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## A Cross-Sectional Study of the Candidal Species Isolated in the Oral Cavities of Type II Diabetic Patients

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## A Cross-Sectional Study of the Candidal Species Isolated in the Oral Cavities of Type II Diabetic Patients

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### Abstract

**Background:** The objectives of this study were to determine the prevalence and colonisation of *Candida* species and to study the factors related to candidal colonisation in the oral cavity among type II diabetic patients. **Methods:** The data of 102 diabetic patients was collected from the Outpatient Diabetic Clinic at the Naresuan University Hospital. Data was collected via a questionnaire and oral examination. The subjects were measured for their fasting blood sugar levels and salivary pH. *Candida* colonisation was assessed using saliva sampling, and was cultured on CHROMagar *Candida* plates. Species and colony forming unit/mL were recorded. **Results:** The results showed that the prevalence of oral *Candida* species in diabetic patients was 73.5%. The most frequent candidal species in diabetics were *Candida albicans* (68.6%), followed by *C. glabrata* (28.4%), *C. tropicalis* (10.8%), and *C. krusei* (2.0%) respectively. There was no statistical significance between the fasting plasma glucose levels and oral *Candida* species colonisation. Logistic regression showed that a decrease of the salivary pH was related to the colonisation of candidal species. Results showed that the use of a dental prosthesis was a related factor to the colonisation of candidal species ( $p < 0.05$ ). **Conclusions:** A high prevalence of candidal species were found in type II diabetic patients. Salivary pH and use of a dental prosthesis are factors that promote candidal infections in type II diabetic patients.

**Keywords:** *Candida*, denture, diabetes mellitus oral health, oral thrush

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### Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that is fast becoming one of the most important chronic diseases worldwide. It has been estimated that the number of adults with diabetes in the world will rise to 300 million by the year 2025.<sup>1</sup> The relationship between diabetes and oral candidiasis has been extensively studied in scientific literature, particularly because diabetic patients are more susceptible to fungal infections than those without diabetes.<sup>2-3,8,11</sup> Yeast adhesion to epithelial cell surfaces is recognised as an essential first step in the process of *Candida* colonisation and subsequent infection.<sup>12</sup> Salivary glucose levels in diabetic patients favours yeast growth, owing to increased numbers of available receptors for *Candida*.<sup>13</sup>

The *Candida* genus presents over 150 species, 10 of which are responsible for infections in humans. Of these species, *Candida albicans* is part of the normal microbiota and is isolated in the greatest frequency from the oral cavity in human beings. When the balance between the host and the microorganism is altered, *Candida* becomes pathogenous and oral candidiasis is manifested. As such, it occurs in diverse populations especially those individuals affected by the human immunodeficiency

virus, those with nutritional deficiencies, malignancies, or with metabolic disorders such DM.<sup>1</sup>

The role of *Candida* in the oral cavity of diabetic patients is a controversial issue as the prevalence of the microorganism in these patients varies greatly from 18-80%.<sup>2</sup> These discrepancies are usually attributed to the methodology used in the laboratory, the number and characteristics of the subjects, and to the sampling techniques used.<sup>3</sup> Although *C. albicans* is the most frequently found species, others like *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, or *C. krusei* are also involved in colonisation and oral candidiasis.<sup>4</sup>

An exhaustive review of the scientific literature revealed a lack of studies on the association between *Candida* and DM in Thailand. As such, it was considered important to carry out an investigation on *Candida* in the oral cavities of Thai patients and study the factors that may facilitate the increase of the microorganism and further understand the relationship between the pathogen and oral health. The objectives of this study were to determine the prevalence and colonisation of candidal species, such as *C. albicans*, *C. krusei*, *C.*

*glabrata*, and *C. tropicalis*, and to study the factors related to candidal species colonisation in the oral cavity among type II diabetic patients.

## Methods

This cross-sectional study was conducted on a sample of 102 outpatients at the Naresuan University Hospital with a prior diagnosis of Type II DM. Patients were required to be under current treatment at the hospital and over 40 years of age. All individuals participated voluntarily and gave signed consent via a form approved by the Human Ethics Committee of Naresuan University (IRB.455/57). The study excluded patients who had a positive diagnosis of HIV, or those who were treated with antifungals within the 30 days prior to the study.

The study population was categorised into controlled and uncontrolled groups according to the level of glycosylated haemoglobin (HbA1c). For the controlled group, values equal to or below 7% were considered for inclusions and for the uncontrolled group, values above 7% were considered.<sup>13</sup> The soft tissue of the oral cavity of each patient was examined in detail with a dental mirror. Possible lesions suggesting candidiasis were recorded, and samples were taken to culture in CHROMagar Candida. Additionally, a fasting blood sugar (FBS) sample was taken, along with stimulated saliva and oral rinse samples. The first permitted HbA1c and FBS measurements, the second was used to determine pH and the salivary flow rate, and the last was used to search for candida species. The samples were taken between 07:00 and 10:00 hours.

Oral rinses were obtained from each patient using 10 mL of saline solution that was retained in the mouth for 30 seconds. It was then deposited into a sterile container that was stored in a portable cooler at 4 °C, and transported to the laboratory in the Faculty of Dentistry, Naresuan University, where it was immediately processed. Each sample was homogenised in a vortex, inoculated in CHROMagar Candida (CHRO Magar Company, Paris, France), and incubated at 30 °C for 48 hours. Thereafter, the colony forming units (CFU) were counted per mL. Preliminary identification of the yeasts and candidal species was completed by observing the growth in the CHRO Magar Candida.<sup>3</sup>

The yeast strains that presented colours different from green tonalities in the CHROMagar Candida were subjected to API 20C Aux identification (bioMérieux; Marcy-l'Etoile, France). Green colonies, which were presumed to comprise of *C. albicans* or *C. dubliniensis*, were subjected to different phenotypic tests that included germ tube formation and production of

chlamydo spores in corn meal agar.<sup>11</sup> Results were also recorded of the growth that occurred at 42 and 45 °C in Sabouraud agar by adding sodium chloride at 6.5%.<sup>11</sup>

The pH was determined as soon as the sample was collected using pH paper. The oral health status of each patient was measured using the decayed-missing-filled (DMF) index outlined by the WHO criteria. A periodontal chart was completed, using the community periodontal index (CPI), to assess the patient's periodontal state and to evaluate their treatment needs.<sup>14</sup>

All results were recorded in a database and analysis was completed using the SPSS version 20.00 statistical program. To measure the association between the different variables the Chi square test was used, when a relationship existed. Logistic regression was used for factors relating to candidal species colonisation in the oral cavity amongst type II diabetic patients.

## Results

Of the 102 type II diabetes mellitus patients who participated in the study, 32 were male and 70 were female, with a mean age of 57.9 ( $\pm 13.6$ ). The mean duration of DM in the subjects was 6.1 years ( $\pm 3.3$ ). Fasting blood sugar, HbA1C, oral salivary pH, duration of DM, number of teeth present, CPI, and denture use of all subjects are outlined in Table 1. The pH of the subject's saliva ranged between 5.0 and 7.5, with a mean salivary pH of 6.2 ( $\pm 0.6$ ). Gender, fasting blood glucose, and duration of disease were not associated with Candida.

Oral examination revealed that the mean number of decayed teeth was 4.1 ( $\pm 2.1$ ) teeth per person. The mean number of missing teeth was 10.0 ( $\pm 9.9$ ) teeth per person and the mean number of filled teeth were 1.2 ( $\pm 2.3$ ) teeth per person. Bleeding on probing was noted in 88 subjects (86.3%), with 4-5 mm periodontal pockets present in 44 patients (43.1%) and 6 mm periodontal pockets present in 37 patients (36.3%). Oral lesions were recorded in 19 patients (18.6%) and removable prostheses were noted in 36 cases (35.3%). Results showed that the prevalence of oral Candida species in diabetic patients was 73.5%. The most frequent candidal species found were *C. albicans* (68.6%), followed by *C. glabrata* (28.4%), *C. tropicalis* (10.8%), and *C. krusei* (2.0%) respectively. We found mixed candidal species in the saliva of patients, as shown in Table 2. There were no statistically significant differences between the prevalence of candidal species in the control group and the uncontrolled group.

Table 1. Characteristics of the Oral Cavities of Both Groups of Diabetic Patients

Characteristic	Total N = 102	Controlled N = 38	Uncontrolled N = 64
Fasting Blood Sugar (mean mg/decilitre)	148.4 ± 55.1	98.5 ± 7.0	175.5 ± 51.8
The level of glycosylated haemoglobin (HbA1c)	7.9 ± 1.7	7.0 ± 1.6	8.2 ± 1.9
Oral pH	6.2 ± 0.6	7.0 ± 2.0	6.0 ± 2.5
Duration of DM	6.1 ± 3.3	5.2 ± 2.7	6.5 ± 2.4
Number of teeth present (teeth/person)	15.4 ± 2.6	16.3 ± 3.1	14.8 ± 2.4
CPI Code 0 (number of patients)	14	8	9
Code 1-4 (number of patients)	88	30	58
Denture Used (number of patients)	52	18	34

Table 2. Species of Candida Isolated in Both Groups of Diabetic Patients

Oral Candida species	Total (Number of patients)	Controlled (Number of patients)	Uncontrolled (Number of patients)
<i>C. albicans</i>	38	14	24
<i>C. glabrata</i>	3	0	3
<i>C. albicans</i> + <i>C. glabrata</i>	10	3	7
<i>C. albicans</i> + <i>C. krusei</i>	1	0	1
<i>C. albicans</i> + <i>C. tropicalis</i>	3	0	3
<i>C. albicans</i> + other	4	1	3
<i>C. glabrata</i> + other	2	0	2
<i>C. albicans</i> + <i>C. glabrata</i> + <i>C. tropicalis</i>	5	3	2
<i>C. albicans</i> + <i>C. glabrata</i> + other	6	4	2
<i>C. albicans</i> + <i>C. krusei</i> + <i>C. glabrata</i> + <i>C. tropicalis</i>	1	0	1
<i>C. albicans</i> + <i>C. glabrata</i> + <i>C. tropicalis</i> + other	2	1	1
No Candida species isolated	27	11	16
	102	37	65

Table 3. Logistic Regression for Candida Infections and its Related Factors

Oral Characteristic	n	OR	Candida infection	p
			Upper - Lower	
<b>Salivary pH</b>				
6.5-7.5 (ref)	38			
<6.5	38	2.61	1.03-6.52	0.041
<b>Denture used</b>				
No (ref)	50			
Yes	52	3.71	1.01-13.81	0.048
<b>Salivary flow rate</b>				
Normal (ref)	42			
Hyposalivation	60	2.54	0.95-4.65	0.869

Logistic regression analysis for Candida infections and its related factors are outlined in Table 3. Use of a denture prosthesis was a significant risk for candidal infections (OR = 3.71) over those patients who did not wear dentures ( $p < 0.05$ ). Logistic regression analysis showed that a salivary pH of  $< 6.5$  was a significant risk for Candida in the oral cavity, versus a salivary pH of  $> 6.5$  (OR = 2.706;  $p < 0.05$ ).

## Discussion

The prevalence of Candida species obtained from our sample group was 74.8%, with the most frequent candidal species found in diabetics being *C. albicans* (68.6%), confirming the variability of the bacteria, which could depend on social factors or the sampling technique. The presence of Candida in the oral cavity of diabetic patients

has been extensively researched all over the world and has resulted in quite diverse values.<sup>2,3</sup> The prevalence of *Candida* species found in this study is similar to a previous study carried out in Thailand using the modified imprint culture technique<sup>4</sup>, which found the most frequent candidal species seen in diabetics was *C. albicans* (81.3%). Similarly, multiple studies also indicate that *C. albicans* is the most commonly isolated yeast in the oral cavities of diabetic patients.<sup>1,3,15</sup> The present study also found a high prevalence of *C. glabrata*, *C. tropicalis* and *C. krusei* in DM patients, results that are also consistent with previous studies showing that species other than *C. albicans* were more prevalent in diabetic patients than their healthy counterparts.<sup>3,15</sup> The higher incidence of *Candida* prevalence in DM patients may be explained by the fact that the normal oral flora is altered by the endocrine abnormalities in DM. The apparent increase in candidal colonisation in diabetic patients may be attributed to the greater adherence of fungi to epithelial cells. This is thought to be facilitated by the increased glucose content in the saliva, a genetic susceptibility to infection, altered cellular and humoral immune defence mechanisms, and local factors such as poor blood supply.<sup>16</sup>

Khosravi *et al.*<sup>8</sup> reported that the degree of yeast colonisation in the oral cavity may be altered by levels of blood glucose, however our study did not reveal any statistically significant differences between the levels of blood glucose and *Candida* colonisation in DM patients. Diabetes mellitus is considered a risk factor for the development of periodontal disease, especially in patients with poor control of the disease.<sup>17</sup> Results from this research did not find an association between the DMF index and the presence of yeasts. Additionally, this study also didn't find any statistical association between CPI and glycaemic control. These results are similar to a study carried out by Arrieta *et al.*<sup>18</sup> in type I and II diabetic patients, which reported that no relationship existed between CPI and the metabolic control of the disease.

We found a high incidence of *C. albicans* in both diabetic and non-diabetic control subjects with low salivary flow rate and pH, results that are consistent with previous studies.<sup>19</sup> A low salivary flow rate may result in decreased cleaning and dilution functions of saliva on the oral mucosa. Results from the present study indicate that oral yeast carriage is not significantly influenced by glycaemic control, duration, or complications of diabetes, gender, or age.<sup>9</sup> However, Kadir *et al.* did report lower pH values in diabetic patients when compared with non-diabetic individuals.<sup>6</sup> *Candida* species are aerobic organisms that require sugar and a neutral to acidic pH for optimal growth.<sup>20</sup> In the current research, the salivary pH variations were independent of glycaemic control, and as such it was considered as a factor that would affect *Candida* growth. The low reactivity of salivary IgA with *C. albicans* cells, grown at acidic pH values, may help to explain the association between acidic saliva and the

carriage of *Candida* in the oral cavity, as well as with oral candidiasis.<sup>21</sup> The pH of saliva is a factor which may influence the growth and carriage of *C. albicans* within the human mouth, and oral thrush has been previously associated with acidic saliva.<sup>22</sup> Salivary dysfunction, reduced salivary pH, and salivary hyperglycaemia can provide a potential substrate for fungal growth in DM patients.<sup>2</sup>

This study found that wearing a dental prosthesis was a risk factor for *Candida* infections. A study carried out by Abu in 1998, found a significant increase in the rate and density of *C. albicans* in denture wearers of the diabetic group compared with the non-diabetic controls.<sup>23</sup> Research has demonstrated that candidal carriage is higher amongst diabetics wearing dentures because *C. albicans* adheres to acrylic and it has been suggested that dentures behave as a reservoir for these yeasts.<sup>22,24</sup> The decrease in salivary pH, the increase in serum glucose, and wearing dentures are all related to an increased rate and density of *C. albicans*.<sup>6</sup> Colonisation of the oral mucosa by candidal species is a risk factor for progression to oral candidiasis, in both immunocompetent and immunocompromised individuals.<sup>25</sup> Oral candidiasis is due to the proliferation of fungi on the mucosal surface and it may come from the microbiota of the same patient in a carrier state.<sup>26</sup> Its growth is explained by fungal mechanisms and the host's defences.<sup>27</sup> The coexistence of different *Candida* species in an infection makes it more persistent and difficult to treat.<sup>28</sup>

The diversity of isolated *Candida* species in the oral cavities of diabetic subjects, and the association between poorly controlled diabetes and species other than *C. albicans*, which generally present less sensitivity to antifungal medications, implies there is a need for further studies to clearly establish the role of *Candida* in the oral cavities of type II diabetic patients.

The limitations of this study were its small sample size, of only 102 patients, and that the majority of the subjects had type II diabetes mellitus. There is need to perform similar research in larger numbers of diabetic subjects including those with type I diabetes mellitus. Additionally, there is a need to assess various alternative treatment protocols that are equally effective against *C. albicans* and other candidal species especially that of *C. glabrata*.

## Conclusions

There was a high prevalence of candidal species found in type II diabetic patients. Salivary pH and the use of a dental prosthesis were risk factors that appeared to promote *Candida* infections in type II diabetic patients. Further knowledge about the prevalence of species distribution, rapid species identification, antifungal susceptibility testing, and the development of new antifungal medications are necessary to achieve a decrease

in Candida infections and an increase in the quality of life of denture wearing individuals with type II diabetes mellitus.

### Conflict of Interest Statement

I confirm that all funding sources that supported the work and all institutions and people who contributed to the work, but do not meet the criteria for authors are acknowledged. I also confirm that all commercial affiliations, stock ownership, equity interests, or patent-licensing arrangements that could be considered to pose a financial conflict of interest in connection with the article have been disclosed.

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