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Incidence of *Candida albicans* in diabetic patients with a dental prosthesis in Northeast Mexico

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Diabetes is an important health problem with a high morbidity and mortality. There are infections that are common in patients with diabetes and others that have greater severity and complications. A study was carried out to determine if there is a higher incidence of *Candida albicans* in patients with diabetes who use total and partial dental prostheses in comparison with patients without diabetes. Samples were obtained from 43 patients seven days after receiving a total or partial dental prosthesis. Afterwards, samples from the dentures were cultured in CHROMagar to determine the presence of *Candida*. The frequency of different types of *Candida* was analyzed and it was found that no significant difference exists between patients with diabetes and those without diabetes with regard to the presence of *C. albicans*.

Key words: *Candida albicans*, dental prosthesis, diabetes mellitus, CHROMagar.

INTRODUCTION

Diabetes is an important health problem with a high morbidity and mortality. It currently affects more than 285 million people worldwide according to the Mexican Federation of Diabetes. Diabetes mellitus is a group of metabolic disorders that are characterized by an increase in blood glucose levels (hyperglycemia). There are infections that are common in patients with diabetes and others which have greater severity and complications (Federación Mexicana de Diabetes, 2012).

A dental prosthesis is an artificial element that is used to restore the anatomy of one or several teeth, also restoring the relationship between the maxillaries, at the same time correcting the vertical dimension and improving both natural dentition and periodontal structures (Rendon, 2007). Prostheses can be classified according to their characteristics, such as the type of support, the material used, and the type of restoration that will be performed; however, they can also be classified as unremovable (fixed prothesis) and removable (removable partial tooth supported, total mucosal supported and over denture prosthesis). The objective of placing a partial or total prosthesis is to provide function, phonation, swallowing, esthetics, and safety for the patient who uses it.

Candidiasis is a frequent disorder, especially in patients with diabetes. It is caused by excessive growth of *Candida* in the mouth, digestive tract, vagina and other tissues. The risk factors for candidiasis are type 1 diabetes mellitus, characterized by autoimmune destruction

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of Langerhans islet B cells by T cells that cause a lack of production of insulin; type 2 diabetes, in which a family history of the disease as well as other factors such as age >40 years, obesity, the use of drugs (corticosteroids) are present, and gestational diabetes with poor glycemic control in which vaginal candidiasis is more frequent (Nowakowska et al., 2004). Immuno-suppressive states and drugs can also cause candidiasis. A prevalence of denture stomatitis of 15% to over 70% has been reported in denture wearers and can be caused by poor denture hygiene, continual and nighttime wearing of removable dentures, accumulation of denture plaque, and bacterial and yeast contamination of the denture surface. Another cause is mucosal trauma produced by poor-fitting dentures. These factors can increase colonization of the denture and oral mucosal surfaces by *Candida albicans*, which can produce an opportunistic infection (Gendreau and Loewy, 2011). *Candida albicans* has been identified as the most frequent etiological agent in oral candidiasis although other studies have isolated *Candida dubliniensis*, *Candida parapsilosis*, *Candida krusei*, *Candida tropicalis* and *Candida glabrata* in the pathogenesis of candida-associated denture stomatitis (Liebana Ureña, 2002; Salerno et al., 2011).

The aim of this study was to determine if there is a greater incidence of *C. albicans* in patients with diabetes in comparison with patients without diabetes when they use a dental prosthesis.

MATERIALS AND METHODS

Study population

We carried out a comparative, open, observational, prospective, cross sectional study in the Total Prosthesis Clinic of the Universidad Autónoma de Nuevo León Dental School. The population consisted of 11250 patients that came for consultation in the prosthesis clinic. To calculate sample size we used the following formula:

$$n = \frac{Nz^2pq}{e^2(N-1)+z^2pq} \quad n = \frac{11250\,(1.96)^2(0.55)(0.45)}{(0.15)^2(11250-1)+(1.96)^2(0.55)(0.45)} =$$

42.10 ≈ 43 patients

with a margin of error of 0.15 and a 95% confidence interval. The population sample consisted of 43 patients (13 men and 30 women) with a mean age of 67 years (range 45 to 85).

The study was approved by the Bioethics Committee of the School of Dentistry of the UANL with registration number SSPI-010613, Page 00008. Patients provided informed consent and were assured of the confidentiality of their personal data and medical history. Men and women with (n =12) and without diabetes (n = 31) with partial or total dental loss and a dental prosthesis evaluated from August to December 2011 were included. Out of town patients and those less than 44 or greater than 85 years of age were excluded. Patients who did not follow or complete the study protocol or who died during the study were removed.

The presence of diabetes was determined by patient interview. Since *C. albicans* is part of the normal flora of the oral cavity, we determined its quantitative presence and that of other species of *Candida*. The independent variables of the study were the presence of diabetes as a nominal qualitative variable. The dependent variables were the quantitative presence of *Candida*, determined by counting the number of colony forming units (CFU), and the identification of the species of *Candida* present.

Protheses were studied at seven days because patients were programmed for revision at that time for examination and necessary adjustments. The opportunity was used to take samples at this time.

Procedures

In the first phase, the prostheses were washed with soap and water, and disinfected with Microdacyn MR for 1 min. They were then rinsed with sterile distilled water to ensure that the prosthesis was clean before giving it to the patient. The device was then placed in the patient´s mouth.

After seven days, the patient returned to the clinic and a sample was obtained using a sterile swab dipped in distilled water. The swab was placed in an Eppendorf tube containing phosphate buffer as a transport medium. Samples were taken to the molecular biology laboratory where 100 µL of the sample were seeded in a Petri dish with previously prepared CHROMagar. Seeding was carried out with a sterile glass pipette.

Samples were incubated at 37°C for 24 to 48 h in CHROMagar™ media and up to 72 h to improve colony pigmentation. Four types of *Candida* were identified by pigmentation. *Candida albicans* colonies appear green in CHROMagar, *C. tropicalis* metallic blue, *C. krusei* fuzzy pink and other species, white to mauve. A macroscopic count of CFU was performed according to the color of the colony.

Statistical analysis

Descriptive statistics were applied to obtain means, medians, standard deviations and 95% confidence intervals of the variable UFC for *Candida*; frequencies and percentages for the presence of diabetes in patients were also determined. Cross tabulation was carried out between the presence of diabetes and the *Candida* values obtained; the mean difference (95% confidence interval) for the presence of *Candida* in each of the groups was subsequently tested. All statistical procedures were performed using Microsoft Excel 2007 and SPSS, achieving a 95% confidence interval for all cases.

RESULTS

We evaluated 13 (30.2%) men and 30 (69.8%) women with (n = 12; 28%) and without diabetes (n = 31; 72%). Forty two patients had upper dentures and 36 lower dentures. The mean pH among patients with and without diabetes was 7.25 and 7.26, respectively (P = 0.4828). Of the total patients 30 (71.4%) had *Candida* in the upper denture. In patients with diabetes mellitus, 9 (75%) had some form of *Candida* in the upper denture. In patients without diabetes mellitus, 21 (70%) had *Candida* in the upper denture. Of all the patients studied, *C. albicans* was present in 11 patients (26.2%). In patients with diabetes, *C. albicans* was present in 3 (25%). Of the

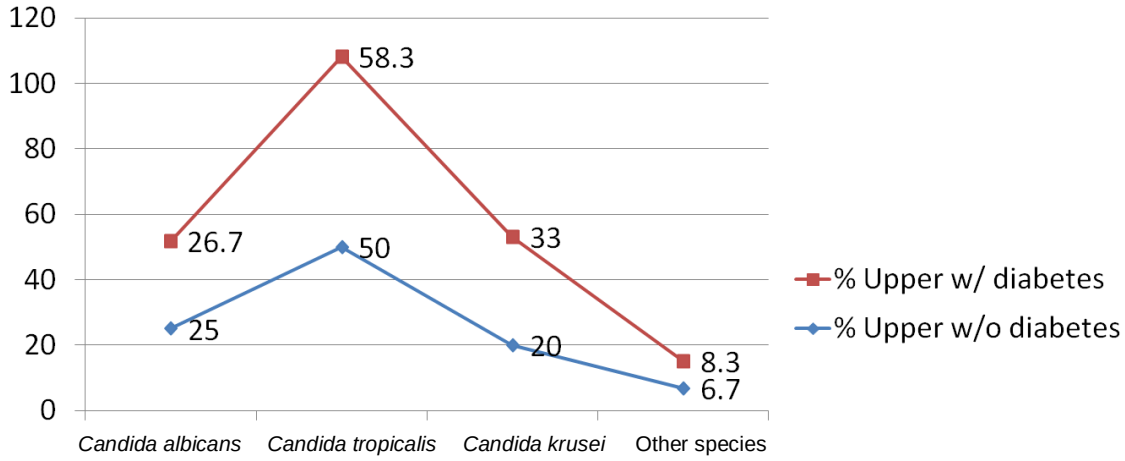

Figure 1. Relationship of percentages of patients with and without diabetes with an upper dental prosthesis from the Total Prosthesis Clinic with different types of *Candida* analyzed after 7 days of use between August and December, 2011.

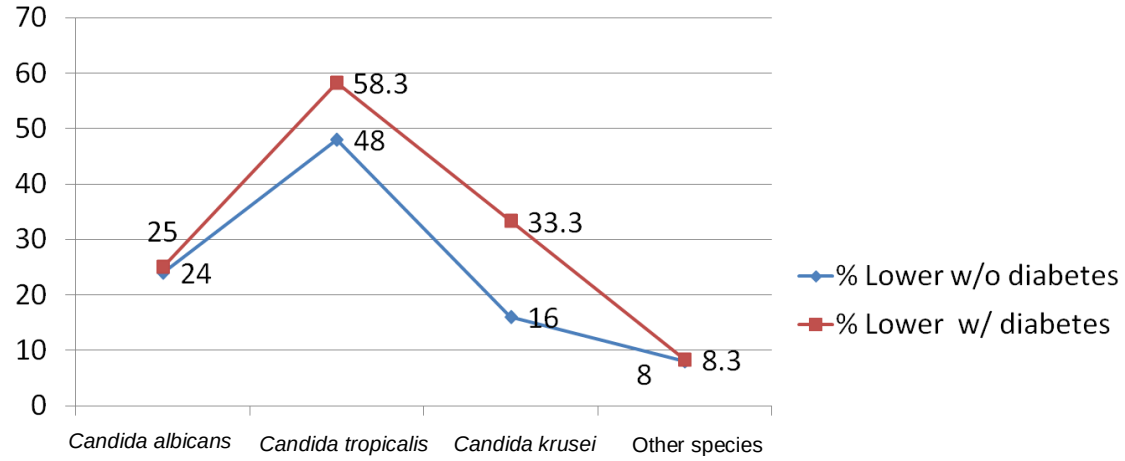

Figure 2. Relationship of percentages of patients with and without diabetes with a lower dental prosthesis from the Total Prosthesis Clinic with different types of *Candida* analyzed after 7 days of use between August and December, 2011.

patients without diabetes mellitus only 8 (26.7%) had *C. albicans*.

In patients with diabetes mellitus, 7 (58.3%) had *C. tropicalis*. *C. krusei* was found in 10 (23.8%) patients. This strain of *Candida* was found in 6 (20%) patients that did not have diabetes mellitus and in 4 (33.3%) patients with diabetes (Figure 1). It was also observed that 3 (7.1%) patients in the study had other species of *Candida*. In patients with diabetes mellitus, only 1 (8.3%) had other species of *Candida*. Of the patients who did not have diabetes mellitus, 2 (6.7%) presented other species of *Candida*.

Regarding those with lower dentures, it was observed that 25 (67.6%) had *Candida*. In patients with diabetes mellitus, 9 (75%) had some form of *Candida*. Of the patients who did not have diabetes mellitus, 16 (64%) had some forms of *Candida*. In patients with lower dentures, 9 (24.3%) had *C. albicans*. In patients with diabetes mellitus, 3 (25%) had *C. albicans*. In patients without diabetes mellitus, 6 (24%) had *C. albicans* (Figure 2).

It was found that 19 (51.4%) patients had *C. tropicalis*. Of the patients who had diabetes mellitus, 7 (58.3%) had *C. tropicalis*. Of the patients who did not have diabetes mellitus, 12 (48%) had *C. tropicalis*.

C. krusei was present in 8 (21.6%) patients. In patients with diabetes mellitus, 4 (33.3%) had *C. krusei*. Of the patients who did not have diabetes mellitus, 4 (16%) had *C. krusei*. As for other species of *Candida*, growth was observed in 3 (8.1%) patients with lower dentures. In

patients with diabetes mellitus, only one (8.3%) had other species of *Candida*. Of the patients who did not have diabetes mellitus, 2 (8%) had other *Candida* species.

DISCUSSION

We found an incidence of *Candida* in 71.4% of samples taken from 43 patients. This is in contrast with Zaremba (2006), who isolated *Candida* in 59.4% of 32 patients with prosthesis and Daniluk et al. (2006), who found an incidence of 66.7% in samples from patients without diabetes, and 43.8% in patients with diabetes. In the present study, 26.2% of the sampled patients had *C. albicans* and in those with diabetes only 25% had the fungus. Pfaller et al. (1996) evaluated the use of CHROMagar as a differential culture medium that allows the isolation of yeasts and simultaneously identifies colonies of *C. albicans, C tropicalis* and *C krusei.* They found that more than 95% of the values and clinical isolates of *Candida* species were correctly identified based on colony morphology and CHROMagar pigmentation. The study by Odds et al. (1994) found that the specificity and sensitivity of the medium in the identification of *C. albicans*, *C. tropicalis* and *C. krusei* exceeded 99% for all three species.

The study by Mata de Henning et al. (2001) showed that 65% of patients had *Candida* species in 20 patients studied by swabbing the prosthesis in the area in contact with the palatal mucosa. However, their study differs from ours because they observed *C. albicans* in 50% of cases and found one case with a species that was not *albicans*.

In a study by Belazi (2005), of the 128 diabetic and 84 nondiabetic patients, *Candida* was observed in the oral cavity of 64% of the diabetic patients. However, in contrast with the present research, they found a higher frequency of *C. tropicalis*. There was also a difference with Daniluk et al. (2006), where it was determined that 43.8% of diabetic patients had *C. albicans*. In the present study, only 25% had a positive result.

We had a small number of patients with diabetes in our clinic and we believed that there would be a greater incidence of *C. albicans*, but this was not the case. It would be convenient to perform this study in a greater number of patients.

Our results indicate that colonization by *C. albicans* is independent of the presence of diabetes mellitus in patients with total and/or partial dentures. Thus, no significant relationship between diabetic and nondiabetic patients with total and/or partial dentures was shown. *C. albicans* was not as frequent as other types of *Candida*, since *C. tropicalis* was found in 52.4% of samples, which was the highest and most significant finding in this study.

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