Prevalence of Oral Candida Infections in Diabetic Patients

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Objective: The purpose of this study was to determine the prevalence, species distribution and antifungal susceptibility profile among oral cavity isolates of *Candida* species from diabetic and non-diabetic subjects. The contribution of smoking and dental status to the prevalence and distribution of *Candida* species was also evaluated.

Design: Retrospective study of oral cadidiasis in diabetic patients between January and October 2003 was undertaken.

Setting: Three private clinics in Amman, Jordan and Department of Biological Sciences at Hashemite University.

Method: A total of 262 individuals were enrolled in the study, 132 were diabetics and 130 healthy controls. None of the non-diabetic controls had any clinical evidence of oral candidiasis, 8.3% of diabetics had clinical evidence of oral candidiasis, of which, 36% were overnight denture wearers and tobacco smokers. An imprint culture method was used to determine the frequency of isolation and density of *Candida* species at up to nine intra-oral sites. Yeast-like colonies were identified by classical methods and CHROMagar *Candida* medium. Broth macrodilution technique was used to determine the antifungal susceptibility pattern of *Candida* isolates.

Results: Positive yeast was detected in 58.3% of diabetics compared with 30% in healthy controls (*P<0.001*). *C. albicans* was the most prevalent species in both diabetics (81.8%) and controls (76.9%) followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. *C. kefyr* and *C. krusei* were isolated only from diabetics at a combined rate of 1.3%. *Candida* was detected more frequently in diabetic denture wearers than in control counterparts in all anatomic sampled sites (*P<0.05*). The frequency of *Candida* isolation was significantly higher in smokers than in the non-smokers in both diabetics and controls (*P<0.001*). All *C. albicans* recovered from diabetics and controls were susceptible to amphotericin B, ketoconazole, itraconazole and fluconazole. Non-*albicans Candida* isolates were shown to have higher azole MIC values than *C. albicans* isolates.

Conclusions: Our findings show that smoking and continuously worn dentures, promote oral candidal colonization in diabetics.

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Oral candidiasis is an opportunistic infection of the oral cavity; it affects various sectors of the world population irrespective of age or health status. Close to 90% of AIDS patients suffer from oropharyngeal or esophageal candidiasis at some stage of the disease[1,2]. In the general population, carriage rates have been reported to range from 20 to 75% without any symptoms[3]. The incidence of *Candida albicans* isolated from the oral cavity has been reported to be 50 to 65% of people who wear removable dentures, 90 to 95% of patients with acute leukaemia undergoing chemotherapy and patients receiving radiation therapy for head and neck cancer[3,4]. It is well established that diabetes mellitus is a predisposing factor to fungal infections, especially those caused by *Candida* species [2]. Several studies have reported that the prevalence of yeast carriage among patients with diabetes could reach up to 54% and that *C. albicans* could account for 25- 69% of the isolates[5-7]. Oral colonization with *Candida* species occurs more frequently in diabetic patients compared with non-diabetic individuals[6]. In some studies, the oral carriage rate of *Candida* has been estimated at around 80%[8]. Several studies have also reported that vulvovaginal candidiasis occurs more frequently in diabetics [9-11].

Studies of oral fungal flora and the prevalence of oral fungal infection in diabetic and non-diabetic Jordanian subjects was previously reported[7]. These studies have indicated that prevalence of *Candida* was significantly higher in diabetics both in healthy controls and complete denture wearers compared to non-diabetics. Moreover, the mean candidal density was higher amongst diabetic denture wearers than non-diabetics[7]. It is worth noting that the findings, which were in the previously reported, were based on a diabetes prevalence rate among Jordanians of around 13.4%, recent epidemiologic data have put diabetes prevalence rates in Jordan at around 32%[12,13]. Therefore, a new survey of the prevalence, species distribution and antifungal susceptibility profile among *Candida* species isolates from the oral cavity of diabetics and healthy controls is warranted. The scope of the study was extended to include the frequency of isolation of *C. albicans* and its intra-oral distribution as well as the contribution of smoking and dental status to candidal prevalence.

METHODS

Patients

The study included 262 Jordanian subjects; 132 diabetics who were attending three private clinics in Amman between January and October of 2003. All patients were receiving treatment for their diabetes mellitus. The control group comprised of 130 healthy volunteers matched for age, sex, dental status, and smoking habits. Patients and controls were examined for signs or symptoms of oral candidiasis. Only those who have not been on antibiotic or corticosteroid therapy during the previous 4 weeks were included in the study. All participants were asked to sign a consent form, on the understanding that collected data will be used for noncommercial research purposes and that names will be kept confidential. All participants were also asked to complete a questionnaire addressing the socio-economic status (age, marital status, and occupation), dental status, smoking habits, and duration of diabetes mellitus. Detailed information pertinent to the demographic and health status data of the sample population is outlined in table 1.

Table 1

Collection of Biological Samples

An imprint culture technique was used to determine the frequency of isolation and density of *Candida* species at up to nine intraoral sites[7, 14]. Briefly, sterile plastic foam pads (2x2 cm) were dipped in Sabouraud`s broth (Difco, Detroit, MI, USA) and placed on the specified anatomic site for 30 seconds. The pad was then placed on Sabouraud`s dextrose agar (SDA) containing 50 mg/L chloramphenicol plate for one hour. The foam pad was then removed and the plate was incubated at 37° C for 48 hours. Candidal density at each site was determined and expressed per unit area. Yeast isolates were grown on SDA slants and stored at 4°C for further testing.

Identification of Candida species

Growth of yeast-like colonies were identified by classical methods using the following tests germ-tube test, hyphae/ pseudohyphae and chlamydospores growth, carbohydrate fermentation and assimilation and urea hydrolysis, and by subculture of 2-3 representative colonies on CHROMagar *Candida* medium (CHROMagar, Paris, France) and incubation at 35°C for 24-48 hours[7,15,16]. *Candida* isolates were identified by detection of various color characteristics on CHROMagar *Candida* plates, and confirmed by using the API 20C *Candida* identification system (Bio-Merieux, Marcy I'Etoile, France). Reference strains of *C. albicans* (ATCC 36082), *C. glabrata* (ATCC 22553), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258), were kindly provided by Dr. M.A.Ghannoum (Center for Medical Mycology, Mycology Reference Laboratory, University Hospital of Cleveland, Ohio, USA), were used as controls.

In vitro Antifungal Susceptibility Testing

Antifungal susceptibility of *Candida* isolates was tested by the broth macrodilution technique with endpoints read at 48 hours in accordance with the standards set by the National Committee for Clinical Laboratory Standards (NCCLS)[17]. Antifungal agents including amphotericin, itraconazole, Ketoconazole and fluconazole were used -as commercially supplied- to prepare stock solutions of 1.25mg/ml final concentration. Minimum inhibitory concentration (MIC) was determined in RPMI medium, pH 7.0 (Sigma Chemical Co., St. Louis, Mo., USA). 3-(N-Morpholinol) propanesulfonic acid (MOPS) (0.165 M, Sigma) and additional glucose (18 g/l) were included in the medium. Using the same media, broth microdilution MIC determinations were performed in microtiter plates, which were inoculated with $0.5x10^3$/ml of *Candida* isolates and incubated at 30ºC. Cell density per plate was measured after 48 hours of incubation. MIC was defined as the lowest concentration of antifungal agent that inhibits growth; MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of *Candida* isolate was inhibited were also determined.

Statistical analysis

Data analysis were carried out by means of one–way analysis of variance (ANOVA) and by multiple comparison using computer statistical analysis software (STATISTICA for Windows (1995), Stat Soft Inc, OK, USA), differences were considered as statistically significant at $P \leq 0.05$.

RESULTS

Clinical evidence of oral candidal infection was seen in 11 (8.3%) diabetic patients, 4 of which were overnight denture wearers and tobacco smokers. None of the controls had any clinical evidence of oral *Candida* infections. A statistically significant difference ($P< 0.001$) was detected

between diabetics and healthy controls in terms of positive yeast culture in that 77 diabetic subjects (58.3%) showed positive yeast compared with 39 (30%) of the healthy controls.

As shown in table 2, *C. albicans* was the most prevalent species in both diabetics (81.8%) and controls (76.9%), followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. Of interest was the isolation of 1.3% of *C. kefyr* and *C. krusei* only from diabetics (Table 2). As shown in figure 1, *Candida* was detected more frequently in diabetic denture wearers patients than in the control denture wearers in all sites which were sampled ($p<0.05$). Floor of the mouth, anterior palate and posterior tongue were the most frequently colonized oral sites. Figure 2 shows that the prevalence of *Candida* was significantly higher in diabetics both in denture wearers and dentate patients compared to healthy controls ($p<0.05$). The frequency of *Candida* isolation was significantly higher in smokers than in the non-smokers both in diabetic and controls ($p< 0.001$) (Figure 2).

Table 2
Figure 1 & 2

All *C. albicans* recovered from diabetic patients and control group were susceptible to the four antifungal drugs tested. *C. albicans* isolates exhibited low antifungal MIC_{90} values: 0.5, 0.06, 0.125 and 0.5 µg/ml to amphotericin B, ketoconazole, itraconazole and fluconazole, respectively (Table 3). Non-*albicans Candida* isolates had higher azole MIC values than *C. albicans* isolates (Table 4). Two isolates identified as *C. tropicalis* and *C. krusei* were resistant to fluconazole (MIC = 64 µg /ml). One *C. tropicalis* isolate was resistant to ketoconazole (MIC = 4 µg /ml) (Table 4). Of interest, resistant isolates of *C. tropicalis* and *C. krusei* were isolated from diabetic patients.

Table 3 & 4

DISCUSSION

Diabetes is rapidly becoming a major public health problem worldwide[12]. The prevalence of oral *Candida* infections among Jordanian patients with diabetes mellitus in the current study is consistent with numerous previous studies, which have shown that diabetes mellitus is a major predisposing factor to symptomatic candidosis, oral or otherwise[5-7,18]. This is also in agreement with numerous previous studies, which have all indicated that diabetes mellitus enhances *Candida* colonization and proliferation[6,7,19-21]. Tapper-Jones *et al*. have shown that 42% of healthy non-diabetics harbor *C. albicans* in their mouths compared to 60% of diabetics[6]. Yarahmadi *et al* have suggested that 16.2% of the controls and 40.2% of the diabetics carry *C. albicans* in the mouth[22]. The threshold of sensitivity has been found to be lower for buccal swabbing than for imprint or saliva collection, therefore, the overall percentage of individuals carrying yeast isolates in the oral cavity may be slightly lower than what is reported in the present study[20]. The mechanisms by which this could occur are numerous, for example, the induction of immune incompetence, the availability of increased levels of sugar in the oral microenvironment and the method used to obtain samples and the site sampled within the oral cavity.

In addition to diabetes mellitus, the prevalence of oral *Candida* infections is influenced primarily by smoking[7, 23-25]. It is also clear from the findings presented in this study that dentate individuals whether diabetics or not are less prone to *C. albicans* colonization. Despite several previous studies, which have looked into this issue more closely, the exact mechanism by which this significant difference between dentate and denture wearers cannot be readily explained[23-25]. It is generally assumed that elderly individuals have higher yeast carriage rate, although, this may be more due to denture wear and increased medication than due to changes in host physiology.

In comparison with previous studies conducted in Jordan, the resistance against these conventional antifungal agents seems to be on the rise[15,26]. Furthermore, in comparison with studies conducted in Lebanon, United States or the United Kingdom, there seems to be a higher rates or resistance to conventional antifungal agents in Jordan[27-29]. This is alarming because the length of time such antifungal agents have been in use in Jordan is significantly lower than that in the US or the UK. It is clear that the overuse/misuse dilemma is rampant. Perturbations in the oral microenvironment sufficient to create niches compatible with the growth of new *Candida* species like the ones listed above might be one of the terrible consequences of current medical practice in the country[15,26].

CONCLUSION

It is clear that diabetics are more susceptible to oral candidiasis than non-diabetics. Furthermore, smoking and denture wears are at high risk of being infected. *C. albicans* is by for the most prevalent among all *Candida* spp. as the cause of oral candidiasis in Jordan.

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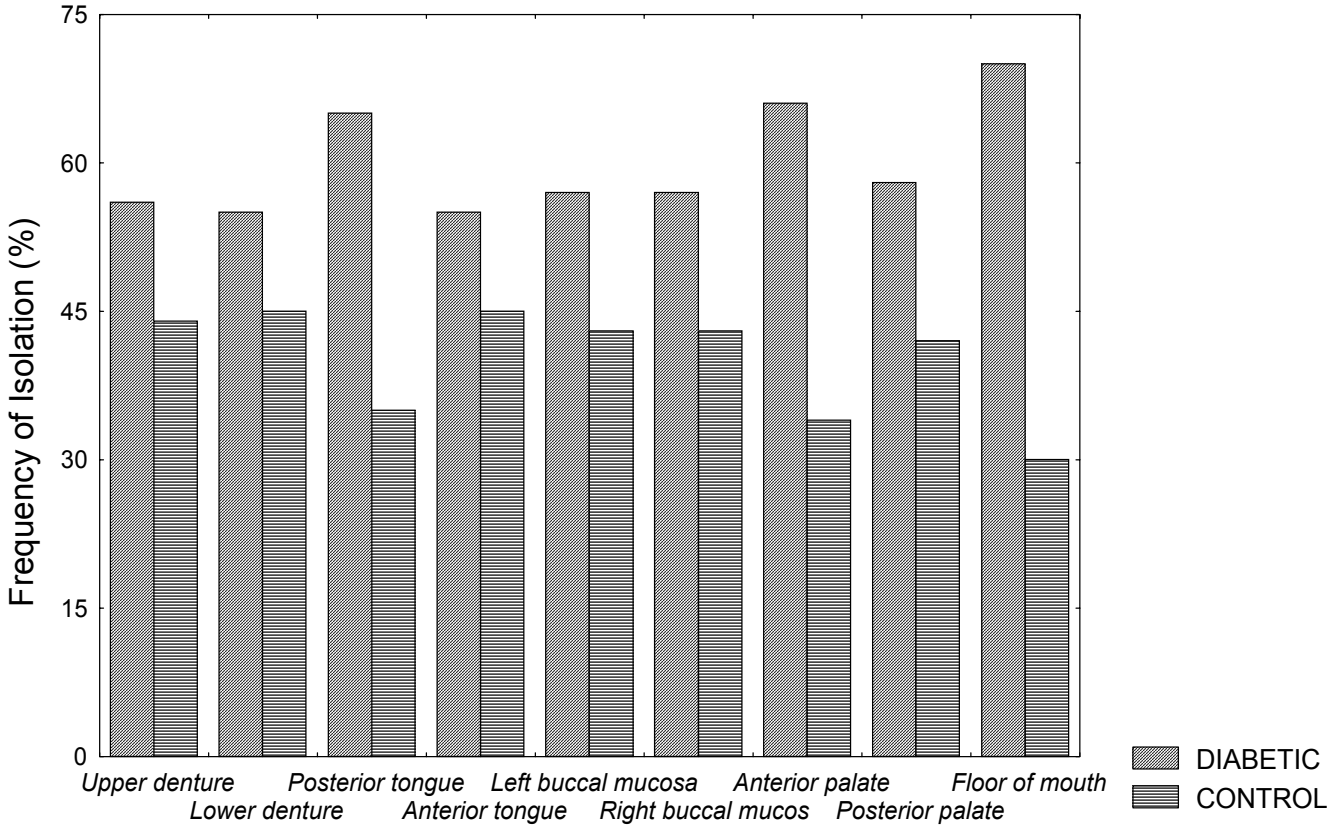

Figure 1. Frequency of isolation of Candida albicans at various sites in diabetic and control patients.

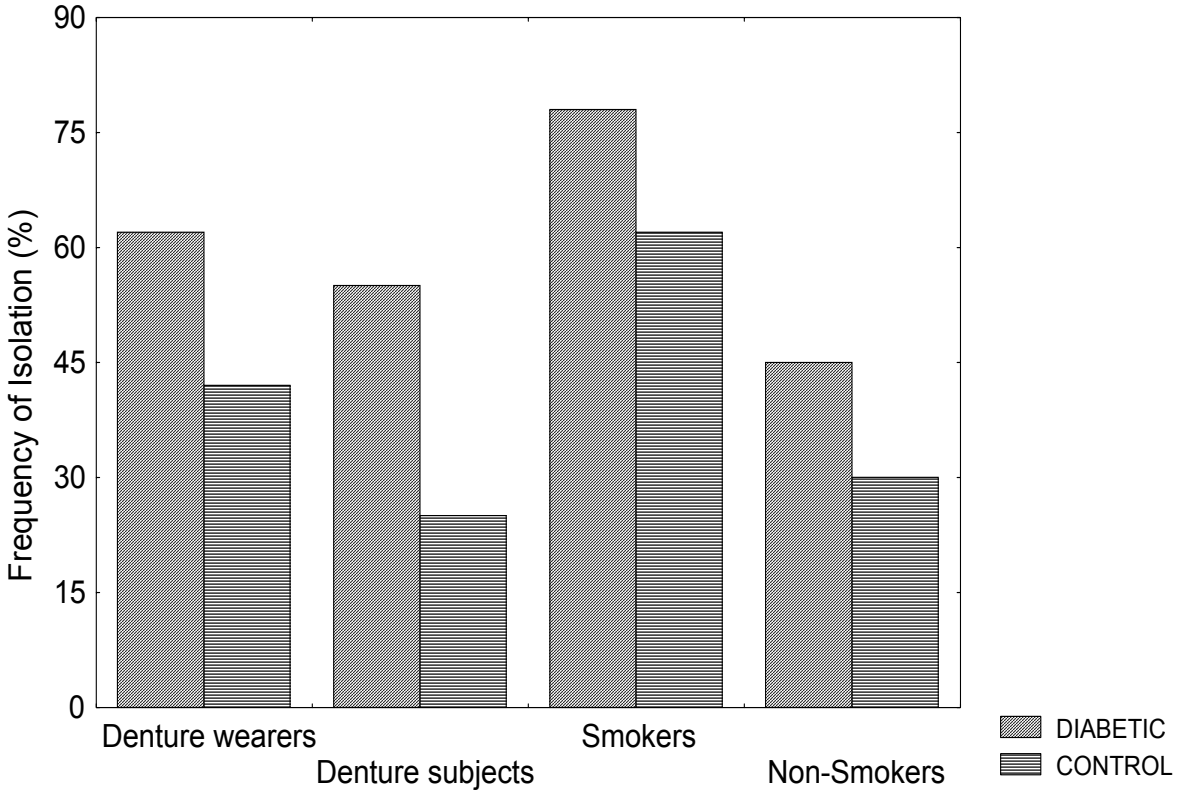

Figure 2. Frequency of isolation of Candida albicans in relation to denture wearing and smoking in diabetic and control patients.