The Oral Biology Parameter of the Diabetes Mellitus Type-2 Patients Relate to the Oral Candida Species Development

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Abstract

To evaluate the o*ral hygiene* index simplified of the diabetes mellitus type-2 patients related to the oral candida species development.

The 38 patients in this cross-sectional descriptive study had type 2 diabetes mellitus. Interviews were used to evaluate medical record data and patient demographic profiles. An oral rinse method was used to collect saliva for candida species culture. The colony counter is used to calculate candida species' isolation using culture on selective CHROM agar media.

The C. albicans was found in 77.27 percent of type 2 diabetes mellitus patients, C. tropicalis in 19.06 percent, C. parapsilosis in 3.50%, C. krusei in 0.17%, and C. glabrata in 0%. Furthermore, diabetes mellitus has a frequency of 28.95 percent in the poor category, moderate 25 65.79%, and good 5.26% in the oral biologic status of the OHIS. Diabetes mellitus with a sugar level of 200 mg/dL had the greatest influence on the development of Candida species, specifically C. albicans (73%), C. tropicalis (22%), C. parapsilosis 4%, and C. krusei 0.3%. Meanwhile, diabetes mellitus with a duration of less than five years favors the development of candida species, specifically C. albicans (75%), C. tropicalis (18%), C. parapsilosis 5%, and C. krusei (0.1%).

The subjects with diabetes mellitus tended to increase the development of oral candida species. Candida albicans was the most dominant (77.27%), followed by C. tropicalis (19.06%), C. parapsilosis (3.50%), and C. krusei (0.17%)). Subjects with a duration of diabetes mellitus under five years and a blood glucose level of more than 200 mg/dL showed an increase in oral candida species development, and type 2 diabetes influences oral health.

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Introduction

Diabetes mellitus is a diverse metabolic disease characterized clinically and genetically by abnormally elevated blood glucose levels, hyperglycemia, and dysregulation of carbohydrate, protein, and fat metabolism[1]. Type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus or adult-onset diabetes) is the most common type of diabetes, accounting for 90-95 percent of all diagnosed cases and an annual incidence of 650,000 new cases [2]. Diabetes types-2 is caused by peripheral insulin resistance, impaired insulin secretion, and increased glucose production in the liver. This type of diabetes usually manifests itself after the age of 40, and its prevalence increases with age, peaking between the ages of 65 and 74 [3].

Oral candidiasis is a common opportunistic infection of the oral cavity caused by a Candida species overgrowth, particularly *Candida albicans*[4]. Candida species growth is significantly increased in type 2 diabetes mellitus patients due to a decreased mucocutaneous barrier and immune response, and it can occur at any intraoral site. In patients with type 2 diabetes mellitus, an increase in salivary glucose concentration combined with a decrease in salivary secretion promotes Candida growth and adherence to oral epithelial cells [5].

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Diabetes mellitus is also reported to worsen oral health, which triggers the development of Candida species. It can reduce the biological function of the oral cavity [6]. Smoking has also been reported to contribute to an increase in oral Candida in patients with diabetes mellitus[7]. Abu-Elteen (2006) noted, in diabetic patients, there were 81.8% species of C. albicans and 76.9% in control patients[8]. Al-Attas et al. found C. albicans (68.9%), C. glabrata (11.1%), C. parapsilosis (6.7%), C. krusei (4.4%), C. tropicalalis (2.2%), and other fungal species (6.7%) from diabetic patients. In contrast, only 40% of C. albicans are present in control patients. Sharma (2017) studied in patients with diabetes mellitus were isolated C. albicans (54.36%), C. tropicalis (14.56%), C. krusei (4.85%), C. parapsilosis (1, 94%) of diabetic patients, and C. albicans (27%) isolated from non-diabetics[9].

The association with the development of oral Candida species in patients with type 2 diabetes mellitus was evaluated in this study, which was confirmed by patient demographic data and medical records, including current blood sugar levels and diabetes mellitus duration. This information can be used as a reference for oral therapy for Candida species infections also possibly used as a reference to the use of antifungal drugs that have sensitivity for each Candida species in patients with type-2 diabetes mellitus. Furthermore, with the oral biology parameter as an indicator of the assessment, a picture of the frequency of each species of oral Candida in patients with diabetes mellitus was obtained.

Materials and methods

The research subjects were 38 patients with type 2 diabetes mellitus treated at the Endocrine Polyclinic of the Internal Medicine Division of RSUZA Hospital, Banda Aceh, Indonesia. The research subjects were obtained through the purposive sampling technique, and the subject's saliva was taken to isolate the oral Candida species. Furthermore, the number of oral candida species was confirmed from the variable status of demographic data and medical record status consisting of gender, smoker, denture wearer, current blood cave levels, duration of diabetes mellitus, frequency of brushing teeth, and oral health status.

Criteria inclusion and exclusion

The following criteria were used to select subjects for this study: type 2 diabetes mellitus patients, both male and female, whose general condition allowed for specimen collection using the oral rinse technique, who were not taking antifungal drugs, and who were willing to participate in research. The subjects in this study were excluded because they were not type-2 diabetes mellitus patients, their general condition did not allow specimens to be taken using the oral rinse technique, they were taking antifungal drugs, and they were unwilling to be research subjects. Demographic data is information about the subject's name, age, gender, and address obtained from the identity sheet completed by the patient. Medical history data were obtained from RSUDZA medical records to determine the patient's type of diabetes, drug history, and disease history.

Informed Consent and Medical Record assessment

Participants in this study were required to sign an informed consent form. Subjects who met the inclusion criteria were informed about the study's benefits, risks, and stages. Furthermore, demographic data such as the subject's name, age, gender, and address were evaluated using the identity sheet completed by the patient. Furthermore, the medical record data was analyzed to determine the type of diabetes, drug history, and disease history.

OHIS Assessment

The *Oral Hygiene* Index Simplified *(*OHIS) performance was adopted by Al-Jasser (2021) [10]. Debris examination is carried out using the Debris Index. Measurements were made with a periodontal probe. The tooth surface is divided horizontally into three parts: cervical 1/3, middle 1/3, and incisal 1/3. The examination was carried out on the index teeth, namely teeth 11, 16, 26, 31, 36, and 46. The analysis was started on 16 buccal teeth, 11 facial teeth, 26 buccal teeth, 36 lingual teeth, 31 labial teeth, and 46 lingual. The periodontal probe was placed on the incisal 1/3 of the tooth and then moved towards the cervical 1/3.46. Furthermore, the examination results were recorded on the examination form.

Calculus examination is performed using the Calculus Index. A periodontal probe was used to examine six teeth, namely teeth 11, 16, 26, 31, 36, and 46. The examination began with 16 buccal teeth, 11 facial teeth, 26 buccal teeth,

36 lingual teeth, 31 labial teeth, and 46 lingual teeth. The gingival margin was swabbed with the periodontal probe both above and below the gingival margin. Direct observation of supragingival calculus is also possible. The inspection results are then recorded on the inspection form. Suppose one of these teeth is missing (it has been extracted, or the root is still present). In that case, an assessment is performed on the replacement teeth that have been assigned to represent it, namely (1) If the maxillary and mandibular M1 teeth are missing, the assessment is performed on the maxillary M2 or mandibular M2 teeth. the lower jaw (2) If the maxillary or mandibular M1 and M2 teeth are missing, the maxillary or mandibular M3 teeth are evaluated. (3) An assessment cannot be performed if the maxillary or mandibular M1, M2, and M3 teeth are missing. (4) If the maxillary right first tooth is missing, the assessment is performed on the first tooth's maxillary left. The examination cannot be performed if the maxillary right and left I1 teeth are missing. (5) If the mandibular left first tooth is missing, the assessment is performed on the mandibular right first tooth. (6) If both the left and right I1 teeth are missing, the assessment cannot be performed.

Collect the Saliva

The collected saliva used to oral rinse approach was adopted by Jo (2019) [11]**.** Saliva samples were collected using the oral rinse technique with the Briefly method. The patient was instructed to rinse his mouth for 60 sec with 10 mL of sterile PBS (phosphate-buffered saline) and then pour the rinse into a 15 mL sterile plastic tube. After that, the plastic tube is sealed and transported to the laboratory. For 10 min, the oral rinse was centrifuged at 3500 rpm to separate the supernatant and pellet. The supernatant was discarded, and only the pellet was kept. After that, the pellets were homogenized in 1 mL of phosphate buffer saline (PBS).

Culture and assessment of oral candida species

Per liter of Chromagar Candida (Paris) consisted of peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g), and a chromogenic mixture (2 g). Chromagar is provided in the form of white flour to be made in a volume of 1000 mL. Chromagar Candida powder was weighed as 4.77 grams with a volume ratio of 100 mL of distilled water. Chromagar powder, distilled water, and stirrer bar were then put into an Erlenmeyer, heated on a hot plate stirrer, and covered with aluminum foil until dissolved. Then pour it into a petri dish with a medium thickness of ± 0.5 cm and wait for it to cool a bit by covering the cup with paper[9].

The tube containing Chromagar was wrapped in black plastic and stored in the refrigerator at 40 ^{0}C. When used, please put it in the water batch first so that the Chromagar is liquid again. Saliva samples were collected using the oral rinse technique with the Briefly method. The patient was instructed to rinse his mouth with 10 mL of sterile PBS (phosphate-buffered saline) for 60 sec and discard the rinse results into a 15 mL sterile plastic tube. The plastic tube is then closed and taken to the laboratory. Oral rinse was centrifuged at 3500 rpm for 10 min to form supernatant and pellet. The supernatant was discarded, and the pellet was taken. The pellets were then homogenized using 1 mL of PBS.

Cultivation was carried out by inoculating 0.1 mL of Chromagar Candida medium. Inoculation was carried out using the streak quadrant technique. A sterile micropipette was used for inoculation on the surface of the medium according to a pattern, and the sample was slowly thinned. The cells separated spatially over all parts of the dish using a loop. The plates were incubated at 37 °C and observed for colony color at 48 h. Candida identification was determined based on the color of each colony species, namely C. albicans appeared in green, C. tropicalis appeared as a dark blue-gray colony, with a purple-red halo circle, C. parapsilosis appeared as a pale white colony. C. krusei was identified as a large colony, has a rough texture, and is pink with white edges, and C. glabrata colonies appear pink.

After identification of the colonies on each plate were counted using a colony counter. Colonies that grow do not always come from a single microorganism's cell because certain microorganisms form groups or chains. Based on this, the term Colony-forming unit (CFU) per ml is used. The colony requirements determined to be counted are that one colony is counted as one colony, two overlapping colonies are counted as one colony, several related colonies are counted as one colony, two colonies that are close together and can still be distinguished are counted as two colonies. Colonies that are too large, i.e., greater than half the cup area, are not

counted, and colonies less than half the plate area are counted as one colony.

Statistical Analysis

The oral growth of Candida from diabetes mellitus subjects with the number and types of Candida from various research variables were analyzed by Kruskal Wallis ($p<0.05$) with Pearson correlation r=1 as a marker of a strong relationship. Meanwhile, the ranking between groups on the number of oral candida species was checked by Wilcoxon Signed Ranks.

Results

This study examined the proliferation of Candida species in the mouths of 38 study subjects.

Variable	Amount (N)	Percentage (%)
Sex		
Male	27	71,05
Female	11	28,95
Smoker		
Yes	12	31,58
No	26	68,42
Denture wearer		
Yes	6	15,79
No	32	84,21
Blood glucose level		
<200 mg/dl	4	10,53
>200 mg/dl	16	42,10
No data	18	47,37
Period of Diabetes Mellitus		
<5 years	14	36,84
5-10 years	9	23,68
11-15 years	10	26,32
>15 years	5	13,16
Tootbrushing		
<1 time a day	0	0
1 time a day	1	2,63
>2 a day	37	97,37
OHIS		
Very good	0	0
Good	2	5,26
moderate	25	65,79
Poor	11	28,95

Table 1. Distribution and frequency of subject of type-2 diabetes mellitus.

According to the study's findings in Table 1, seven variables relating to the growth and type of candida species were examined in this study, including gender variables, smokers, denture wearers, blood sugar levels, duration of type 2 diabetes mellitus, frequency of brushing teeth, and oral cavity health. Each variable has a sub-variable that defines the link between the primary variable and the second variable that will be evaluated to determine the frequency and distribution of oral candida species. Additionally, type 2 diabetes in 38 participants tended to be associated with poor OHIS conditions, with 11 subjects (28.95%), 25 subjects (65.79 percent), and just two subjects (2. (5.26%). These findings demonstrate that diabetes affects OHIS. The criteria evaluated of OHIS used to the category, very good (0), good (0.1-1.2), moderate (1.3-3,0), and poor (3.1-6.0).

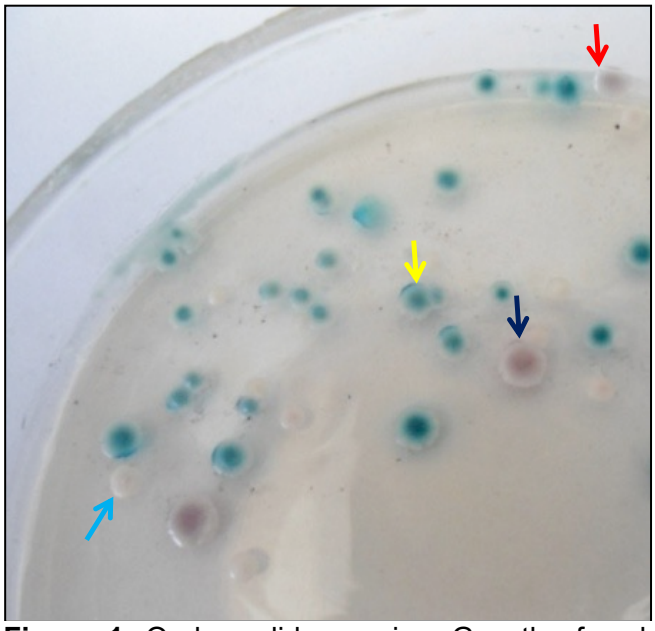

Figure 1. Oral candida species. Growth of oral candida species on CHROM agar selective medium. C. albicans is green (yellow arrows), C. tropicalis is dark blue-gray with a purple-red halo (red arrows), C. parapsilosis is white (black arrows) and C. krusei is pink with pale margins (blue arrows).

Oral Candida	N	Oral Candida Development (CFU/mL)					Friedman Test
		Mean	S.Dev	Total	Freq	Mean Rank	p
C. albicans	38	242	68.861	2209	77%	3.62	p<0.05 (0.00)
C. tropicalis	38	119	31.015	545	19%	2.53	
C. parapsilosis	38	58	10.663	100	3%	2.00	
C. krusei	38	4	0.665	5	0.2%	1.86	

Table 2. The frequency and distribution of oral candida species of type-2 diabetes mellitus patients.

The kind of oral candida species isolated from the saliva of a subject with type 2 diabetes mellitus is depicted in Figure 1. The color of the candida colony indicates the species of oral Candida. The diversity of candida species isolated from the saliva of persons with diabetes

mellitus (Table 2) provides insight into the association between diabetes mellitus and oral illness, particularly the prevalence *of C. albicans* as a risk factor for developing oral candidiasis.

Variable	Oral Candida species (N)				
	Colony (CFU/mL)	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. krusei*
Sex					
Male	2006	1688 (24)	272 (9)	42 (3)	4 (1:0.1)
Female	853	521 (10)	273 (5)	58 (1)	1 (1)
Smoker					
Yes	833	666 (12)	134 (3)	33 (2)	0 (0)
No	2026	1543 (20)	411 (10)	67 (2)	5 (2)
Denture wearer					
yes	579	498 (6)	49 (4)	32 (1)	0 (0)
No	2280	1711 (27)	496 (10)	68 (2)	5 (2)
Blood glucose levels					
<200 mg/dl	211	190(5)	21(2)	0 (0)	0 (0)
>200 mg/dl	1524	1110 (14)	342 (7)	67 (1)	5 (1)
No data	1124	909 (14)	182 (6)	33 (2)	0 (0)
Period Diabetes Mellitus					
<5 years	1115	852 (11)	203 (6)	59 (2)	1 (1)
5-10 years	602	509 (7)	93 (4)	0 (0)	0 (0)
11-15 years	837	674 (7)	131 (3)	32 (1)	0 (0)
>15 years	305	174 (5)	118 (2)	9 (1)	4 (1)
Dental brushing					
< 1 time a day	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 time a day	102	102 (1)	0 (0)	0 (0)	0 (0)
>2 time a day	2757	2107 (37)	545 (15)	100 (2)	5 (2)
OHIS					
Very good	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Good	27	12 (2)	15 (1)	0 (0)	0 (0)
Moderate	1684	1372 (21)	209 (10)	99 (2)	4 (1)
Poor	1148	825 (10)	321 (4)	1 (1)	1 (1)

Tabel 3. Distribution of the colony of oral candida species of type-2 diabetes mellitus.

The distribution of Candida species colonies in patients with diabetes mellitus based on research characteristics is shown in Table 3. The generation of candida species from each sub variable revealed distinctions within and between candida species. It can be explained by the fact that each variable evaluated affects the development of candida species. The *C. albicans* development was significantly greater than that of other candida species in people with diabetes mellitus for less than five years and a blood glucose level of more than 200 mg/dL. It suggests that the severity and duration of diabetes mellitus affect *C. albicans* development. Although there is no significant difference between and among the variables evaluated, there is a difference between and amongst the variables being analyzed.

Kruskal Wallis analysis revealed a significant difference in the quantity of oral candida species colonies between individuals with and without diabetes mellitus ($p<0.05$: 0.000), with an inverse connection (negative) r=-0.972. Meanwhile, the analytical factors revealed no significant difference in the growth of oral Candida species colonies between patients with and without diabetes mellitus ($p>0.05$: 0.96) with a negative inverse connection (r= - 0.202). It is suggested that oral Candida species did not affect the development of diabetes mellitus. Still, diabetes mellitus alters the oral biology status, including OHIS anomalies and Candida species development. The Wilcoxon Signed Ranks Test revealed significant differences in the number of colonies and variables investigated for each species ($p<0.05$; 0.00).

Discussion

The most often encountered *C. albicans* strain in this study was 2209 colonies (77.27%). Additionally, several publications reported that *C. albicans* are the most often overlooked species in the oral cavity of diabetic and healthy persons because *C. albicans* species have a higher virulence than non-*C. albicans* species, which results in increased adherence to the mucosal surface, resulting in colonization and infection. *C. albicans* also assimilate glucose, maltose, sucrose, and galactose, but not lactose [12].

The *C. tropicalis* was the study's second most abundant species, with 545 colonies (19.06 percent). *Candida tropicalis* is the second most pathogenic species after *Candida albicans*, possessing the ability to attach to epithelial cells in vitro and secrete a modest amount of proteinases[13]. Diabetes-induced rats were found to be more susceptible to *C. tropicalis* than normal rats. The *C. tropicalis* can ferment and assimilate glucose, maltose, sucrose, and galactose, but not lactose[14].

In our investigation, *C. parapsilosis* was the third most often seen species, accounting for 100 colonies (3.50%). The *C. parapsilosis* had a lower amount of adhesion and secretion to buccal epithelial cells than *C. albicans*. Numerous investigations have demonstrated that *Candida albicans* adheres to buccal epithelial cells 80–95 percent more strongly than *Candida parapsilosis*[15].Additionally, *C. parapsilosis's* biofilm development is less qualitatively and structurally complicated than *C. albicans*. In contrast to infections caused by Candida albicans and *C. tropicalis*, which are preceded by early colonization and are transmitted vertically

from mother to child, *C. parapsilosis* infection can occur without initial colonization and is frequently transmitted horizontally via contaminated external sources such as instruments and medical fluids, health personnel hands, prosthetic devices, and catheters[16]. The *C. parapsilosis* ferments only glucose; it does not ferment sucrose, lactose, maltose, or galactose. The *C. parapsilosis* also assimilates glucose, maltose, sucrose, and galactose, but not lactose[17].

The *C. krusei* was the least abundant species discovered in this investigation, with only five colonies (0.71%). The *C. krusei* may exist as a temporary commensal on mucosal surfaces or as a mucosal dweller in healthy persons. *C. krusei* had a weaker adhesion to buccal epithelial cells than *C. albicans*. Colonization of *C. krusei* on buccal epithelial cells may be increased due to antifungal medication therapy and is frequently observed in immunocompromised patients[18].The individuals who received preventive fluconazole had a higher rate of *C. krusei* colonization than those who did not. The *C. krusei* is unable to ferment or absorb sucrose, lactose, maltose, or galactose[19].

No colonies of *C. glabrata* were discovered in this investigation. It could be because *C. glabrata* has a low virulence level and produces insufficient hyphae, inhibiting tissue attachment and penetration. The *C. glabrata* demonstrated the least adherence in an in vitro examination of vascular endothelial adhesion than *C. albicans*, *C. tropicalis*, *C. krusei*, *C. kefir*, and *C. parapsilosis*. The Colonization with *C. glabrata* was linked with disease severity, prolonged hospital stay, and a history of antibiotic usage. The *Candida glabrata* is unable to ferment sucrose, maltose, lactose, and galactose. The *C. glabrata* also assimilates glucose and maltose, but not sucrose, lactose, or galactose.

Candida species colonization of the mouth was more prevalent in persons with diabetes mellitus than in non-diabetic subjects. According to some research, the Candida colony prevalence in diabetic people reaches almost 80%. In contrast, others state that most Candida in diabetic subjects can get more than 54%, and Candida albicans can reach 25–69%[20]. The increase in Candida colonies is associated with an increase in salivary glucose levels in people with diabetes. Increased glucose levels in the saliva of diabetes mellitus patients might increase the amount of food available to Candida, resulting in Candida overgrowth[21]. Candida performs cell metabolism in an aerobic environment by converting glucose to CO_2 and H_2O. Lactic acid, ethanol, and CO_2 are the fermentation products under anaerobic circumstances in oxidation and respiration processes also, the candida species use glucose as a carbon and energy source during the absorption process[22].

Additionally, the oral epithelium of diabetic people is a more favorable site for Candida adherence and colonization than the oral epithelium of non-diabetic subjects. It is due to inherently qualitative alterations in cell surface receptors in diabetes patients that modulate Candida adhesion. Number the 24 male individuals were located in 2006 colonies with an average score of 83.58, whereas ten female subjects were found in 853 territories with an average score of 85.30. Lao (2020) reported no significant difference in the number of Candida colonies between men and women in a study involving 103 people with diabetes, 49 of who had type 1 diabetes and 54 of whom had type 2 diabetes[23]. Twelve smokers discovered 833 colonies with an average of 69.42, while 22 nonsmokers discovered 2026 colonies with an average of 92.09. According to this study, smoking did not increase oral Candida species colonization in healthy persons. Sanita (2011) reported that smokers had a considerably greater Candida prevalence than nonsmokers in diabetes patients[24].

Candida infection and smoking are not entirely known mechanisms. According to some writers, smoking can result in localized epithelial alterations that favor colonization. In smokers, oral mucosal alterations can be caused by irritants, poisons, carcinogens created by smoking tobacco, high temperatures, changes in the oral cavity's pH, and a weakened immune system[25]. Smoking and nicotine can impair neutrophil activity, phagocytosis, and oxidative burst, impairing the body's regular fight against infection. Additionally, smoking is related to elevated plasma levels of adrenaline and noradrenaline, which results in peripheral vasoconstriction and a failure of the mouth cavity's wound healing systems[26].

Six patients using dentures discovered 579 colonies with an average of 96.50, while 28 subjects not wearing dentures found 2280 colonies with 81.43. Candida albicans was the

most often detected Candida colony in denture-wearing participants in this study, with 498 colonies, followed by *C. tropicalis* with 49 colonies, *C. parapsilosis* with 32 colonies, and no *C. krusei* or *C. glabrata* colonies. *C. albicans* was likewise shown to be the most prevalent pathogen among non-denture wearers, with 1711 colonies, followed by *C. tropicalis* (496 colonies), *C. parapsilosis* (68 colonies), *C. krusei* (5 colonies), and no *C. glabrata* colonies.

Lyon (2006) found that 64.2 percent of denture wearers and 19.4% of non-denture wearers in Brazil had Candida species. Additionally, the investigation discovered 71 isolates of Candida albicans, 15 isolates of *C. glabrata*, 12 isolates of *C. tropicalis*, 8 isolates of C*. parapsilosis*, and 3 isolates of *Candida krusei* [27]. Dentures promote the growth of Candida albicans, *Candida tropicalis*, and *Candida glabrata* [28]. *C. albicans* was the most isolated species, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. guillermondii*, according to Lotfi-Kamran research of 92 denture wearers, 46 diabetic patients, and 46 non-diabetic patients [29].

Dentures can promote Candida biofilm colonization and production in people with diabetes mellitus, resulting in candidiasis[30]. In denture wearers, increased colonization of oral Candida species can be attributed to various causes, including porous dentures induced by prolonged usage and the absence of a cleaning mechanism beneath the dentures. Salivary antimicrobial agents also have little effect on fungal proliferation beneath dentures[31]. Additionally, lowering the pH under the denture to 4-5 encourages Candida adhesion.[32]

In this investigation, four people with a glucose level of less than 200 mg/dL discovered 211 colonies with an average of 52.75. In contrast, fifteen subjects with a glucose level of greater than 200 mg/dl found 1524 colonies with an average of 101.60. At the1124 oral Candida colonies with an average of 74.93 were detected in 15 patients who did not have glucose level data. Odds et al. previously assessed glucose level in 204 diabetic individuals over two hours postprandial (pp), with one value for each mmol/l increase in blood glucose. A 0-5 indicates reasonable control, 6-10 indicates moderate control, while 11-15 indicates poor control. *C. albicans* was shown to be substantially less prevalent in well-managed diabetes patients than in poorly controlled diabetic patients. Additionally, the study revealed that the number of oral yeast colonies dramatically dropped within a few days when hyperglycemia was adequately controlled[33].

Tapper-Jones (1981) observed that patients with poorly controlled diabetes had more significant fungal counts than moderate or good management[34]. Diabetic patients with an assessment based on glycated blood hemoglobin (HbA1c) levels where less than 10% is regarded acceptable, 10%-12% is deemed moderate, and >12% is considered poor. The study concluded that while there was no correlation between the quality of diabetes control and the frequency or quantity of Candida isolation, the number of Candida was considerably higher in individuals with poor management than in those with good power [6]. In this study, 12 participants with type 2 diabetes for five years discovered 1115 oral Candida colonies with an average of 92.92, and 8 patients with type 2 diabetes for 5-10 years discovered up to 602 oral Candida colonies with an average of 75.25. There were 837 colonies with an average of 93.00 in 9 patients with a duration of 11-15 years and 305 colonies with an average of 61.00 in 5 subjects with a period of >15 years.

Diabetes' duration is frequently connected with irreversible and permanent functional and structural alterations in various cells. In the blood, glucose is converted to glycosylate products and tissue proteins. In patients with diabetes mellitus, the amount of glycosylating or other protein products at the bottom of the membrane of endothelial cells is significantly increased[35]. As a result, changes in the glycation process in diabetic individuals, may disrupt the carbohydrate moiety of epithelial cells, hence altering fungal cell attachment[36]. There were no subjects in this study who did not wash their teeth.

In this investigation, oral Candida was obtained in 102 colonies with an average of 102.00 against a single patient who washed their teeth once daily and 2757 colonies with an average of 83.55 against 32 subjects who brushed their teeth >2 times daily. Prevalence of oral Candida was much higher in participants who did not wash their teeth than in subjects who did brush their teeth, precisely 45.5 percent of 75 subjects who brushed their teeth 72.0 percent of the 25 non-brushing subjects[37].

There were no patients in this study who had an excellent OHIS. Oral Candida was detected in this investigation in 27 colonies from

two people with normal OHIS, with an average of 13.50 oral Candida colonies. In 22 subjects with moderate OHIS, 1684 colonies with an average of 76.55 were discovered; in ten subjects with severe OHIS, 1148 colonies with 114.80 were discovered. Inadequate OHIS is frequently cited as a risk factor for oral Candida colonization. According to this study, the dental plaque index and gingival condition did not affect the number and abundance of oral Candida. The previous study showed that 52% of 50 non-hospitalized people with signs and symptoms of oral candidiasis said that having a poor OHIS was one of the risk factors for oral Candida species overgrowth[38].

In conclusion, the diabetes mellitus subjects tended to enhance the candida species development in the oral cavity. *Candida albicans* was the most prevalent pathogen (77.27%), followed by *Candida tropicalis* (19.06%), *Candida parapsilosis* (3.50%), and *Candida krusei* (3.50%). (0.17%). Subjects with diabetes mellitus for less than five years and a blood glucose level greater than 200 mg/dL had a greater likelihood of developing oral Candida species. Additionally, type diabetes affects the biology of the oral cavity, as 11 people (28.95%) have a poor OHIS, 25 people (65.79%) have a good OHIS, and only two people have a good OHIS (5.26%).

Ethical approval

This study was reviewed and approved by the Ethics Committee of the Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia, reference number: 068/KE/FKG/2020

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Declaration of Interest

The authors report no conflict of interest.

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