Journal of Dental Applications

Austin Publishing Group

Open Access Full Text Article

Research Article

# Identification of *Candida* Spp. In Patients with Denture Stomatitis: Relationship with Gender, Age, Time of Denture use and Newton's Classification

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**Received:** June 25, 2014; **Accepted:** August 23, 2014; **Published:** August 25, 2014

**Abstract**

Epidemiological researches are important to understand the distribution and etiology of oral diseases. The actual researches that show the relationship between patient ages, denture status and denture stomatitis are scarce. So, the aim of this study was to identify of *Candida* spp. in patients with Denture Stomatitis (DS) and to correlate with gender, age, time of denture use and Newton's classification. 204 complete denture patients (46 males and 158 females) were selected. DS was classified according to Newton's classification and it was related to gender, age and time of denture use. Samples from the palatal mucosa and the surface of the upper denture of patients with DS were evaluated using PCR test for identification of *Candida* species. T-test, chi-square and Fisher's exact tests were used for statistical analysis. DS was evidenced in 54.4% of the sample. According to gender 41.3% of the males and 58.3% females had the disease and the differences were statistically significant ($p = 0.032$). The type of DS was directly influenced by the time of denture use ($p<0.001$), but it was not significantly related to the age of the participants ($p>0.05$). *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. dubliniensis* were identified by PCR test. DS is more prevalent in women and the prevalence of DS was influenced by the time of denture use (years). *C. albicans* was identified as the most frequent specie in patients with DS.

**Keywords:** Denture Stomatitis; *Candida* spp.; Epidemiology; Denture

## Introduction

The colonization by microorganisms and subsequent biofilm formation on denture surface are relevant factors in the development of Denture Stomatitis (DS) [1-4], which may be considered a public health problem. This disease is the most frequent oral mucosal lesion in elderly people [1]. DS is an inflammatory condition found usually in partial or full dentures users [2-4]. It is characterized by diffuse erythema, which may have homogeneous appearance or reddish focal points or areas, besides showing various changes in texture and surface of the palatine mucosa. DS is most often asymptomatic. Only few patients have experienced pain or burning sensation [5,6].

DS is considered as a multifactorial disease. It has been strongly correlated with denture hygiene, reaction to oral biofilms, trauma and presence of *Candida* spp. [7-10]. Although *Candida spp.* are often present as benign commensal organisms in healthy individuals, these kind of microorganisms produce a broad range of serious illnesses in compromised hosts [11,12]. Different *Candida* spp have been frequently isolated from the oral cavities of patients with DS. The most commonly recovered species is *C. albicans* with a prevalence of up to 80%. This oral fungal pathogen is the most virulent of the *Candida* species and is able to grow as biofilm. Other *Candida* spp such as *C. tropicalis, C. krusei* and *C. glabrata* were also identified, but in smaller percentages [13-18].

Elderly population is increasing in many developed and developing countries and this increase can result in potential health problems in certain populations [19]. In dentistry, there is an increasing emphasis on maintaining oral health into the old age, when alterations in oral tissues are associated with various conditions [1]. In addition, in the elderly population, it can expect reduce in tissue regeneration and higher susceptibility to diseases, which may favor the appearance of the DS. Systemic complexity, aging process, metabolic changes, nutritional factors, medications, prosthetic use, psychobiological habits and alcohol or tobacco use could explain the high susceptibility to diseases in the elderly population [20].

Epidemiological researches are important to understand the distribution and etiology of oral diseases. The epidemiology of DS has great clinical variability depending on the population studied and diagnostic methods [21,22]. Oral mucosal alterations, including stomatitis, were observed in 60% of the elderly population in Brazil [23]. On the other hand, Cueto et al. [24] observed that denture-induced stomatitis corresponded to 37.1% of the population. In spite of these studies, the researches that show the relationship between patient ages, denture status and denture stomatitis are scarce. So, the aim of this study was to identify of *Candida* spp. in patients with DS and to correlate with gender, age, time of denture use and Newton's classification [25].

## Material and Methods

This study was carried out after the approval by the Committee of Ethics in Research of the State University of Ponta Grossa (Protocol: 17572/10). 204 denture patients (46 males and 158 females) were




**Citation:** Tay LY, Herrera DR, Gomes BP, dos Santos FA and Jorge JH. Identification of *Candida* Spp. In Patients with Denture Stomatitis: Relationship with Gender, Age, Time of Denture use and Newton's Classification. J Dent App. 2014;1(3): 46-50.

selected according to the inclusion criteria for evaluation. Patients with or without DS were selected (n=204). The inclusion criteria were male or female adults who frequently go to the long-term care institutions of Ponta Grossa city and maxillary complete denture wearers for at least one year. Women of childbearing age, patients with impaired hepatic or renal function, diabetes, xerosthemia, hypoparathyroidism, immune alterations, chemotherapy and radiation therapy and patients who had received any recent treatment with antibiotics, antifungal or steroidal agents within four weeks before the study and poorly fitting dentures were excluded.

In the first, the patients were interviewed using a structured questionnaire. Demographic data (name, age, gender, educational level) and medical records were recorded using the attendant's files in the Prosthodontic Clinic of the State University of Ponta Grossa, as well as the duration of maxillary denture experience, the age of the last denture, their wearing frequency and denture hygiene habits (after each meal, once per day, less often than once per day).

The subjects were examined using a portable high-intensity light, a dental mirror and a tongue blade. All parts of the oral cavity were examined. A clinical examination was performed by two trained examiners after interexaminer calibration (k = 0.9). The information obtained was registered on a clinical record specially designed for this purpose. The clinical examination included the assessment of the oral mucosa covered by the maxillary denture. The presence of denture stomatitis according to Newton's classification was observed as follow: pinpoint hyperaemia (type I), diffuse erythema confined to the mucosa under the denture base (type II) and inflammatory papillary hyperplasia of the palate (type III). Of the 204 selected patients, 54,4% had DS. All the patients were given instruction on appropriate oral and denture hygiene techniques and they were provided with hygiene aids such as toothbrush, toothpaste and dental floss. It was recommended to the patient to remove the prosthesis to sleep. Patients with clinical symptoms of DS were referred for treatment to the oral medicine clinic.

Presence and type of DS was correlated with gender (male and female), age (60 years old or younger and older than 60 years old) and time of use of the last denture (1-3 years old, 3-10 years old and older than 10 years). Statistical analysis was performed using SPSS 13.5 (SPSS Inc, Chicago, IL, USA). T-test, chi-square and Fisher's exact tests were used for comparative analysis. A p-value below 0.05 was considered to indicate statistical significance.

Only for patients with clinical symptoms of denture stomatitis (54,4%), recovery of *Candida spp*. was performed by rubbing oral swabs along the palatal mucosa and the tissue surface of the upper denture. Only one visit was need to this procedure. Each swab was placed into a test tube containing 5 mL of Brain-Heart Infusion (BHI) and vortexed for 1 minute to suspend the organisms from the swab. These samples were taken for recognition of *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi* and *C. dubliniensis* presents in the sample with simple PCR test (Polymerase Chain Reaction). These *Candida* species were correlated with the patient's DS type.

DNA extraction was performed using the QIA amp DNA kit (QIAGEN, Chatsworth, CA, USA) according to the manufacturer's instructions from each patient sample. Besides the samples, purified genomic DNA of each microorganism was used as positive control and Mili Q sterile water was used as the negative control.

*Candida* spp. were identified by using a nested amplification with species-specific primers for *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi* and *C. dubliniensis.* Primer sequence and cycles are shown in Table 1. The reactions were performed in a total volume of 20 µL containing 2 µL of DNA sample; 2 µL of 10X PCR buffer; 0.6 µL of 50 mmol/L $MgCl_2$; 1.6 µL of a mixture of each deoxynucleoside triphosphate (2.5 mmoL $L^{-1}$); 1 µL of each species-specific primer (10 µmol); 0.1µL of 5U/mL Platinum Taq DNA Polymerase and 11.6 µL of sterile water.

The PCR products were electrophoresed on 1% agarose gel and tri-acetate-EDTA buffer stained with 0.5µL $mL^{-1}$ ethidium bromide (Invitrogen® - Life Technology do Brasil) and visualized under ultraviolet light. Positive reactions were determined by the presence of bands of the appropriate size. A 1kb DNA ladder (Invitrogen) was used as size marker for universal PCR.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, United States). Although continuous data were shown as mean±standard deviation, nominal data were expressed as the number of cases and percentage. The prevalence of each lesion and 95% confidence intervals were calculated. Whether the differences in lesion prevalence regarding age and gender groups were statistically significant or not were evaluated by the Chi-square test. A P-value less than 0.05 was

**Table 1:** PCR species-specific primer pairs used for detection of six *Candida* ssp.

| Candida | Primer pairs (5' – 3') | Amplification | Cycles |
|---|---|---|---|
| ***Candida albicans*** | F: TTTATCAACTTGTCACACCAGA<br>R: ATCCCGCCTTACCACTACCG | 273 bp | Initial denaturation at 96°C for 5 min and 40 cycles of: 94°C for 30s, 58°C for 30s, 72°C for 30s and a final step 72°C for 15min. |
| ***Candida tropicalis*** | F: CAATCCTACCGCCAGAGGTTAT<br>R: TGGCCACTAGCAAAATAAGCGT | 357 bp | Initial denaturation at 96°C for 5 min and 40 cycles of: 94°C for 30s, 58°C for 30s, 72°C for 30s and a final step 72°C for 15min. |
| ***Candida krusei*** | F: GAGCCACGGTAAAGAATACACA<br>R: TTTAAAGTGACCCGGATACC | 227 bp | Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 94°C for 60s and a final step 72°C for 15min. |
| ***Candida glabrata*** | F: CCCAAAAATGGCCGTAAGTATG<br>R: ATAGTCGCTACTAATATCACACC | 674 bp | Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 94°C for 60s and a final step 72°C for 15min. |
| ***Candida guillermondi*** | F: CCCAAAATCACAAAGCTCAAGT<br>R: TACGACTTGAAGTTGCGAATTG | 205 bp | Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 74°C for 60s and a final step 72°C for 15min. |
| ***Candida dubliniensis*** | F: AAATGGGTTTGGTGCCAAATTA<br>R: GTTGGCATTGGCAATAGCTCTA | 816 bp | Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 74°C for 60s and a final step 72°C for 15min. |

**Table 2:** DS prevalence according to distribution of personal characteristics.

| | | Denture Stomatitis | | | |
|---|---|---|---|---|---|
| | | Not prevalent | Prevalent | total | p |
| Gender | Male | 27 (58.7%) | 19 (41.3%) | 46 (100%) | 0.032 |
| | Femele | 66 (41.8%) | 92 (58.2%) | 158 (100%) | |
| Age (years) | <= 60 | 14 (34.1%) | 27 (65.9%) | 41 (100%) | 0.070 |
| | > 60 | 79 (48.5%) | 84 (51.5%) | 163 (100%) | |
| Time of denture use (years) | 1-3 | 40 (67.8%) | 19 (32.2%) | 59 (100%) | 0.0001 |
| | 3-10 | 31 (43.7%) | 40 (56.3%) | 71 (100%) | |
| | > 10 | 22 (29.7%) | 52 (70.3%) | 74 (100%) | |

Chi-square test

considered statistically significant.

## Results

Denture stomatitis was evidenced in 54.4% of the sample (Table 2). According to gender, 41.3% of the males and 58.3% females had the disease and the differences were statistically significant (p = 0.032). Pinpoint hyperaemia was recorded in 19.1% of the sample (males: 3.43%, females: 15.68%), diffuse erythema in 22.1% (males: 3.93%, females: 18.13%) and papillary hyperplasia in 13.2% (males: 1.96%, females: 11.27%). Although all three types of denture stomatitis were more prevalent in females, this difference was not statistically significant (p = 0.75) (Table 3).

The mean age of the 204 patients was 65.9 ± 8.6 years. No differences were found comparing the patients 60 years old or younger (65.9%) and patients older than 60 years (51.5%) (Table 2). Neither the types of denture stomatitis were related to the age of the patients (p = 0.39) (Table 3).

Participants had been edentulous in the maxilla for 17.4 ± 12.2 years. The statistically significant associations using Chi-square test are presented in Table 2. The mean age of the last denture was 12.21 ± 12.1 years (range: 1-45 years). The prevalence of denture stomatitis significantly increased with the increasing age of the denture (p<0.001). DS was observed in 32,2% of the participants dentures users of 1 to 3 years, increasing to 56,3% subjects dentures users of 3 to 10 years, but increased to over 70,3% of the participants dentures users more than 10 years. When the three Newton types were separately investigated, the age of the last denture was significantly related (p<0.05). 15.3% of the subjects wearing dentures less than 3 years had dentures stomatitis type I, 6.8% type II and 10.2% type III. In subjects wearing denture more than 10 years, it was found DS type I in 14.9% patients, type II in 33.8% and in type III 21.6% (Table 3). PCR test was randomly performed in 56 patients with DS for identification of different *Candida spp*. Table 4 shows the number and the frequency distribution of the patients who had presence of *Candida spp*. Only two patients with DS type I did not present *Candida spp*. *Candida albicans* was present in the three types of DS, the others species of *Candida* were present along with *Candida albicans*, none of them had an isolated growth.

## Discussion

Prevalence studies of denture stomatitis have been conducted over the years [18,19,22,24]. Contradictory results are found, which may be a result of the variety of sampling techniques employed and population studied. Conventional techniques from oral sites include swabbing, oral rinses, imprint cultures, and saliva sampling. However, this may not be a concern for qualitative analysis. In addition, the differences may be due to the origin of the examined patients and training of examiners in the diagnosis of oral mucosal lesions [24]. For example, in this study, the data were collected from Brazilian patients. On the other hand, in the studies of Evren et al., Rabiei et al. and Cueto et al., the data were collected from Turkey, Iran and Chile, respectively. Because of this, the comparison of the findings of this study with those of other epidemiological studies is difficult.

For the clinical diagnosis of DS, should consider some clinical signs, such as changes in color and texture of the mucosa. In addition to clinical examination, other diagnostic methods available are cell culture and histopathological examination. These methods are used for diagnosis of chronic hyperplastic candidiasis [26,27]. In this study, denture stomatitis was assessed clinically and the patients were classified according to Newton, based on clinical lesions: class I, class II and class III [25].

**Table 3:** Prevalence of denture stomatitis types (Newton).

| | | Denture Stomatitis | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | total | p |
| Gender | Male | 7 (15.2%) | 8 (17.4%) | 4 (8.7%) | 19 (100%) | 0.754 |
| | Femele | 32 (20.3%) | 37 (23.4%) | 23 (14.6%) | 92 (100%) | |
| Age (years) | <= 60 | 7 (17.1%) | 13 (31.7%) | 7 (17.1%) | 27 (100%) | 0.397 |
| | > 60 | 32 (19.6%) | 32 (19,6%) | 20 (12.3%) | 84 (100%) | |
| Time of denture use (years) | 1-3 | 9 (15.3%) | 4 (6.8%) | 6 (10.2%) | 19 (100%) | 0.021 |
| | 3-10 | 19 (26.8%) | 16 (22.5%) | 5 (7.0%) | 40 (100%) | |
| | > 10 | 11 (14.9%) | 25 (33.8%) | 16 (21.6%) | 52 (100%) | |

Chi-square test

**Table 4:** Frequency and distribution of *Candida spp.* in patients with DS.

| Candida spp. | Number of patients (%) |
|---|---|
| *Candida albicans* | 54 (96.4%) |
| *Candida tropicalis* | 7 (12.5%) |
| *Candida glabrata* | 4 (7.14%) |
| *Candida krusei* | 2 (3.5%) |
| *Candida dubliniensis* | 1 (1.7%) |
| *Candida guillermondi* | 0 |

**Table 5:** *Candida spp.* distribution in the three types of DS.

| Candida spp. | DS type I | DS type II | DS type III |
|---|---|---|---|
| *Candida albicans* | 37 | 45 | 27 |
| *Candida tropicalis* | 1 | 3 | 2 |
| *Candida glabrata* | 0 | 3 | 1 |
| *Candida krusei* | 0 | 1 | 1 |
| *Candida dubliniensis* | 0 | 0 | 1 |
| *Candida guillermondi* | 0 | 0 | 0 |

Curiously, in general, *non-Candida albicans Candida* species have been isolated only from patients with DS type II and III (Table 5). The synergic relationship that exists between the *Candida* species can favor the colonization of more resistant strains, enhancing the infection process and the severity of the disease [12].

The prevalence of DS in the study population was 54.4%, in compliance with other studies [19,22]. Cueto et al. [24] observed that denture-induced stomatitis corresponded to 37.1% of the population. The factors that can increase the risk of DS are poor denture fit, poor denture hygiene and colonization of the denture surface and oral mucosa [21]. These factors were not assessed in this study. Thus, future studies should be performed to associate DS with these factors.

The survey showed statistically significant difference in the prevalence of DS between males (41.3%) and females (58.3%). These results are in agreement with previous studies [9,10]. Hormonal differences between men and women could explained these results. It has suggested that during or after menopause, there is atrophy of the oral cavity mucosa, increasing the probability to develop an inflammatory reaction [3]. In contrast, the results of Evren et al. [19] showed that there were no differences between males and females incidence of DS. Furthermore, for classification of Newton [25], in this study was observed prevalence similar for all kinds regardless of gender.

Age may influence the prevalence of DS. Older patients have less manual hability to hygiene the denture and cleaning oral mucosa [28]. Other problems associated with aging may favor the onset of disease such as oral epithelium thins, decrease synthesis of collagen in connective tissues, decrease in tissue regeneration and lower resistance to diseases [24]. Contrarily, in relation to age, the results of this study showed no difference between patients younger than 60 years (65.9%) or older than 60 years (51.5%). This shows that other factors not related to age, may favor the appearance of DS, such as systemic diseases, inadequate hygiene habits and very old prostheses [19]. However, as previously reported, further studies are needed to correlate the etiological factors with the prevalence of DS.

In this study, the factor that most influenced the prevalence of denture stomatitis was the time of use of the denture. The quality of the dentures was not evaluated in this study, but the results point to more prevalence of denture stomatitis with the increasing time of the denture use. The prevalence of the disease was 70% for users who used the same prosthesis for more than 10 years. More than 50% of the patients wearing their prosthesis from 3 to 10 years had DS, which represents a high prevalence of the disease in these cases. Over time, changes occur in the surface acrylic base, such as increased surface roughness [29], favoring the accumulation of biofilm and thus the appearance of DS.

The ability of adherence of *Candida spp.* to saliva-coated acrylic resin may play a role in establishing itself on the denture surface [30]. The majority of the denture wearers in our study was low-income individuals or no income elderly. Nearly half of the maxillary dentures worn by these patients were acrylic-based and were defective. Also, the acrylic-based dentures are less stable than the cobalt chrome based dentures causing accumulation of plaque or mucosal lesions [31].

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida spp*., the commonest being *C. albicans* [32]. When it is associated to the use of dental prosthesis, it is called denture stomatitis. Not surprisingly 96.8% (54 patients) harbored *C. albicans*, which was by far the predominant yeast isolated. This finding is in agreement with earlier reports. Coco et al. [15] demonstrated that *C. albicans* was isolated from 75% of patients without denture stomatitis and from 81% of those with clinical signs of the infection. In addition, *C. albicans* has the ability to adhere to mucosal and denture surfaces, which is considered to be the first step in the pathogenesis of DS.

Although *C. albicans* is still the most frequently isolated species from patients with *Candida* infections, the growing prevalence of non-albicans species is clearly a concern. In the present study, *C. tropicalis* and *C. glabrata* were isolated from 12.5% and 7.14% of patients with denture stomatitis. *C. krusei* and *C. dubliniensis* were isolated from 3.5% and 1.7%, respectively. In terms of frequency distribution, some studies have shown that *C. tropicalis* was the second most prevalent species identified [33,34]. However, contrasting results have been found in other studies, in which *C. glabrata* was the most common yeast after *C. albicans* [35,36]. However, multiple isolations of *Candida* spp. in the biofilm were evidenced in patients with oral candidiasis and the yeast associations most commonly observed were *C albicans* together with *C tropicalis, C glabrata,* or both [37].

Despite maxillary and mandibular denture were evaluated, DS lesions were found only in the maxillary mucosa. Maxillary dentures cover a greater area of oral mucosa than mandibular dentures; therefore a greater chance of plaque and yeast retention as well as mechanical injury would be expected [30]. This study confirmed that high prevalence of *C. albicans* and the subsequent infection in the palatal mucosa is due to poor hygiene of full denture and the time of use of it, careful instruction should be provide to elderly people with more emphasis in women. In addition, the high prevalence of DS pointed in this study is one of the motives why oral examination of patients using total denture is of significant importance, so it is recommended to reevaluate the prosthesis every three years to assess hygiene and the need of relining or replacement of it.

## Conclusion

According to the findings in this study can conclude that:

- There is a high prevalence of dentures stomatitis in the patients evaluated in this study, being DS type II the most prevalent;
- DS is more prevalent in women;
- There are no differences according to the age of the patients on the prevalence of DS;
- DS is more prevalent in patients that wear their dentures for more than 10 years;
- *C. albicans* was identified as the most frequent specie in patients with DS.

## Acknowledgments

In addition, this publication was supported by FUNDUNESP - Fundação para o Desenvolvimento da UNESP.

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