agents, the cause of candida infection shifted from *Candida albicans* to *Candida glabrata* and other non albicans species, as *Candida glabrata* and *Candida krusei* develop resistance to fluconazole [1,5]. As normal flora is the source of many opportunistic infections and candida species are important causes of severe invasive disease in immunocompromised persons [12]. We made an attempt to know the carriage rate of candida in healthy individuals, diabetics and HIV seropositive persons in our area.

Materials and Methods

Persons who were attended to diabetic clinic, ICTC and medical OP were included in the study. The study was done between January 2011 and March 2011.

Exclusion criteria:

1. Individuals wearing dentures.
2. Individuals with oropharyngeal candidiasis.
3. Those on antibiotic treatment, steroid treatment and antifungal treatment and those using antiseptic mouth wash.

Inclusion criteria:

1. Type 2 diabetics who are on oral anti diabetic drugs.
2. HIV positive individuals on ART.

HIV status was determined by doing three tests using three different antigen kits as per NACO (National AIDS Control Organization) guidelines. CD4 counts of the HIV seropositive individuals were done by FACS counter. Blood sugar level was determined by using glucose oxidase-peroxidase method. Samples were collected from 50 HIV seropositive individuals (non diabetics), 50 diabetics (HIV seronegative) and 50 healthy individuals (males between 20-40 years age group in all three categories) after obtaining written consent. Sample was collected by swabbing palatal mucosa, dorsum of tongue and buccal mucosa. Swabs were inoculated on HiChrome agar Candida medium immediately and incubated at 22-26°C in BOD. Inoculated media were examined daily for seven days. Gram's staining was done to all the isolates with

mucoid and yeast like growth and observed for gram positive oval budding yeast cells 4-6 microns. Germ tube test: All candida isolates were tested for germ tube formation. A colony was inoculated in human serum and incubated at 37°C. After 2-4 hrs. wet mount was prepared and observed for germ tubes. Chlamydospore formation: All candida isolates were tested for production of chlamydospores on corn meal agar. After inoculation and incubation at 25°C the plates were examined under low power objective of microscope for the presence of chlamydospores (Fig.1).

Growth was identified by Gram's staining and speciation was done by observing the colour of the growth on HiChrome agar and confirmed by germ tube test, chlamydospore formation on CMA, pellicle on SDA broth and growth at 45°C as shown below.

Table II showing properties of candida species [13].



Figure 1. Chlamydospore formation on CMA

Results

Among 50 healthy group, 12 persons carried candida in their oral cavity with *Candida albicans* (Fig.2) as the most common (41.66%) followed by *Candida tropicalis* (Fig.3,5), *Candida glabrata* (Fig.4), *Candida parapsilosis* (each one in 16.66%) and *Candida dubliniensis* (8.33%) (Fig.5).

In diabetics out of 50, 22 persons carried candida in their oral cavity (44%). 19 persons with single species and 3 persons with combination of two species (6%). Most common species was *Candida albicans* in 68% followed by *Candida glabrata* in 16%, *Candida parapsilosis* and *Candida tropicalis* in 8% cases.

Out of 50 HIV seropositive individuals, 27 carried candida. Single species was found in 22 persons and combination of two or more species in 5 persons (10%). *Candida albicans* in 42.42%, *Candida tropicalis* and *Candida parapsilosis* in 21.21%, *Candida dubliniensis* in 9.09%, *Candida glabrata* and *Candida krusei* in 3.03% cases (Tabl. II).

P value between healthy persons and HIV seropositives is less than 0.05% (significant). P value between healthy and diabetics is less than 0.05% (significant).

Study group	No. tested	No. + ve	Singles + combination	Species
Healthy	50	12 (24%)	Only single species	<i>C.albicans</i> (41.66%) <i>C.tropicalis</i> (16.66%) <i>C.glabrata</i> (16.66%) <i>C.parapsilosis</i> (16.66%) <i>C.dubliniensis</i> (8.33%)
Diabetics	50	22(44%)	19(38%) +3(6%)	<i>C.albicans</i> (68%) <i>C.tropicalis</i> (8%) <i>C.glabrata</i> (16%) <i>C.parapsilosis</i> (8%)
HIV seropositive	50	27(54%)	22(44%)+5(10%)	<i>C.albicans</i> (42.42%) <i>C.tropicalis</i> (21.21%) <i>C.glabrata</i> (3.03%) <i>C.parapsilosis</i> (21.21%) <i>C.dubliniensis</i> (9.09%) <i>C.krusei</i> (3.03%)

Table I. Percentage & species of Candida in study group

Species	Color on HiChrome agar	Germ tube test	Chlamydo spores on CMA	Pellicle in SDA broth	Growth at 450°C
<i>C.albicans</i>	Light green	+	+	no	+
<i>C.tropicalis</i>	Purple halo in agar, dark blue color	-	-	small	
<i>C.parapsilosis</i>	Pale color	-	Pineforest appearence	NA	-
<i>C.glabrata</i>	Dark pink	-	-	NA	-
<i>C.dubliniensis</i>	Dark green	++	++	NA	-
<i>C.krusei</i>	Pale pink centre with white edge, rough, spreading colony	-	-	Thick pellicle	-

Table II. Properties of Candida species [12]



Figure 2. Colonies of Candida albicans on HiChrome agar



Figure 3. Colonies of Candida tropicalis on HiChrome agar



Figure 4. Colonies of Candida glabrata on HiChrome agar



Figure 5. Colonies of Candida dubliniensis and Candida tropicalis on HiChrome agar

Discussion

Candida species colonize mucosal surfaces of human beings during or soon after birth and risk of endogenous infection is ever present [13-22]. Patients with compromised host defenses are susceptible to ubiquitous fungi to which healthy people are exposed but usually resistant. As members of normal microbial flora candida and related yeasts are endogenous opportunistic organisms [22]. The carriage rate of candida in oral cavity was different in various studies. This could be due to different methods of sampling. The carriage rate of Candida in oral cavity of diabetic subjects is claimed to be higher. Candidal density also be reported higher in diabetics than in non diabetics [3]. Candida is one of the most common opportunistic fungi in HIV/AIDS cases [9]. Infections with *Candida albicans* appear when CD4 is below 500-200/cumm and may be the first indication of immunodeficiency [5-7]. Today's concern about candidiasis is emergence of fluconazole resistant *Candida albicans* in AIDS patients with recurrent attacks of oral thrush and less susceptibility of *Candida krusei* and *Candida glabrata* to fluconazole [13-15].

As diabetics and HIV seropositive individuals are vulnerable to develop opportunistic infections because of high glucose levels in tissues in diabetics and decreased immunity in HIV seropositives, it is necessary to know carriage rates of candida in oral cavities. Moreover reviews have shown that candidal esophagitis may occur frequently without thrush [22]. So by studying the prevalence of colonization of oropharynx among HIV individuals, we can assess the risk of esophageal candidiasis.

Comparative studies in oral carriage rate of Candida between healthy and diabetics and between healthy individuals and HIV seropositive persons are available. But comparative studies between healthy individuals, diabetics and HIV seropositive individuals are rare. To the best of our knowledge our study is the first of that kind from our geographical area. In the present study age matched males of healthy, diabetics and HIV seropositives – all 50 in number, were taken. 42% of healthy persons, 44% of diabetics and 54% HIV seropositives were shown to carry Candida in their oral cavities. *Candida albicans* was the most common species in all groups. *Candida dubliniensis* was seen among healthy and HIV sero positives, but not present in diabetics. Only single species were isolated in healthy persons whereas more than one candida species were found in diabetics (6%) and in HIV seropositive persons (10%). *Candida krusei* was present only in HIV seropositive persons.

Jianping XU and Thomas G Mitchell tried to compare rate of commensalism of candida in oral cavity of Asians (Chinese) and North Americans from Canada and USA. 66.94% of Chinese and 39.5% of North Americans carried candida in their oral mucosa. *Candida albicans* was the predominant species in North Americans and *Candida parapsilosis* and *Candida guilliermondii* were the commonest in Chinese [23]. Eun Seop Shin et al found that oral carriage was 45% in healthy individuals [19]. Zeng X et al found 20.31% carriage rate in healthy individuals from China [24].

Margerida Martins et al from Portugal isolated candida from 54.6% from a dental clinic [6]. Present study findings correlated with Zeng X et al in the oral carriage rate of candida in healthy individuals with 24%. Whereas carriage was more in other studies.

Carriage rate of 68.52% in type 2 diabetics, 83.67% in type 1 diabetics and 27% in healthy individuals was found in a study by Kumar BV et al from North India [3]. Fisher BM et al reported single candida species in 51% and more than one species in 6% of diabetics and *Candida albicans* as the commonest (89%) [20]. It was 36% in type 2 diabetics (Chinese) and 23.80% in healthy persons according to Tsang CSP et al [18].

Safia A. AL-Attas and Soliman O. Amro from Jeddah, Saudi Arabia observed 33.3% carriage rate in diabetics with *Candida albicans* in 68.9% and 14.3% in healthy individuals with *Candida albicans* in 40% [10].

Carriage rate in diabetics in our study (44%) was a little higher than in some studies [3-10,18-23]. Combination of *Candida species* in our study was 6% as in other studies [18-20].

Candida albicans was commonest in the present study (68%) like other studies [3-10,20-23]. Our results, in oral carriage rate of candida in healthy individuals were in agreement with other studies [3,18-23].

In a study from India by Gugnani HC et al the oropharyngeal carriage of *Candida species* in HIV infected patients was 65.3% for *Candida albicans* and 2.7% for other species including one case of *Candida dubliniensis* [21], which was also isolated in the present study. Arati Mane et al from India found 58.7% carriage rate in HIV positive persons and 22.4% in healthy individuals [14]. Pavithra A Jain from Karnataka, India found 68% of HIV positive persons and 40% of healthy persons carried candida in their oral cavities in one study [8]. 53.7% and 33.07% in another study [9]. All three studies were from India.

Carriage rate in HIV positive and healthy persons 75% and 68% in a study from South Africa by Catherine Hester Johanna with *Candida albicans* in 56% [17] and 28.6% and 18% in another study from China by Liu X et al [4]. Rodrigues Costa et al found 62.6% of HIV positives carried candida in their oral cavities with *Candida albicans* in 50% in a study from Brazil [16]. Where it was 57% in HIV seropositives with *Candida albicans* in 44.4% and 24% in healthy persons with *Candida albicans* in 41.66% in the present study. Carriage rate of candida in HIV seopositives in the present study correlates with Arati Mane study where as it was a little higher in other studies. But *Candida albicans* was the commonest species in all studies.

Conclusions

1. Carriage rate was high in HIV seropositive individuals (54%) when compared to other two groups, but in comparison with healthy persons, it was found high in diabetics.
2. *Candida albicans* was the predominate species in all three groups, but more so in diabetics, needs further study.
3. Other common species isolated were *Candida tropicalis*, *Candida glabrata*, *Candida tropicalis*.
4. *Candida dubliniensis* was recovered from healthy persons and HIV seropositive individuals and *Candida krusei* was from HIV seropositive individuals only.
5. Combination of species was found in diabetics (6%) and in HIV seropositive individuals but not.
6. We did not find a significant correlation between oral carriage of *Candida species* and CD4 counts above 200, as well as blood glucose levels.

Though oral carriage rates in different studies, were highly variable, but higher carriage were observed in diabetics and HIV seropositive individuals consistently in all studies. By that preventive measures like improvement in general health and oral hygiene discriminate use of antibiotics can be taken to reduce morbidity.

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