

## Relationship between oral *Candida* species infection and glycemia levels of diabetic patients at the Bafoussam Regional Hospital in Cameroon

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### Abstract

Diabetes mellitus is a chronic metabolic disease characterized by high blood sugar levels due to insulin abnormalities. In poorly controlled diabetic patients, candida species are more prevalent. This study aimed at evaluating the relationship between oral *Candida* species infection and the management of glycemia in diabetic patients. A hospital based cross sectional study was carried out in diabetic patients within the period of 3 months at Bafoussam Regional Hospital. The study questionnaires were submitted to 124 eligible participants and later, mouth swabs were collected using sterile cotton wool swabs, fasting blood sugar levels were detected using a glucose meter and 4 ml venous blood samples were collected for determination of the levels of glycated hemoglobin (HbA1c). *Candida* species were isolated and identified. The study consisted of 124 diabetic patients, and a total of 27 (21.7%) patients were screened positive for oral candida species infections. The isolated *Candida* species were; *Candida albicans* (74.1%), *Candida glabrata* (7.4%), *Candida tropicalis* (3.7%), *Candida krusei* (7.4%), *Candida dubliniensis* (3.7%), and *Candida parapsilosis* (3.7%). *Candida albicans* was shown to significantly increase with elevated levels of glycated hemoglobin ( $p=0.043$ ) and *Candida* species colonization was substantially linked with fasting blood glucose, urine catheter usage, type of diabetes, level of education and the wearing of dental prosthesis ( $p < 0.05$ ). No statistically significant association between *Candida* colonization and factors like poor oral hygiene, smoking, alcohol intake, being hospitalized, being on long term antibiotics or experiencing dry mouth was observed. These findings indicate that high blood glucose levels promote the growth of *Candida*. None of the predisposing factors of oral candidiasis impacted the development of *Candida tropicalis*. While *Candida albicans* was the most isolated species in all the groups, *Candida krusei* was mostly isolated in severe cases of exposure.

**Keywords:** *Candida* species, glycemic control, predisposing factors, Bafoussam Regional Hospital

### 1. Introduction

Diabetes mellitus is a chronic metabolic condition defined by elevated blood glucose levels (hyperglycemia) caused by insulin insufficiency or insulin resistance.<sup>1</sup> There are three primary forms of diabetes: type 1, type 2, and gestational diabetes. Type 1 diabetes mellitus is a condition where the body fails to produce insulin, requiring insulin injections or pumping, type 2 diabetes mellitus, also known as adult-onset diabetes results from insulin resistance while gestational diabetes, a rare condition, can occur during pregnancy without prior diabetes history.<sup>1</sup>

Diabetes prevalence is predicted to rise significantly by 2030, with 552 million people affected, primarily in low- and middle-income countries. The incidence varies by region and age, with type 2 diabetes becoming more common. Diabetes is a major risk factor for visual loss, amputation, and end-stage renal disease detection and prevention. Additionally, it poses a significant risk for atherosclerotic disease, the primary cause of morbidity and death in individuals with diabetes.<sup>2</sup>In Cameroon, diabetes prevalence was estimated at around 6% in 2018. This prevalence is increasing in the general population, rising from 2.0% in 1999 to 4.7% in 2002 and 5.8% in 2018. There is a regional disparity between rural and urban areas, with a rural prevalence of diabetes lower than the urban one but rising with time.<sup>3</sup>

Oral candidiasis, also known as "thrush," is a fungal disease caused by *Candida albicans*, a highly adaptable organism. Its survival depends on co-adhesion with oral bacteria, innate immunity, and the T helper 17 adaptive immune response. OC can cause various symptoms in healthy and immunocompromised individuals<sup>4</sup>, causing a range of clinical symptoms from moderate acute surface infections to deadly disseminated illness.

Over 80% of oral candidiasis lesions involve *Candida albicans*, the most prevalent type of *Candida*. These dysmorphic yeasts coexist peacefully in the oral cavity together with other species like; *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida pseudotropicalis*, and *Candida tropicalis*. Risk factors for pathologic colonization include metabolic disease, immunocompromising conditions, malnourishment, age extremes, concomitant infections, long-term steroid treatment, antibiotic treatment, and salivary gland hypofunction.<sup>5</sup> Immunocompetent and immunocompromised persons can get oral candidiasis, although immunocompromised hosts are more likely to contract it. Oral candidiasis affects more than 90% of HIV patients at some time throughout their illness. Both men and women can get oral candidiasis. Consequently, immunosuppression such as diabetes, dentures, steroid use, malnutrition, vitamin deficiencies, and recent antibiotic use often leads to the disease.<sup>5</sup> Oral candidiasis are influenced by local and systemic factors such as poor oral hygiene, untreated dental caries, smoking, HIV, diabetes, anemia, iron deficiency, malnutrition, neutropenia, and oral prostheses, with HIV immunosuppression being the most common.<sup>6</sup> Diabetics are more susceptible to infections, particularly fungal ones, due to immune system disruption and salivary composition alterations.<sup>7</sup> Reoccurring candida infections in patients are a significant sign of uncontrolled diabetes, potentially aiding in the identification of pre-diabetic conditions.<sup>8</sup> Diabetes Type 2 is characterized by insulin resistance, lower mitochondrial density, and severe tissue alterations, leading to secondary vascular disease, cardiac, renal, and ophthalmic complications. Previous studies reported relationship between diabetes and candidiasis, with diabetic patients being more susceptible to fungal infections compared to those without diabetes.<sup>9</sup> *Candida* species propensity in diabetic patients is influenced by factors like yeast adherence, increased salivary glucose levels, and lower salivary flow, which can lead to infection and xerostomia.<sup>10</sup> Improved glycemic control in type 2 diabetes can reduce gingivitis and periodontitis, requiring measures like quitting smoking, limiting sugar intake, reducing inflammation, and maintaining good oral hygiene.<sup>11</sup> Diabetes mellitus increases the risk of oral infections, especially those caused by *Candida* species, so diabetics with poor glycemic control may be more likely to develop *Candida*-colonization. This study aimed at evaluating the relationship between oral *Candida* species infection and glycemic control management in diabetic patients in order to contribute to the improvement of the healthcare of diabetic patients.

## 2. Methods

**2.1. Study design and study area:** this study was a hospital based cross sectional study carried out within a period of three months, starting from March to June 2024. At enrolment, an informed consent and assent form was obtained and

each diabetic participant was asked to complete a questionnaire which consisted of socio-demographic and personal details, history of present illness, clinical signs and symptoms, and so forth. The study area was the Bafoussam Regional Hospital in the West region of Cameroon.

**2.2. Sample size:** Diabetic patients in most units of the hospital were approached, and those willing to participate in this study were recruited as well. Sample size was calculated from the Lorenz formula:

$$n = \frac{Z^2 P(1 - P)}{d^2}$$

Where n is the sample size, Z is the confidence interval (95%) Z=1.95, P is the pre-estimated prevalence of 12.1% obtained from a previous study in Cameroon<sup>12</sup> and d is the margin error (5%). So, sample size was estimated n =164

**2.3. Study population:** The study population consisted of eligible patients suffering from diabetes who agreed to participate in the study and the target population were diabetic patients present at the Bafoussam Regional Hospital at the time of study.

**2.4. Inclusion and exclusion criterion:** The inclusion criteria consisted of all diabetic patients who came for consultation or who were hospitalized at the Bafoussam regional hospital at the time of study. Non-inclusion criteria consist of participant who did not agree to donate their samples, pregnant women and mentally unstable patients. Exclusion criteria consisted of patients who did not agree to fill the consent form.

**2.5. Ethical considerations:** Ethical review and clearance were obtained from the *Comité Régional d'Ethique pour la Recherche en Santé Humaine de l'Ouest (CRERSH-Ouest)* with reference number N°/437/27/03/2024/CE/CRERSH-OU/VP and the administrative authorization was obtained from the Delegation of Public Health West Region (N°281/L/MINSANTE/SG/DRSPO/CBF) and from the Bafoussam Regional Hospital (Ref N°216/L/MINSANTE/SG/DRSPO/HRB/D).

**2.6. Sample collection:** Mouth swab samples were collected from 124 participants using sterile cotton wool swabs to be used for the isolation of candida species, fasting blood sugar levels were determined using a drop of blood on the fingertip and 5ml venous blood samples were collected for the determination of the levels of HbA1c.

## 2.7. Laboratory diagnosis

**2.7.1. Fasting blood glucose measurement:** A code-free glucose meter was used to measure blood glucose levels using a small blood sample. It uses an electrochemical enzymatic reaction to produce an electrical current proportional to glucose concentration. This was done immediately after participants consent and the results were displayed on the screen.

**2.7.2. Glycated hemoglobin (HbA1c) analysis:** The blood specimen was used for determination of glycated hemoglobin. HbA1c is a form of hemoglobin that is bound to glucose. The measurement of HbA1c provides an average blood glucose level over the past two to three months and is a key marker for long-term glycemic control in diabetic patients. The AccuCare reagent uses a method known as ion-exchange resin chromatography combined with absorbance measurement to determine HbA1c levels.

**2.7.3. Microscopy:** Oropharyngeal swab samples were examined on a clean, grease-free glass slide at 10X and 40X magnification for the presence of pus cells, budding yeast cells, and branching pseudo hyphae typical of Candida. Smears were prepared, Gram stained, and then examined for fungal components at a magnification of 100X.<sup>13</sup>

**2.7.4. Culture:** Oropharyngeal swab samples were inoculated on Sabouraud dextrose agar (SDA) impregnated with Chloramphenicol and incubated at 35°C ( $\pm 2^\circ\text{C}$ ) within 24 hours under aerobic conditions. Colonies were white to cream-colored, smooth, glabrous, and yeast-like in appearance.<sup>13</sup> Colonies were Gram stained and subculture on chrom agar Candida medium, and SDA was impregnated with Chloramphenicol for identification and antibiogram respectively [13,14].

**2.7.5. Germ tube test:** *Candida albicans* can be identified presumptively by a simple germ tube test. Single colony was inoculated in human serum and incubated for 2-4 hours at 37°C and observed under the microscope using the 40x objectives for germ tube formation.

**2.7.6. CHROMagar Candida medium:** The CHROM agar Candida (Paris, France) is a selective and differential chromogenic medium used to identify diverse *Candida* species. This medium is based on the direct detection of certain enzyme activity by adding numerous chemical dyes that function as fluorochromes substrates to the media. Because of the chromogenic substrates added to the medium, *Candida* colonies of distinct species create different colours, allowing them to be identified directly on the isolation plate.<sup>14</sup> Discrete colonies were selected from the Sabouraud dextrose agar impregnated with chloramphenicol and emulsified in distilled water in a sterile test tube and inoculated on chrom agar Candida medium by streaking the whole surface of the test plate and cultured for 18-24 hours, after 24 hours colour change was observed which indicate a particular type of *Candida species*.<sup>15</sup> This is represented in (table1).

**2.8. Statistical analysis:** Data was collected using registration forms, entered into a Microsoft Excel database 2016 version on a secure computer, and analyzed using SPSS version 25. Data were statistically characterized using frequencies and percentages. To evaluate relationships between *Candida* species colonization and HbA1c levels, various antidiabetic treatments, oral hygiene, and other predisposing variables, a Pearson Chi-square test with Fisher's exact test was employed with a minimal significance threshold of  $p < 0.05$ . Regression analysis was used to investigate risk variables linked with *Candida* colonization. For dichotomous outcomes, the odds ratio (OR) and 95% confidence interval (CI) were applied.

### 3. Results

#### 3.1. Demographic characteristics of diabetic patients at Bafoussam regional hospital:

The study comprised of a total number of 124 diabetics patients, among which 68(54.8%) males and 56(45.2%) females with age ranging from 13 to 86 years old and mean age of  $31.47 \pm 13.57$  years. Majority (60.5%) of the participants falls within the age group 35-64 years. Type 2 diabetes was more prevalent, with a frequency of 114 and percentage of 91.9%, than DT1 with frequency 10 and percentage of 8.1%. Majority (43.2%) of the participants ended their education in primary school, 39.2% in secondary school and 16.8% in the university. Most of the participants (66.9%) said that they wash their mouth only once per day (Table2).

#### 3.2. Prevalence of oral candidiasis in diabetic patients:

Out of the overall 124 diabetic patients screened for the presence of oral candidiasis, 27(21.7%) patients were positive for oral candidiasis and males were more likely to develop oral candidiasis (12.9%) than females (8.8%) (table3)

#### 3.3. Frequency of *Candida* species identified:

From 124 patients screened for the presence of oral candidiasis, 27(21.7%) patients developed oral candidiasis, and six different species of *Candida* were identified; *Candida albicans*, *Candida krusei* and *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida dubliniensis*. The highest frequency of isolated species

was *Candida albicans* (27), followed by the non albicans species; *Candida krusei* (2), *Candida glabrata* (2), *Candida parapsilosis* (1), *Candida tropicalis* (1), and *Candida dubliniensis* (1) as shown in (table 3).

#### 3.4. Distribution of the prevalence of oral candidiasis according to the level of control of diabetes

In order to get the prevalence of the candida species with respect to the level of control of diabetes, we divided the diabetic population into four groups with six species of isolated candida. These groups were; normal controlled, well controlled, fairly controlled and poorly controlled.

Out of the 27 (21.7%) patients with positive candida species identified, those with poorly controlled glycemia experienced more positive candida cases (8.9%) than those with fairly controlled (6.4%), well controlled (5.6%), and normal glycemia (0.8%), (table 4). Using binary regression test, we evaluated the significance of association between the isolated species and the different levels of glycemic control. The association between glycated hemoglobin and the prevalence of oral candidiasis was statistically significant ( $\chi^2=7.935$ ,  $P=0.043$ ). With respect to the species distribution, *Candida krusei* was found in the fairly controlled and poorly control groups, *Candida dubliniensis* was only in the fairly controlled group, *Candida albicans* was in all the groups of participants but in different frequency, *Candida glabrata* was only present in the well-controlled group, *Candida parapsilosis* only found in the poorly controlled group, and *Candida tropicalis* only in the well controlled. Thus, uncontrolled individuals presented a significantly higher percentage of yeast different from *C. albicans*. This result was presented as P- value, odds ratio and confidence interval (95% C.I, lower and upper) as shown in (table 7).

According to fasting plasma glucose, among the candida species identified, 15 of candida species was identified in diabetics group, 7 in normal group and 5 in the prediabetes group as shown in (table 4). *Candida krusei* was found in the diabetic and prediabetes participants, *Candida dubliniensis* was only present in the diabetic group, *Candida albicans* was in all the groups of participants and in different frequencies, *Candida glabrata* was found in the diabetic and prediabetes group, *Candida parapsilosis* was only in the prediabetes group, *Candida tropicalis* was only in the normal controlled group, ( $\chi^2=13.713$ ,  $P=0.002$ ). This test result showed that there was a statistically significant relationship between oral candida species infection and the variation of plasma blood glucose levels or a disequilibrium in glycemic control. Using binary regression test, we evaluated the significance of association between the oral candida species and the different levels of plasma glucose control as shown in (table 7).

#### 3.5. Risk factors associated with oral candidiasis in diabetic patients in Bafoussam Regional Hospital:

The wearing of dental prosthesis, wearing of urinary catheter, type of diabetes and the level of education were significantly associated with the development of oral candidiasis in diabetic patients.

Oral candidiasis was frequent among participants not wearing urinary catheter (17.7%) than those wearing them (4.0%) (table 5). However, the association between urinary catheter and the prevalence of OC was statistically significant. The Pearson chi square ( $\chi^2$ ) test also suggested that, there was a statistical significant difference between development of oral candidiasis and urinary catheter usage ( $\chi^2=10.126$ ,  $P=0.006$ ) (table 8). Oral candidiasis was frequent among individuals not wearing dental prosthesis (16.9%) than those wearing them (4.8%) (table 5). However, the association between dental prostheses and the prevalence of oral candidiasis was statistically significant ( $P=0.042$ ) (Table 8). The Pearson chi square ( $\chi^2$ ) test suggested that, there was a statistical significant association between oral candidiasis and dental prosthesis ( $\chi^2=4.616$ ,  $P=0.042$ ). Also, oral candidiasis was frequent among type 2 diabetes participants with 22 (17.7%) than type 1 diabetes with 5 (4.0%) (table 6). However, the association between type of diabetes and the prevalence of oral candidiasis was statistically significant. The Pearson chi square ( $\chi^2$ ) test suggested

that, there was a statistically significant difference between oral candidiasis and type of diabetes, ( $\chi^2=4.678$ ,  $P=0.046$ ) (table 8). Oral candidiasis was more frequent among individuals with primary level of education (14.5%) than secondary (5.6%) and university (1.6%). However, the association between the level of education and the prevalence of oral candidiasis was statistically significant ( $P=0.035$ ) (table 7).

### 3.6. Distribution of the prevalence of oral candidiasis according to other risk factors:

Gender, age, dry mouth, oral hygiene, long term antibiotics consumption, alcohol consumption, cigarette smoking, duration of diabetes, disease comorbidities, hospitalization and antidiabetic treatment type were considered risk factors for the development of oral candidiasis.

The frequency of oral candidiasis was more among males (16) than females (11) in this study (Table 8). However the association between gender and the prevalence of oral candidiasis was not statistically significant ( $P=0.523$ ). The frequency of oral candidiasis was highest among individuals in the aged group 35-64 years (13) than those within 65-95 years with 12 (9.6%) and within 10-34 years with 1.6%. However there was no significant association between infection rate with age ( $P=0.295$ ). Oral candidiasis was more frequent among individuals who do not brush their mouth more than once per day (16.1%) than those who did brush (5.7%) (Table 8). However, the association between oral hygiene and the prevalence of oral candidiasis was not statistically significant ( $P=0.566$ ). The frequency of oral candidiasis was more among those not under long-term antibiotics (16) than those under who were 11 (8.8%), but no significant association between infection rate with antibiotics use ( $P=0.658$ ) was observed. There was a higher frequency for those who do not consume alcohol (21) than those who consumed (6), but no significant association between infection rate with alcohol consumption ( $P=0.142$ ).

The frequency of oral candida species was more among non-smokers (23) than smokers (4), but with a  $p=0.920$  which is not significant. Those taking both insulin and oral antidiabetic had more frequency (14) than those taking only insulin (7) or oral antidiabetics (6), however there was no significant association between infection rate with antidiabetic drugs ( $p=0.292$ ). The frequency of oral candidiasis was more among those not hospitalized (16) than those who were hospitalized (11) (Table 8). However the association between hospitalization and the prevalence of oral candidiasis was not statistically significant ( $P=0.217$ ). Those with the duration of diabetes less than 10 years were more likely to develop oral candidiasis (14.3%) than those with diabetes duration of more than 10 years (7.4%), however the association between duration of diabetes and the prevalence of oral candidiasis was not statistically significant ( $P=0.646$ ).

**Table 1: Identification of candida species using CHROMagar medium )**

Sample number	Candida species	Colour on CHROM agar medium
1	<i>Candida albicans</i>	Light green
2	<i>Candida glabrata</i>	Pink
3	<i>Candida krusei</i>	Large, fuzzy purple
4	<i>Candida parapsilosis</i>	Creamy white
5	<i>Candida tropicalis</i>	Steel blue
6	<i>Candida dubliniensis</i>	Dark green

**Table 2: Demographic characteristics of diabetic patients in Bafoussam Regional Hospital (N=124)**

Variable	Frequency	Percentage (%)
<b>Age (years)</b>	Mean±SD (31.47±13.57)	
[10-34]	11	8.9
[35-64]	75	60.5
[65-95]	38	30.6
<b>Gender</b>		
Female	68	54.8
Male	56	45.2
<b>Education</b>		
Primary	54	43.2
Secondary	49	39.2
University	21	16.8
<b>Type of diabetes</b>		
DT1	10	8.1
DT2	114	91.9

**Table 3: Distribution of different candida species isolated**

<i>Candida species</i>	Frequency	Percentage (%)
<i>Candida albicans</i>	20	74.1
<i>Candida glabrata</i>	2	7.4
<i>Candida krusei</i>	2	7.4
<i>Candida tropicalis</i>	1	1.6
<i>Candida parapsilosis</i>	1	1.6
<i>Candida dubliniensis</i>	1	1.6
Total	27	100

**Table 4: Relationship between oral candida species with glycated hemoglobin (HbA1c) and blood glucose**

<i>Candida species</i>	HbA1c		Blood glucose	
<i>Candida albicans</i> (20)	Well control	5(4)	Diabetic	12(9.7)
	Fair control	6(4.8)	Prediabetes	2(1.6)
	normal	1(0.8)	Normal	6(4.8)
	Poor control	8(6.5)		
<i>Candida glabrata</i> (2)	Well control	1(0.8)	Diabetic	1(0.8)
	Fair control	0(0)	Prediabetes	1(0.8)
	normal	0(0)	Normal	0(0)

	Poor control	1(0.8)		
<i>Candida krusei</i> (2)	Well control	0(0)	Diabetic	1(0.8)
	Fair control	1(0.8)	Prediabetes	1(0.8)
	normal	0(0)	Normal	0(0)
	Poor control	1(0.8)		
<i>Candida tropicalis</i> (1)	Well control	1(0.8)	Diabetic	0(0)
	Fair control	0(0)	Prediabetes	0(0)
	normal	0(0)	Normal	1(0.8)
	Poor control	0(0)		
<i>Candida parapsilosis</i> (1)	Well control	0(0)	Diabetic	0(0)
	Fair control	0(0)	Prediabetes	1(0.8)
	normal	0(0)	Normal	0(0)
	Poor control	1(0.8)		
<i>Candida dubliniensis</i> (1)	Well control	0(0)	Diabetic	1(0.8)
	Fair control	1(0.8)	Prediabetes	0(0)
	normal	0(0)	Normal	0(0)
	Poor control	0(0)		
<b>Total (27)</b>	Well control 7(5.6)		Diabetic 15(12.1)	
	Fair control 8(6.4)		Prediabetes 5(4.0)	
	Normal 1(0.8)		Normal 7(5.7)	
	Poor control 11(8.9)			
Glycated haemoglobin(HbA1c): Well control [7.6-8.9%], Fair control [9-10%], Normal [4.5-7.5%], Poor control [>10%]				
Plasma glucose: Diabetic[>125(g/L), Prediabetes[1.1-1.25(g/L), Normal[0.7-1.0(g/L]				

Table 5: Relationship between oral candida species and some predisposing factors

Candida species	Predisposing factors of oral candidiasis					
	Factors	Urinary catheter	Dental prostheses	Oral hygiene	Dry mouth	Hospitalization in ICU
<i>Candida albicans</i> (20)	No	17(13.7)	17(13.7)	15(12.1)	11(8.9)	12 9.7
	Yes	3(2.4)	3(2.4)	5(4)	9(7.3)	8 6.5
<i>Candida glabrata</i> (2)	No	2(1.6)	1(0.8)	2(1.6)	1(0.8)	2 1.6

	Yes	0(0)	1(0.8)	0(0)	1(0.8)	0(0)
<i>Candida krusei</i> (2)	No	2(1.6)	0(0)	2(1.6)	0(0)	1(0.8)
	Yes	0(0)	2(1.6)	0(0)	2(1.6)	1(0.8)
<i>Candida tropicalis</i> (1)	No	1(0.8)	1(0.8)	0(0)	0(0)	1(0.8)
	Yes	0(0)	0(0)	1(0.8)	1(0.8)	0(0)
<i>Candida parapsilosis</i> (1)	No	0(0)	1(0.8)	1(0.8)	0(0)	0(0)
	Yes	1(0.8)	0(0)	0(0)	1(0.8)	1(0.8)
<i>Candida dubliniensis</i> (1)	No	0(0)	1(0.8)	0(0)	0(0)	0(0)
	Yes	1(0.8)	0(0)	1(0.8)	1(0.8)	1(0.8)
<b>Total (27)</b>	No	22(17.7)	21(16.9)	20(16.1)	12(9.7)	16(12.9)
	Yes	5(4.0)	6(4.8)	7(5.6)	15(12.1)	11(8.8)

Table 6: Relationship between oral candida species and types of diabetes

Type of diabetes	Freq OC Negative	<i>Candida krusei</i>	<i>Candida dubliniensis</i>	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Candida parapsilosis</i>	<i>Candida tropicalis</i>	Freq OC Positive	Total Freq
DT1	5(4.0)	1(0.8)	0	4(3.2)	0	0	0	5(4.1%)	10(8.1%)
DT2*	92(74.2)	1(0.8)	1(0.8)	16(12.9)	2(1.6)	1(0.8)	1(0.8)	22(17.6%)	114(91.9%)
Total	97(78.2)	2(1.6)	1(0.8)	20(16.1)	2(1.6)	1(0.8)	1(0.8)	27(21.7%)	124(100%)
<b>Percentage candida(N=27)</b>		7.4%	3.7%	74.1%	7.4%	3.7%	3.7%	27(100%)	27(21.7%)
Significance	<b>P-Value= 0.041</b>		<b>OR= 3.957</b>		<b>CI= (1.056-14.830)</b>				

Freq OC: Frequency of oral candida

Table 7: Relationship between oral candida species infection and its predisposing factors

Factors	Frequency/ percent	P- values	Odds ratio( 95% C.I)	P- Value	Odds ratio (CI)
<b>HbA1c (%)</b>					
Normal [4.5-7.5]	35(29.8%)	0.052	1	<b>0.042</b>	6.810 (1.522-30.476)
Fairly control [9-10]	23(16.9%)	<b>0.010</b>	8.800 (1.664-46.531)		
Well control [7.6-8.9]	32(25.8%)	0.070	4.620 (0.883-24.181)		
Poorly control [>10]	34(27.4%)	<b>0.011</b>	7.891 (1.597-39.005)		
<b>Blood glucose intervals( g/L)</b>					
Normal[0.7-1.0]	73(61.3%)	<b>0.002</b>	1	<b>0.002</b>	5.243(2.079-13.217)
Diabetic[>1.25]	35(27.4%)	<b>&lt;0.001</b>	6.094 (2.256-16.461)		

Prediabetic[1.1-.25]	16(11.3%)	<b>0.047</b>	3.693 (1.020-13.379)		
<b>Treatment type</b>					
Oral Antidiabetics and Insulin	45(36.3%)	<b>0.048</b>	1	0.292	0.574 (0.238-1.387)
Oral Antidiabetics	51(41.1%)	<b>0.014</b>	0.267 (0.093-0.765)		
Insulin	28(22.6%)	0.452	0.667 (0.232-1.917)		
<b>Levels of education</b>					
Secondary	49(39.5%)	<b>0.044</b>	1		
Higher	21(16.9%)	0.461	0.539 (0.104-2.787)	<b>0.035</b>	0.333 (0.139-0.801)
Primary	54(43.5%)	<b>0.048</b>	2.562 (0.995-6.596)		

Table 8: Relationship between oral candida species infection and other risk factors

Factors	Frequency/ percent	P- Value	Odds ratio (CI)
<b>Wearing of urinary catheter</b>			
NO	117( 94.4%)	<b>0.006</b>	10.217 (1.863-56.043)
YES	7( 5.6%)		
<b>Wearing of dental prostheses</b>			
NO	111( 89.5%)	<b>0.042</b>	3.468 (1.059-11.354)
YES	13( 10.5%)		
<b>Type of diabetes</b>			
DT1	10(8.1%)	<b>0.041</b>	3.957 (1.056-14.830)
DT2	114(91.9%)		
<b>Oral hygiene</b>			
NO	83(66.9%)	0.566	1.310 ( 0.521-3.292)
YES	41(33.1%)		
<b>Hospitalization</b>			
YES	37(29.8%)	0.217	1.742 (0.721-4.204)
NO	87(70.2%)		
<b>long term antibiotics</b>			
NO	80(64.5%)	0.633	1.235(0.519-2.941)
YES	44(35.5%)		
<b>Alcohol consumption</b>			

NO	83(66.9%)	0.142	0.475 (0.176-1.284)
YES	41(33.1%)		
<b>Cigarette smoking</b>			
NO	107(86.3%)	0.920	0.940(0.280-3.149)
YES	17(13.7%)		
<b>Gender</b>			
Male	68(54.8%)	0.479	01.364 (0.578-3.216)
Female	56(45.2%)		

#### 4. Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels (hyperglycemia) due to defects in insulin production, insulin action, or both.<sup>1</sup> Diabetes mellitus is associated with an increased risk of oral infections, including those caused by *Candida* species. Poor glycemic control may predispose diabetic patients to higher rates of *Candida* colonization.<sup>7</sup>

This study was conducted in order to evaluate the relationship between different oral *Candida* species infections and glycemia levels as measured by HbA1c in diabetic patients. Additionally, we assessed the association of *Candida* species with other predisposing factors such as oral hygiene, alcohol consumption, smoking, dry mouth, and the use of dental prostheses. For this study, 124 diabetic patients were recruited after giving their consent, with more males 68(54.8%) than females 56(45.2%) and age ranging from 13 to 86 years old; mean age of 31.47±13.57 years, similar result was observed by Mubarak et al.<sup>16</sup> Type 2 diabetes was more prevalent 114(91.9%) than type 1 diabetes 10(8.1%), this is due to the limited number of type 1 diabetic participants.

Among the 124 patients screened for oral candidiasis in this study, 27(21.7%) patients had oral candidiasis, with *Candida albicans*(74.1%) being the most frequently isolated specie. This result correlates with the previous studies by Mohammadi et al.<sup>10</sup>, Mubarak et al.<sup>16</sup> and Samnieng et al.<sup>17</sup> who indicated that *C. albicans* was the most commonly isolated yeast in the oral cavities of diabetic patients. This high prevalence of *C. albicans* can be attributed to biological, ecological, and environmental factors as it can outcompete other *Candida* species and commensal bacteria for nutrients and space, undergo phenotypic switching to adapt to different environmental conditions, and also form biofilms with significant resistance to antifungal agents, making it difficult to eradicate the yeast.<sup>18</sup>

With respect to HbA1c, the uncontrolled group (fairly and poorly controlled HbA1c) showed a statistically significant relationship with oral candidiasis whereas the control group (normal and well controlled HbA1c) showed no statistically significant association with oral candidiasis. Nevertheless, the overall  $p=0.043$  indicated a statistically significant relationship. The odds of 6.810 (1.522-30.476) shows that patients with uncontrolled glycemia (HbA1c >9%) were 6.8 times more likely to have oral candidiasis as compared to those with better glycemic control (with prevalence of 7(5.6%) for well control, 8(6.4%) for fairly control, 1(0.8%) for normal control, 11(8.9%) for poorly control). Similarly, with the results from fasting plasma glucose, both the uncontrolled participants (prediabetic and diabetic) and the controlled participants (normal blood sugar) showed a statistically significant association with oral candidiasis, and the overall  $p$  value of 0.002 and odd ratio of 5.243(2.079-13.217) signified that uncontrolled participants were 5.2 times more likely to develop oral candidiasis than the control. Similar findings were obtained by Mubarak et al.<sup>15</sup> where in

their study, they found that *Candida* infection levels were increased in uncontrolled diabetics (HbA1c > 9%) compared to the well-controlled subjects (HbA1c < 6). Kumar et al.<sup>19</sup> also showed that the salivary candidal carriage was significantly higher in uncontrolled diabetics when compared with controlled diabetics and nondiabetic controls. Also, Ganapathy et al.<sup>20</sup> showed that there was a positive correlation between the blood glucose levels (post prandial sugar and fasting blood sugar) and the candidal colonization.

With respect to species distribution, this study found that *Candida krusei* and *Candida dubliniensis* were only present in the uncontrolled groups (HbA1c > 9.0% and FBS > 1.1g/L), i.e. severe cases of hyperglycemia, *Candida albicans* and *Candida glabrata* were present both in the uncontrolled and controlled groups. *Candida tropicalis* was only present in control groups (HbA1c < 9.0% and FBS < 1.1g/L) since it does not adapt well to these conditions and is more commonly linked to other forms of infections like bloodstream infections. Also, *Candida krusei* and *Candida albicans* resistance to common antifungals, ability to thrive in high glucose environments, improved biofilm formation and adherence properties can contribute to its higher prevalence in people with poorly controlled glycemia.<sup>9</sup> This result is in accordance with that of Bhuyan et al.<sup>21</sup> which showed a significant difference in frequency of *Candida* in poorly controlled diabetes when compared to moderately controlled, well controlled and normal control diabetic patients, with a higher number of colonies count among poorly controlled diabetes than well controlled, and a comparatively low number of non-albicans in the normal control individuals. This finding is in contrast with that of Suárez et al.<sup>22</sup>, Manfredi et al.<sup>23</sup> who showed that subjects with controlled diabetes presented more yeasts than the uncontrolled and no significant differences were noted among the presence of yeasts and HbA1c level of control of blood glucose. They suggested that growth of yeasts in the oral cavities of diabetic patients depends on other factors.

Based on other predisposing factors of oral candidiasis, patients who experienced dry mouth (xerostomia), were more likely to develop oral candidiasis, even though not statistically significant as P value of 0.472. This result partly correlates with that of Katebi et al.<sup>24</sup> as all 6 species of *Candida* were identified in patients with xerostomia. Those who did not experience dry mouth were less likely to develop oral candidiasis as just two species identified; *Candida albicans* and *Candida glabrata*. Similarly, Molek et al.<sup>25</sup> found that patients with xerostomia and hypo salivation had greater risk of developing oral candidiasis than the control groups, this can be because saliva is vital for maintaining oral health, cleansing food particles including *Candida*. Reduced saliva production can allow *Candida* to colonize and also lead to an acidic environment, promoting *Candida* growth.<sup>26</sup>

In this study, participants wearing urinary catheter were more likely to develop oral candidiasis than no catheter wearers. Similarly, Saint et al.<sup>27</sup> showed that the increased risk of oral candidiasis in patients with urinary catheters is multifactorial, involving a compromised immune system, antibiotic use, prolonged hospitalization and exposure to various medical interventions increase the risk of nosocomial oral candidiasis.

Concerning dentures, three species were identified for those wearing dentures; *C. albicans*, *C. glabrata*, and *C. krusei*. Five species were identified for those not wearing dentures; *C. dubliniensis*, *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*, this variable did not have a favorable influence on *C. tropicalis*. This result was significant with p-value of 0.042. Similarly, Lira et al.<sup>28</sup> and Rodríguez-Archilla et al.<sup>7</sup> indicated a positive connection between the prevalence of *Candida* and the usage of prosthesis (p-value 0.00\*), as oral candidiasis was linked to improperly fitted prostheses, poor oral hygiene, and continuous use of them. This result can be explained by the fact that dental prosthesis can facilitate *Candida* colonization due to rough surfaces, small porosities, and limited saliva flow, which can host germs and provide an ideal environment for *Candida*.<sup>26</sup>

Concerning oral hygiene, those who did not perform oral hygiene were more likely to develop oral candidiasis, as four species were identified *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. Those performing oral hygiene three species were identified; *C. dubliniensis*, *C. albicans* and *C. tropicalis*, no significant association was obtained. Similarly, Gaconet et al.<sup>29</sup> obtained no association between oral hygiene and fungal microbe growth rates, contradicting Muzurovic et al.<sup>30</sup> findings. This result can be explained by that; poor oral hygiene accumulates dental plaque and food particles, creating a nutrient-rich environment for *Candida* growth and biofilm formation, shielding the fungus from antifungal therapies.<sup>26</sup>

Furthermore, patients under long term antibiotics had an increased odd (1.2) of developing oral candidiasis than those who were not. This can be because, antibiotics intake may destroy the bacteria that prevent *Candida* from growing out of control.<sup>26</sup> Concerning alcohol consumption, nonalcoholic were more likely to develop oral candidiasis than alcoholics with an insignificant p value of 0.142. This result was partly in accordance with Khalili et al.<sup>31</sup> and Sheth et al.<sup>32</sup> who found no significant association between opium and alcohol consumption with the odds of oral candidiasis. This can be explained by that; non-drinkers may be on medications that disrupt oral flora and immune response and also due to diabetes. Also, Nonsmokers of cigarette were more likely to develop oral candidiasis, with p=0.920 even though not significant, this can be due to the limited sample size of this study. Similarly, Sheth et al.<sup>32</sup> found that even though smokers harbor elevated levels of *C. albicans*; however, there was no observed effect on the carriage of other species investigated, contradicted by Khalili et al.<sup>31</sup> who found a positive significant association. A plausible theory suggests that cigarette smoking reduces salivary flow rate and consequently decreases pH of saliva. This acidic environment may increase candidiasis development.

Concerning antidiabetic treatment as a risk factor, those taking both oral antidiabetic and insulin were more likely to develop oral candidiasis than those taking insulin or oral antidiabetics only. Even though the overall p value shows no significant association between oral candidiasis and treatment type, this finding can be explained by the fact that drugs like sulfonylureas and metformin lower glucose levels but can influence immune responses and cause side effects like dry mouth, which reduces saliva production and promotes *Candida* growth.<sup>32</sup> This study further showed no statistical significant relationship between hospitalization in intensive care unit and the development of oral candidiasis. Contradicted by Freire et al.<sup>33</sup> who found that clinical and subclinical forms of oral candidiasis are frequent in intensive care unit patients. This can be due to the fact that intensive care unit patients compromised immune systems, severe illness, and immunosuppressive drugs increase their susceptibility to oral candidiasis, with antibiotics, invasive procedures, and prolonged hospitalization exacerbating the risk.

Type 2 diabetes patients were more likely to develop oral candidiasis than the type 1 diabetes patients. This can be due to the limited number of type 1 diabetes participant recruited for this study. Contradicted by Rodríguez-Archilla et al.<sup>7</sup> that neither gender nor diabetes mellitus type conditioned fungal infections. Also, the level of education conditioned the probability of *Candida* species detection. This can be because lower levels of education are often associated with limited knowledge about proper oral hygiene practices. Individuals may not be aware of the importance of regular brushing and dental check-ups, leading to poor oral hygiene and risk of oral candidiasis.

According to gender, males were more likely to develop oral candidiasis than the females. However, gender did not condition the probability of *Candida* species oral infection or detection, with no statistically significant association. Similarly, to another study, neither gender nor diabetes mellitus type of diabetes conditioned fungal infections as determined by Rodríguez-Archilla et al.<sup>7</sup> Moreover, age groups did not condition the probability of *Candida* species in oral candidiasis, with no statistically significant association. The relationship between oral candida species and the duration of diabetes did not condition the probability of *Candida* species oral infection, with no statistically significant

association. Result similar to Shenoy et al.<sup>34</sup>, as a negative correlation of duration of the disease in years with the colonies of candida in diabetics can also be attributed to the better degree of oral hygiene because of improved awareness of the disease.

This study was unable to attain the required sample size due to time limit. Also, the limited number of health care facility could not enable the generalized characterization of the prevalence of these candida species in the entire population of diabetic patients.

## 5. Conclusion

From this research study carried out at the Bafoussam Regional Hospital on diabetic patients, with the main objective of evaluating the relationship between oral candida species infection and glycemic control management in diabetes patients, it can be noted that: The species of *Candida krusei*, *Candida parapsilosis* and *Candida dubliniensis* were only present in the uncontrolled groups (poorly controlled and fairly controlled). *Candida albicans* and *candida glabrata* were present both in the uncontrolled and controlled groups, and *Candida tropicalis* was just present in control groups (well controlled and normal controlled). Candida specie prevalence was lower for well controlled and for normal controlled, but higher for poorly controlled and fairly controlled. Several factors like glycated hemoglobin levels, the presence of a urinary catheter, fasting blood glucose levels, presence of dental prosthesis, type of diabetes and education levels were significantly associated with greater oral candida colonization. A shift in oral environment in diabetic patients results in the diversification of the Candida species. Patients with several risk factors (poor glycemic control and poor oral hygiene) had the highest rate of oral Candida infections. This underscores the importance of a multifaceted approach to treating and preventing these infections in people with diabetes. Addressing glycemic control alone may not be adequate; comprehensive care, including oral hygiene education programs, is required. Understanding these risk factors helps in focused prevention, early intervention, individualized therapy, and improved patient outcomes.

### Limitations of study

The calculated minimum sample size for this study was 164 participants. However, only 124 participants were ultimately recruited and included in the final analysis. This discrepancy was mainly due to limited availability of eligible participants during the study period, in addition to incomplete records that led to the exclusion of some cases and in some instances, refusal to participate. Despite not reaching the calculated sample size, all eligible participants available during the study period were included in order to minimize selection bias and preserve the integral validity of the study. Nevertheless, the achieved sample size still allowed meaningful analysis and interpretation in relation to the study objectives

### Future Perspectives

Based on the obtained results, we plan to expand study by increasing hospital facilities with diabetes care units and also study the population size to better assess glycemic control and identify factors link to poor control, conduct longitudinal studies on a large sample size, following patients over an extended period. These studies could provide more precise information on the evolution and complications in diabetic patients for a better understanding of risk factors and effective prevention.

### Conflicts of Interest

There were no conflicts of interest.

### Author Contributions

In this research project, all authors played integral roles across every phase of the work. Their contributions encompassed refining the manuscript's content to ensure clarity, coherence, and accuracy, as well as meticulously editing the language for consistency and readability. Additionally, they diligently verified data and references, enhancing the overall structure and flow of the document. This collaborative effort underscores the collective commitment of all authors to the quality and integrity of the research presented.

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### Additional information

Data management and analysis: After quantitative analysis of the results of glycated hemoglobin and blood plasma glucose, continuous variables are often converted into clusters, as they are much easier to analyze in blocks and make decisions. All data will be available upon demand from author.

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