Presence of Different Candida Species at Denture Wearers With Type 2 Diabetes and Clinically Healthy Oral Mucosa-Pilot Study

SUMMARY

Background/Aim: *The aim of this study was to examine prevalence of different Candida spp. at diabetics and nondiabetics wearing dentures without clinical signs of Denture Stomatitis (DS) and to study if some local and systematic factors are confounders for harboring Candida at these subjects.* **Material and Methods:** *Total of 60 subjects wearing partial or complete upper acrylic denture having at least half of palatal mucosa covered by denture were selected and stratified into three experimental groups: systematically health subjects; patients with diagnosed Type 2 Diabetes (T2D) and good glycoregulation; and T2D subjects with poorly regulated blood sugar level. Cotton swab samples were obtained from each patient from hard palate mucosa and denture surface. Swab cultures were made on Sabouraud dextrose agar and ChromAgar Media for distinciton of various Candida spp. Density growth was also measured.* **Results:** *Frequency of Candida spp. findings were similar between groups. At healthy subjects, only C.albicans was detected. At diabetics, C.albicans was the most common isolated species, followed by C.glabrata and C.tropicalis. Negative finding of yeasts on palatal mucosa, but positive on denture surface were detected at all groups, with the highest frequency (33.4%) at diabetics with poor glycoregulation. Denture surface was heavier colonized than hard palate mucosa. Duration of diabetes in years were only independent predictors for harboring Candida spp. at denture surface (Exp B=1.186, CI=1.047-1.344, p=0.007).* **Conclusions:** *Prosthesis of denture wearers without DS may serve as reservoir of Candida spp. Presence of more pathogenic and resistant non-albicans species are related to diabetics, even without clinical signs of DS.*

Key words: Stomatitis, Denture; Candida albicans; Diabetes Mellitus Type 2; Denture, Complete; Denture, Partial, Removable

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Introduction

Denture stomatitis (DS) is an inflammatory process of the mucosa located under partial or complete removable dentures. Prevalence of DS at denture wearers varies among studies from 14.3% up to 72% depending on study population[1,2]. This condition is often asymptomatic and of multifactorial ethiology. Systematic illness (diabetes mellitus)[3,4], vitamin A deficiency and cigarette smoking[5], gender (females) and age[6], are thought to be some of the systematic factors related to DS. Diabetes mellitus is considered to be the most important systematic factor related to Candida infection. Although precise mechanisms responsible for higher candida carriage/infections, as well as higher pathogenicity of yeasts at diabetic subjects are not defined, high salivary glucose level, low pH and low salivary flow rate are presumed to be some of them[7]. Also, accumulation of glycated proteins in epithelial oral cells may facilitate adhesion of yeasts to these cells, which is considered as the essential first step for candida

colonization[8]. Mentioned mechanisms, together with decreased phagocyte functions, may somewise explain influence of diabetes on *Candida* carriage/infections. Local factors are predominant in etiology of DS, and the commonest are amount of tissue covered with denture and constant denture wearing[5], poor denture hygiene[9], nocturnal dental wearing[6,10,11] low salivary/palatal mucosa pH[3,6,11], hyposalivation[6], and microorganisms. Among different microbes involved in pathogenesis of DS, *Candida* spp. are thought to be the most frequently isolated microorganism and also the commonest etiological factor responsible for DS[3, 12-14]. On the other hand, presence of *Candida* on palatal mucosa and/or denture surface of prosthesis wearers do not always mean DS[3,5,6,15]. Also, cofactors for DS are not always same as for presence of *Candida* spp.[6]. Although DS or/and *Candida* presence are often asymptomatic, prothesis may be a portal of entry for further infections, especially at immunocompromised subjects. According these facts, it is important to study prevalence and diversity of *Candida* spp. at denture wearers, and to explore potential cofactors facilitating colonisation of yeasts at palatal mucosa and dentures.

The aim of this study was to examine prevalence and variety of *Candida* spp. at diabetics and nondiabetics wearing partial/complete acrylic dentures without clinical signs of DS. Also, we studied if some local and systematic factors are risk factors for harboring *Candida* spp. at these subjects.

Material and Methods

This case-control observational study was approved by Ethical Committee of the School of Dental Medicine, University of Belgrade (Ethics Approval no. 36/8, 20th February 2013). Total of 60 subjects wearing partial or complete upper acrylic denture having at least half of palatal mucosa covered by denture were selected and stratified into three experimental groups:

- Group A: 14 patients free of systematic diseases, referred to Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade.
- Group B: 16 patients with T2D with satisfactory metabolic control (HbA1c≤ 7.5%). T2D was diagnosed by measuring glycaemia using oral glucose tolerance test (OGTT), as well as glycated hemoglobin (HbA$_1$c) values[1].
- Group C: 30 patients with diagnoses T2D and with poor metabolic control (HbA1c>7.5%). Diabetic patients (B and C groups) were referred to the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia as well as Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade.

Informed consent was signed by every subject participated in the study.

Inclusion and exclusion criteria

The subjects included in the study wore upper denture more than a year, did not made modifications of denture in this period, and lacked any of the following criteria: presence of any disease except T2D and its chronic complication, aggressive periodontitis, presence of diseases or oral mucosa (Lichen planus etc.), clinical visible erythema of palatal mucosa, usage of medication that might affect presence of *Candida* spp., e.g. corticosteroids, antibiotics, antiseptics, usage of antiseptics for denture hygiene, pregnancy, lactation and periodontal treatment in the last 1.5 year.

Anamnesis of the patients, clinical and biochemical examinations

Anamnestic data

Anamnestic data were done by a mean of medical questionnaire which included information about parameters that could potentially modify or predict clinical parameters of T2D or presence of *Candida* spp., but which could not be defined as exclusion criteria. This included identification of demographic and social data (gender, age, educational level), medical history (presence of T2D complications, xerostomia, glossodyniae and glossopyrosis, blood type (O vs. A+B+AB blood type[16]), ill-fitting denture, number of years wearing denture, and habits (smoking habits defined as "smokers" and "non-smokers", alcohol consumption, everyday intake of carbohydrates, nocturnal wearing of denture, continuous wearing of dentures, hygiene habits for dentures- usage of tooth brush, tooth paste). Patients who ceased smoking in period 6 months prior to study were not included in the study.

Clinical examination

Clinical examination included examination of oral mucosa and denture. Examination of oral mucosa were done by two trained doctors, according to WHO criteria[17]. Lips, buccal mucosa, tongue, sublingual area, hard and soft palate were examined using appropriate dental light and dental mirror. Clinical variations of mucosa of tongue (fissured tongue, glossitis migrans etc.) were not exclusion criteria. Rhomboid glossitis and angular cheilitis, as common pathologic changes associated with DS, were exclusion criteria. Subjects with atrophic mucosa of dorsum of tongue or any clinical signs of candidiosis were excluded from the study. Present teeth were also examined at six sites per tooth (distobuccal, med-buccal, mesiobuccal, distolingual, med-lingual and mesiolingual) by two experienced and calibrated doctors, one performing the clinical measurements by Williams' probe calibrated in millimeters (Hu-Friedy Chicago, IL) and another recording the results. Following periodontal

parameters were assed: plaque index-Silness Loe (PI), dichotomous bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL).

Dentures were also directly examined in means of: type of dentures, retention and stability, presence of fractures, modifications, roughness or tooth abrasion.

Biochemical analysis

Biochemical analysis were done in order to measure following parametres: fasting plasma glucose levels (FPG), glycated hemoglobin (HbA1c), hematological parameters (RBC, Hgb, HCT, MCV, MCH, MCHC, RDW) and sedimentation rate.

Sample collection and cultivation

Samples were collected a day after clinical examination. Patients were suggested not to brush their teeth and to wear denture all night before the swabs were taken. Swabs were collected by swabbing ten times from the mucosa of hard palate or from palatal surface of denture with the help of a dry sterile cotton stick. Swab cultures were immediately inoculated on Sabouraud dextrose agar (SDA) (Oxoid, Basingstoke, UK) at 37°C for 48 h. After incubation, one calibrated microbiologist counted the growth density. The yeast growth density was define as rare, medium or dense.

Further analysis were done using germ-tube production test, carbohydrate assimilation test and by using ChromAgar medium. Distinction of different species of *Candida* genus by ChromAgar medium were done according to colony color: green color for *C. albicans* and *C. dubliniensis*, steel blue color for *C. tropicalis*, white for *C. parapsilosis* and pink for *C. glabrata*[18,19]. Distinction between *C. albicans* and *C. dubliniensis* were determined by carbohydrate assimilation test[19]. Microbiologist were blinded for any clinical and anamnestic information.

Statistical analysis

Statistical analyses were carried out by using SPSS 18.0 software package for Windows (SPSS inc., Chicago, USA). Descriptive data were presented as Mean ± SD or the percentage for discrete measures. T-test and One Way ANOVA were used for normally distributed data. Non-parametric data were analyzed using the Kruskall Wallis and Mann-Whitney test. Categorical variables were compared using the Chi Square Test ($\chi 2$). Spearman's correlations were done in order to assess correlation between presence of yeasts and measured parameters. The linear regression model was used to determine predictors of the presence of *Candida* spp. Subjects with missing data were not included in the study. Differences were considered significant when p-value was < 0.05.

Results

Groups were matched according to gender, age, smoking status, ABO blood type and duration of denture wearing in years. Also, presence of glossodyniae and glossopyrosis and self-reported halitosis were similar between the groups (Table 1).

Frequency of xerostomia in anamnesis were higher at diabetics with poor glycoregulation than in healthy subjects (Chi Square, p=0.000). None of the patients reported presence of sialorrhea. Anamnestic data about everyday consumption of carbo-hydrates, showed that significant difference exists between group A (N=8) and group B (N=0), (Chi, p=0.018). Groups of patients with diabetes were similar according diabetes duration (Independent sample T test, p=0.071) and the diabetes treatment (Chi, p=0.194). These groups were similar according to the presence of microvascular and macrovascular chronic diabetic complications.

Table 1. Demographic and clinical data of subjects

	Group A	Group B	Group C	
Gender (male (%))	2 (28.6)	4 (50)	10 (66.7)	[a]0.272
Age	53.14±4.221	50.25±8.844	47.93±6.216	[b]0.243
Xerostomia in anamnesis	12 (28.6)	14 (50%)	28 (70%)	[a]0.001
Glossodyniae at glossopyrosis	1 (2.4)	3 (10.7)	4 (10)	[a]0.298
Self-reported halithosis	14 (33.3)	11 (39.3)	10 (25.)	[a]0.445
Presence of O blood type	8 (19.0)	6 (21.4)	4 (10.0)	
Duration of denture wearing (years)	10±9.871	12.46±11.354	6.55±5.891	[c]0.746
Smokers N (%)	16 (38.1)	9 (32.1)	10 (25.50)	[a] 0.445

[a]$\chi 2$, [b]ANOVA, [c]Kruskall-Wallis

Presence of positive dental mirror stick test, denture wearing during the night, and presence of coated tongue were similar between the groups (Data not presented).

HbA1c and FPG levels were significantly different between all three groups (Bonferroni, p=0.000 for all comparisons). The most of hematological parameters were similar between the groups: Hgb (Kruskall-Wallis, p=0.998), Erythrocyte count (ANOVA, p=0.757), MCV (Kruskall-Wallis, p=0.960), MCH (Kruskall-Wallis, p=0.102). Only HCT differed between healthy control groups and two groups with diabetes (Mann-Whitney Test, p=0.000 for both comparisons).

Frequency of isolation of *Candida* spp. from palatal mucosa were similar between the groups (Fig. 1) ($\chi 2$=0.762). In group A, only *C. albicans* were isolated. In group B, two samples (2/16=12.50%) showed mixed isolates, while in group C, 20% of samples (6/30) exhibited mixed *Candida* species (presented in Fig 1. as 6.7%+13.3%). *C. albicans+C. glabrata* were only combination at diabetics with good glycoregulation. On the other hand, at diabetics with poorly regulated blood

sugar level, 6.7% (2/30) of positive mixed isolates showed mentioned combination of yeasts, while 13.3% (4/30) exhibited *C. albicans+C. tropicalis*. Rare density of yeasts growth was present at all positive samples in groups A and B, and at 50% of isolates from group C. The rest of positive samples from group C exhibited medium (16.7%) and dense (33.3%) growth.

Positive finding of yeasts on the surface of the denture were 42.9% (6/14) at group A, 37.5% (6/16) at group B, and 66.7% (20/30) at group C ($\chi 2$=0.153) (Fig 1). Presence of multiple yeasts isolation were 12.5% (2/16) of positive sample at group B and 20% (6/30) at group C (also presented as 6.7%+13.3% in Fig.1). *C. albicans+ C. glabrata* were only isolated combination in group B, while in group C, this combination presented 13.3% (4/30). Another combination of yeasts in group C was *C. albicans+C. tropicalis*. Rare yeasts growth from denture surfaces was not detected. Medium growth was detected at 100% positive samples in groups A and B, and 80% of samples in group C.
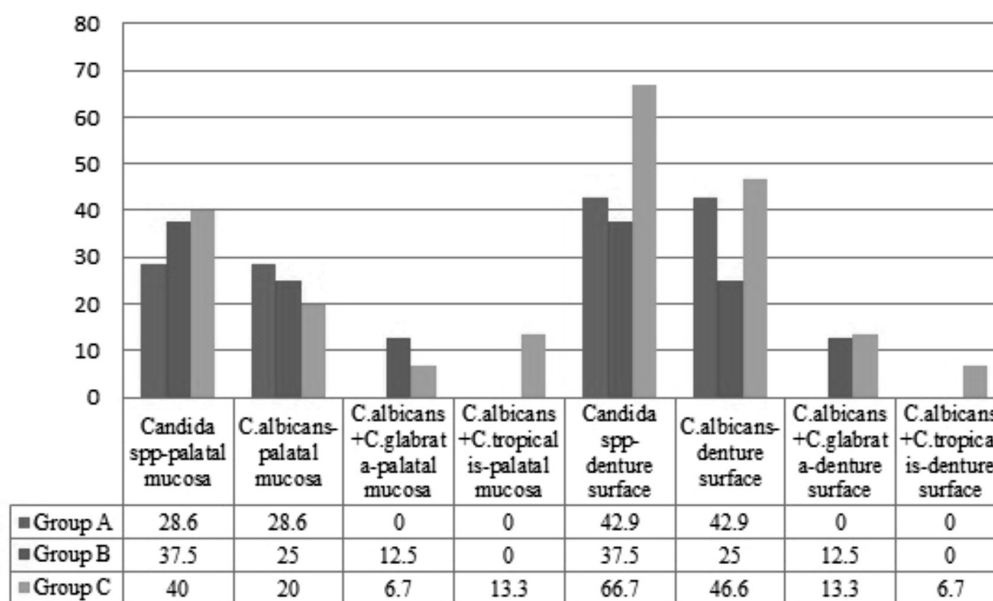

	Candida spp-palatal mucosa	C.albicans-palatal mucosa	C.albicans +C.glabrata-palatal mucosa	C.albicans +C.tropicalis-palatal mucosa	Candida spp-denture surface	C.albicans-denture surface	C.albicans +C.glabrata-denture surface	C.albicans +C.tropicalis-denture surface
■ Group A	28.6	28.6	0	0	42.9	42.9	0	0
■ Group B	37.5	25	12.5	0	37.5	25	12.5	0
■ Group C	40	20	6.7	13.3	66.7	46.6	13.3	6.7

Figure 1. Frequency and diversity of yeasts on palatal mucosa/denture surfaces in groups (values are expressed as %)

Different finding of yeasts' presence from swabs of palatal mucosa and denture surface existed. Negative finding of yeasts on palatal mucosa, but positive on denture surface were detected at 14.3% (2/14), 12.0% (2/16) and 33.4 (10/30) in groups A, B and C respectively. There were no samples with positive yeasts finding on palatal mucosa, but negative on denture surface.

Presence of yeasts on palatal mucosa correlate significantly (Spearman correlation) with HbA1c level neither at all patient (r=0.033, p=0.804) nor at diabetics (r=0.090, p=0.556). Correlations between positive finding

of yeasts on denture and HbA1c also were not significant at all subjects (r=0.106, p=0.483) and at diabetics only (r=0.137, p=0.507).

Univariate regression models were done in order to assess if some of parameters (age, gender, xerostomia, glossodyniae at glossopyrosis, blood type, everyday consumption of carbohydrates, smoking, period of wearing denture measured in years, night wearing of dentures, ill-fitting dentures, duration of diabetes in years, dental mirror stick test, HbA1c, Hemoglobin level, Erythrocyte number, MCV, MCHC, MCH, Plaque Index)

can predict presence of *Candida* spp. on palatal mucosa or denture surface. None of the examined parameters were predictors of presence of *Candida* spp. on palatal mucosa. Presence of diabetes (Exp B= 1.243, CI=1.159-1.762, p=0.045), duration of diabetes in years (Exp B=1.169, CI=1.038-1.316, p=0.010) and Erythrocyte count (Exp B= 0.304, CI=0.103-0.901, p=0.032) were assessed as predictors of finding of *Candida* spp. on denture surface in univariate logistic regression. In multivariate logistic regression analysis, only duration of diabetes were predictor of Candida spp. presence on denture surface (Exp B=1.186, CI=1.047-1.344, p=0.007).

Discussion

Candida spp, commonly found yeast in humans, can be isolated in about 50% of healthy population without clinical signs of infection[20]. In the case of diabetics, this prevalence is even higher.

Among oral *Candida* infections, Denture stomatitis is described as the commonest form of oral Candidiosis[21]. Placement of a denture in the oral cavity leads to big changes of the environmental conditions because the denture, as non-shedding surface, becomes colonized with oral microorganisms and additionally cuts off the underlying mucosa from the mechanical cleaning of tongue and the flow of saliva[22]. Colonization of acrylic surface of different roughness by yeasts cells and hyphae has been proven and is the initial step for the further accumulation of yeasts against supporting mucosa[23]. Except this role in initial colonization, it is thought that dentures may keep microorganisms in long contact with the mucosa and permit the microbial metabolic products to initiate an inflammatory reaction[24]. What is more, yeast may form biofilm, which is an essential strategy for their survival in oral milieu[25,26]. Beside biofilm formation, this genus is able to produce exoenzimes, proteinases and metabolites in order to adhere and inhibit the function of polymorphonuclears[27-29].

In our study, we detected presence of different *Candida* spp. at patients with clinically health oral mucosa, incuding mucosa of hard palate. Unlike a lot of studies researching presence of yeasts at subjects with DS, there are limited number of researches dealing with yeasts at subject without signs of infections. At our study, frequency of *Candida* spp. finding was 36.6% at all subjects and 28.6%, 37.5% and 40.0% at groups A, B and C respectively. Overall prevalence is in agreement with findings of Figueiral et al. (27.2%)[6], but is different from studies which revealed higher 51% or lower frequency (15.5%) at subjects without DS[3,30].

A study conducted in Serbia, presented positive Candida finding at 36.5% systematically health subjects without DS[11], which is quite similar to frequency of

yeasts at our group A. Furthermore, number of studies that considered different *Candida* spp. at subjects without DS is very low. Although results of frequency of isolation of *Candida* spp. are inconsistent, all studies describe unique results about most commonly isolated species- *Candida albicans*[6,12,30,31]. The second common isolated species from palatal mucosa at our study was *C. glabrata* and afterward *C. tropicalis*, which is also in agreement with other studies[32-35]. Recovery of non- albicans species is important because of its high level of resitance to some antifungal drugs[36]. *C. tropicalis* was isolated only at T2D patients with poor glycoregulation at our sample, which is in agreement with fact that this species is commonly isolated from immunocompromised patients[37] and not at healthy[38]. Similarly, *C.glabrata* is also considered as second or third most common isolate of *Candida* spp.[34], and is increasingly connected with mucosal and systematic infections, especially at diabetics[39].

It is well-known that principal site for Candida colonization is the surface of denture, and that colonization of prosthesis surface is heavier than colonization of supporting mucosa[22], as we showed in this study by measuring growth density of yeasts. Overall prevalence of yeasts at denture surface at our sample was 53.3%, and 42.9%, 37.5% -and 66.7% at groups A, B and C respectively. Some studies report even 68% or 78% of overall prevalence of Candida spp. on denture[3,40], but it should be noted that these studies included subjects with wider variety of diseases than our study, and more sensitive methodology for detection of yeasts. Distribution of different Candida spp. on surface of denture was same as on palatal mucosa- *Candida albicans* being the most frequently isolated, followed by *C. glabrata* and then *C. tropicalis*, which is in agreement with previous studies[6,32].

In contrast to some other studies, presence of *C. glabrata* or *C. tropicalis* at our sample were always associated with *C. albicans*[35, 40]. When comparing results of yeasts finding with other researches, variations of isolation of Candida species depending on the geographical region and/or patient group[41] should be taken into consideration. It is proven that in some countries, even non-albicans forms could be more frequently isolated than *C. albicans*[41]. In Serbia, there are not sufficient number of studies examining presence of different *Candida* species. A study in Serbia examining prevalence of *C. albicans* and non-albicans species at oral lesions[42] and observing their extraoral prevalence[43] showed also highest prevalence of *C. abicans*, followed by *C. glabrata* and *C. tropicalis*.

As it was expected, some of the subjects who exhibited yeasts on denture surface had negative finding on palatal mucosa. Although nonsignificant, percentage of such finding was higher at diabetics with poor glycoregulation. This could be explained by reliability of these two sampling methods (false negative results of swabs from palatal mucosa), or on the other hand,

dentures may be considered as potential reservoirs of yeasts. Last is particularly important, because although presence of yeasts at subjects without DS or even with DS are often asymptomatic, it may serve as a portal of entry for further infections, e.g. aspiration pneumonia[44]. This is especially important at immuncompromised patients, such are diabetics, especially with poorly regulated blood sugar, who are anyhow more prone to yeasts colonisation/ infection. Relating to these facts and common findings of more pathogenic[37], and resistant[45], forms of *Candida* at such patients, screening of DS or even yeasts presence at denture wearers should be done regulary.

According that oral candida carriage may be influenced by many factors nonrelated to diabetes (gender, smoking, medications, saliva, site, blood type…), we considered as much as possible factors which can be potentially confounders for *Candida* carriage. Surprisingly, any of our examined factors were not predictor for carriage of *Candia* spp. on palatal mucosa. Independent predictors for candida carriage on denture were only presence and duration of diabetes, but with rather low risk ratio. On the other hand, it should be stressed that there are not a lot of studies which deal with regression models in order to access parameters which could facilitate presence of yeast.

Although glycoregulation at diabetics has often been correlated to presence of yeasts/candidiosis[38,46], at our sample we failed to show that. This can be explained by different definition of "good" and "poor" glycoregulation among studies. Some studies defines non-satisfactory glycemic control limit up to 12%, while others define "gap" in HbA1c value between subjects with satisfactory and poorly regulated blood sugar, what were not case in our study.

Unfortunatelly, usage of removable partial/ complete dentures to rehabilitate partially/complete edentulous patients is the very common option due to the low cost. As is it mentoined, placement of denture, as non-shedding surface, causes changes in oral cavity. Oral cavity itself is a compound environment that is continually exposed to numerous opportunistic microbes, which are controlled by a vigorous arsenal of immune factors that maintain a healthy oral environment, prevent development or spreding of disease[40]. In state of disturbed immunological state, as at diabetics, this is not always mantained succesfully. As it is said before, diabetics and other immunocompromised denture wearers should be periodically examined for presence of DS or *Candida* carriage in order to prevent potential complications. It should be stressed that treatement of DS/yeast colonisation should be taken seriosuly, according that after antifungal therapy the infection often reestablish, because of resistens species or if predisposing factors persists[22].

Conclusions

Prosthesis of denture wearers without DS may serve as reservoir of Candida spp. Presence of more pathogenic and resistant non-albicans species are related to diabetics, even without clinical signs of DS.

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