

HbA1c Levels and Oral *Candida albicans* Burden in Patients with Type 2 Diabetes Mellitus

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Abstract: Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, which may increase salivary glucose levels and facilitate the growth of *Candida albicans* in the oral cavity. Glycated hemoglobin (HbA1c) is a widely used indicator of long-term glycemic control and may be associated with oral *Candida* colonization in patients with Type 2 Diabetes Mellitus. This study aimed to analyze the association between HbA1c levels and *Candida albicans* colony counts in oral-rinse specimens among patients with Type 2 Diabetes Mellitus. This analytical observational study used a cross-sectional design and included 33 patients with Type 2 Diabetes Mellitus at the Posyandu of Puskesmas Guntung Manggis, Banjarbaru, South Kalimantan, using total sampling. Data collection included blood glucose, triglyceride, and HbA1c measurements, as well as identifying and quantifying *Candida albicans* in oral-rinse specimens using CHROMagar and colony-forming unit (CFU/mL) counts. We assessed data normality using the Shapiro-Wilk test, followed by Spearman's rank correlation analysis. Most respondents were female (60.6%) and aged 55–64 years (48.5%). A total of 63.6% of respondents had HbA1c levels in the diabetic category ($\geq 6.5\%$). Regarding presumptive *Candida albicans* colony counts, 12.1% of respondents had elevated counts (400–1000 CFU/mL), while 9.1% had a high *Candida* burden (>1000 CFU/mL). Spearman's rank correlation analysis demonstrated significant positive correlations between *Candida albicans* colony counts and blood glucose ($r = 0.470$, $p = 0.006$), triglyceride levels ($r = 0.366$, $p = 0.036$), and HbA1c levels ($r = 0.382$, $p = 0.028$). These findings indicate that higher HbA1c levels are associated with higher presumptive *Candida albicans* colony counts in oral-rinse specimens among patients with Type 2 Diabetes Mellitus.

Keywords: *Candida albicans*; candida burden; glycemic control; HbA1c; type 2 diabetes mellitus.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia and is associated with various oral complications, including oral candidiasis. Hyperglycemia may increase salivary glucose levels, thereby creating a favorable environment for the growth and colonization of *Candida* species, particularly *Candida albicans*, in the oral cavity. Patients with DM may also experience alterations in salivary gland function and oral defense mechanisms, which can further increase

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susceptibility to oral *Candida* infection^{1,2}.

Oral candidiasis (thrush) is an opportunistic fungal infection of the oral cavity that commonly presents with white pseudomembranous lesions, particularly on the tongue and palate³. *Candida albicans* is one of the most frequently identified *Candida* species in the oral cavity and can exist as part of the normal oral microbiota. However, under favorable conditions, including metabolic disturbances associated with diabetes, *Candida* colonization may increase and contribute to clinical oral candidiasis⁴. Therefore, the presence and increased growth of *Candida albicans* are important microbiological findings in evaluating oral fungal conditions among patients with DM.

Glycated hemoglobin (HbA1c) is an important laboratory parameter for assessing long-term glycemic control in patients with diabetes. HbA1c reflects the average blood glucose concentration over approximately the preceding two to three months and is widely used to monitor and evaluate glycemic control in patients with diabetes mellitus^{5,6}. Unlike a single blood glucose measurement, HbA1c indicates prolonged glycemic exposure and is associated with the risk of diabetes-related complications^{5,7}. Persistent hyperglycemia may alter the oral environment and host defense mechanisms, potentially facilitating the growth of opportunistic microorganisms such as *Candida albicans*⁸. Thus, HbA1c levels may provide an important indicator for examining the relationship between long-term glycemic control and oral *Candida albicans* burden in patients with Type 2 Diabetes Mellitus.

Previous studies have reported *Candida albicans* in saliva among patients with Type 2 Diabetes Mellitus, including studies conducted in Indonesia^{9,10}. However, information regarding the association between HbA1c levels and the quantitative *C. albicans* burden among patients with Type 2 Diabetes Mellitus remains limited, particularly in the local population of Banjarbaru, South Kalimantan. Investigating this association may provide additional information regarding the relationship between long-term glycemic control and oral *Candida albicans* burden in this population. Based on this background, this study aimed to analyze the association between HbA1c levels and *Candida albicans* colony counts in oral-rinse specimens among patients with Type 2 Diabetes Mellitus.

MATERIALS AND METHODS

Study Design and Period

This study employed an observational analytic cross-sectional design to examine the association between glycemic and metabolic parameters and presumptive *C. albicans* colony counts in processed oral-rinse suspension among patients with Type 2 Diabetes Mellitus (T2DM). The study was conducted from January to March 2026 at the Health Polytechnic of the Ministry of Health Banjarmasin and the Posyandu of Puskesmas Guntung Manggis, Banjarbaru, South Kalimantan, Indonesia.

Study Population and Participants

The study population consisted of patients diagnosed with Type 2 Diabetes Mellitus who attended the Posyandu of Puskesmas Guntung Manggis during the study period. Participants were selected according to the predetermined eligibility criteria. The inclusion criterion was a previous diagnosis of Type 2 Diabetes Mellitus. A total of 33 respondents were included in the study and underwent blood and microbiological examinations.

Study Variables

The primary outcome variable was the *Candida albicans* colony count in the oral-rinse specimen, expressed as CFU/mL. The independent variables were blood glucose, blood triglyceride, and HbA1c levels. We considered HbA1c the primary metabolic parameter. We included blood glucose and blood triglyceride levels as additional metabolic parameters to provide a broader assessment of participants' glycemic and metabolic status. We recorded participant characteristics, including sex, age, occupation, and T2DM duration, as descriptive variables.

We analyzed blood glucose, blood triglyceride, and HbA1c levels as continuous variables for correlation analysis. We also categorized HbA1c levels for descriptive purposes as normal (<5.7%), prediabetes (5.7–6.4%), and diabetes (≥6.5%) according to the diagnostic categories established by the American Diabetes Association Professional Practice Committee for Diabetes¹¹. Because all participants had previously been diagnosed with T2DM, we used these categories only to describe the distribution of HbA1c values and not to establish a new diagnosis of diabetes.

Data Collection

We obtained data on participant characteristics using a structured questionnaire and participant records. The recorded characteristics included sex, age, occupation, and duration of T2DM. The laboratory examination data included blood glucose, blood triglyceride, and HbA1c measurements. Microbiological examination was performed using oral-rinse specimens to determine the presence and quantitative colony count of presumptive *C. albicans*.

Laboratory Examination

Blood glucose, blood triglyceride, and HbA1c levels were measured to assess participants' glycemic and metabolic status. Blood glucose levels were measured using an AutoCheck point-of-care testing (POCT) system. Blood specimens were collected according to the laboratory's standard blood collection procedure and analyzed using the AutoCheck system according to the manufacturer's instructions. The results were expressed in mg/dL.

Blood triglyceride levels were determined using the glycerol-3-phosphate oxidase-peroxidase (GPO-PAP) enzymatic colorimetric method with BioMaxima reagents. We collected and processed blood specimens according to the laboratory's standard operating procedure. We determined triglyceride concentrations using the GPO-PAP method and expressed them in mg/dL. We determined HbA1c levels using the VivaDiag point-of-care testing (POCT) system. We analyzed whole-blood specimens according to the manufacturer's instructions and expressed results as percentages (%).

Oral-Rinse Specimen Collection and Processing

We collected oral-rinse specimens using an oral-rinse sampling procedure. After completing the questionnaire, participants sat in a comfortable, relaxed position. Each participant rinsed their mouth with 10 mL of sterile distilled water for 30 seconds. Participants then expectorated the oral-rinse specimen into a sterile container. Each specimen container was labeled using a participant identification code and transported to the laboratory in a cool box for further processing. The oral-rinse sampling procedure was adapted from a previously reported oral-rinse sampling method¹².

The oral-rinse specimen was transferred into a sterile centrifuge tube and

centrifuged at 3,000 rpm for 15 minutes. Following centrifugation, the supernatant was discarded, and the remaining pellet was resuspended in 1 mL of sterile distilled water. The resulting suspension was subsequently used for microbiological examination and quantitative enumeration of *Candida* colonies.

Gram Staining

Gram staining was performed as a preliminary microscopic examination of the processed oral-rinse specimen. A smear was prepared from the processed specimen on a clean glass slide and fixed. The preparation was sequentially stained with crystal violet, Lugol's iodine, 95% acetone-alcohol as the decolorizing agent, and safranin as the counterstain. The stained preparation was examined microscopically using a 100× oil-immersion objective. The Gram-stained preparation was used to observe yeast-like fungal cells and provide preliminary morphological information.

Culture and Presumptive Identification of *Candida albicans*

A 25-μL aliquot of the processed oral-rinse suspension was transferred onto CHROMagar and evenly distributed using a sterile glass spreader. The inoculated plates were incubated aerobically at 37°C for 48 hours. Following incubation, colonies with morphology and coloration consistent with *C. albicans* were presumptively identified and enumerated. *Candida albicans* was presumptively identified based on the characteristic green to light-green colony coloration produced on CHROMagar Candida Differential Agar. According to the manufacturer's specifications, different *Candida* species produce characteristic colony colors on the medium. Therefore, colonies exhibiting the characteristic morphology and coloration of *C. albicans* were included in the presumptive quantitative enumeration.

Enumeration and Classification of *Candida albicans* Colony Counts

The number of presumptive *C. albicans* colonies was determined after incubation and expressed as CFU/mL of the processed suspension. Because 25 μL (0.025 mL) of the processed suspension was inoculated onto the agar plate, the colony count was calculated using the following equation:

$$\text{CFU/mL} = \frac{\text{Number of } C. \textit{albicans} \text{ colonies}}{0.025 \text{ mL}}$$

Because we inoculated 25 μL (0.025 mL) of the processed suspension, one recovered colony corresponded to 40 CFU/mL of the processed oral-rinse suspension. We adapted the quantitative culture procedure from a previously reported oral-rinse method for *Candida* enumeration¹².

For descriptive purposes, we operationally categorized presumptive *C. albicans* colony counts into three groups: low *Candida* burden (<400 CFU/mL), elevated *Candida* count (400–1000 CFU/mL), and high *Candida* burden (>1000 CFU/mL). The 400 CFU/mL threshold was derived from previous quantitative studies reporting higher salivary *Candida* counts among individuals with oral candidiasis compared with asymptomatic carriers^{4,13}. Previous studies have also used approximately 400 CFU/mL as a quantitative threshold for increased *Candida* burden¹⁴. However, quantitative *Candida* thresholds are not universally standardized and may vary by sampling procedure, culture method, study population, and clinical context⁴.

This study operationally defined the >1000 CFU/mL category as a high *Candida* burden, based on previous reports describing higher *Candida* concentrations in individuals with oral candidiasis than in asymptomatic carriers^{4,13}. We used this

classification for descriptive microbiological interpretation only and did not consider it a standalone diagnostic criterion for oral candidiasis.

Statistical Analysis

We analyzed data using IBM SPSS Statistics version 25. We performed descriptive analysis to summarize participant characteristics and laboratory findings. We presented categorical variables as frequencies and percentages, and summarized quantitative variables according to their distribution. We assessed the normality of continuous variables using the Shapiro-Wilk test. All variables showed p-values <0.05 , indicating deviation from normality. We then used Spearman's rank correlation test to assess associations between blood glucose, blood triglyceride, and HbA1c levels and *C. albicans* colony counts. We reported correlation coefficients (r) and corresponding p-values. We defined statistical significance as $p < 0.05$.

Ethical Considerations

The study was conducted after obtaining ethical approval from the Ethics Commission of the Faculty of Medicine, Poltekkes Kemenkes Banjarmasin, Indonesia, under approval number No. 118/KEPK-PKB/2026. All participants were informed about the study's purpose and procedures before data and specimen collection. We obtained written informed consent from each participant before participation. We kept participant identities confidential and assigned each specimen a unique identification code.

RESULTS AND DISCUSSION

Based on the study conducted, the characteristic data of the 33 Type 2 DM respondents were obtained as Table 1.

Table 1. Characteristics of the Study Participants (n = 33)

Characteristics	n (%)
Sex	
Male	13 (39.4)
Female	20 (60.6)
Age (years)	
25-54	12 (36.4)
55-64	16 (48.5)
>64	5 (15.2)
Employment status	
Not employed	20 (60.6)
Employed	13 (39.4)
Duration of diabetes (years)	
0-5	26 (78.8)
6-10	4 (12.1)
>10	3 (9.1)

Most respondents were female (60.6%) and in the 55-64 age group (48.5%). Most respondents were not employed (60.6%) and had a history of DM for 0 to 5 years (78.8%).

Laboratory Examination Results

Furthermore, laboratory examinations assessed blood glucose, triglyceride, and HbA1c levels, as well as *Candida albicans* colony counts in oral-rinse specimens. Blood glucose and triglyceride levels were measured as clinical and metabolic parameters, while HbA1c levels were assessed as an indicator of long-term glycemic control. We examined oral-rinse specimens for the presence and quantitative enumeration of *Candida albicans*. On CHROMagar, *Candida albicans* colonies were characterized by green coloration according to the manufacturer's interpretation¹⁵. Colony counts were subsequently determined and expressed as CFU/mL in the respondents' oral-rinse suspension. Below is some documentation of the plates showing the growth of *Candida albicans* colonies

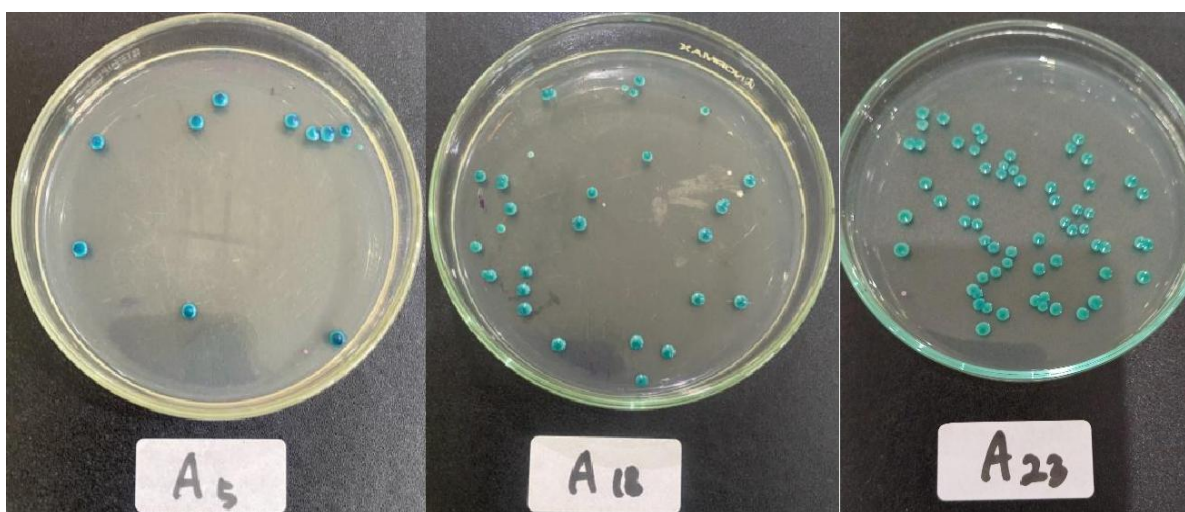


Figure 1. Greenish presumptive *C. albicans* colonies observed on CHROMagar. (A) Sample A5, (B) Sample A18, and (C) Sample A23.

HbA1c levels were distributed across three descriptive categories: normal ($<5.7\%$), prediabetes ($5.7\text{--}6.4\%$), and diabetes ($\geq 6.5\%$). We classified presumptive *C. albicans* colony counts into three operational categories: <400 CFU/mL, $400\text{--}1000$ CFU/mL, and >1000 CFU/mL. In this study, we used these thresholds as operational criteria to classify presumptive *C. albicans* colony counts in the processed oral-rinse suspension. The distribution of these findings is presented in Table 2.

Based on Table 2, more than half of the respondents (54.5%) had abnormal blood glucose levels, and 51.5% exhibited abnormal triglyceride levels. HbA1c evaluation showed that 63.6% of respondents fell into the Diabetes category. In the microbiological examination, most respondents (78.8%) had presumptive *C. albicans* colony counts in the low *Candida* burden (<400 CFU/mL). In comparison, 12.1% had elevated *Candida* counts ($400\text{--}1000$ CFU/mL) and 9.1% had a high *Candida* burden (>1000 CFU/mL).

Table 2. Distribution of Laboratory and Microbiological Findings Among Study Participants (n = 33)

Laboratory parameter	Category	n (%)
Blood glucose	Normal	15 (45.5)
	Abnormal	18 (54.5)
Triglycerides	Normal	16 (48.5)
	Abnormal	17 (51.5)
HbA1c	Normal (<5.7%)	9 (27.3)
	Prediabetes (5.7-6.4%)	3 (9.1)
	Diabetes (≥6.5%)	21 (63.6)
<i>C. albicans</i> colony count (CFU/mL)	Low Candida burden (<400 CFU/mL)	26 (78.8)
	Elevated Candida count (400 – 1000 CFU/mL)	4 (12.1)
	High Candida burden (> 1000 CFU/mL)	3 (9.1)

CFU/mL; colony-forming units per milliliter of processed oral-rinse suspension.

Statistical Analysis

We analyzed the laboratory data to determine correlations between blood glucose, blood triglycerides, HbA1c, and *Candida albicans* colony counts in oral-rinse specimens. Before the correlation analysis, we performed a normality test to determine the appropriate statistical method.

Table 3. Data Normality Test Results

Variable	Shapiro-Wilk p-value
Blood Glucose Level	<0.001
Blood Triglyceride Level	<0.001
HbA1c Level	0.004
<i>Candida albicans</i> Colony Count	<0.001

The Shapiro-Wilk normality test showed that all variables had p-values below 0.05, indicating that the data were not normally distributed. Therefore, we used the non-parametric Spearman's rank correlation test for subsequent analysis.

Table 4. Spearman's Rank Correlation Analysis between Laboratory Parameters and *Candida albicans* Colony Counts

Dependent Variable	<i>Candida albicans</i> Count	
	Correlations	Significance
Blood Glucose Level	0.470	0.006*
Blood Triglyceride Level	0.366	0.036*
HbA1c Level	0.382	0.028*

*Statistically significant at $p < 0.05$.

Based on Table 4, Spearman's rank correlation analysis showed statistically significant positive correlations between *Candida albicans* colony counts and blood glucose levels ($r = 0.470$, $p = 0.006$), blood triglyceride levels ($r = 0.366$, $p = 0.036$), and HbA1c levels ($r = 0.382$, $p = 0.028$). The correlation coefficient was highest for blood glucose, followed by HbA1c and triglyceride levels.

The demographic distribution showed that most participants with Type 2 Diabetes Mellitus (T2DM) were female (60.6%) and aged 55–64 years (48.5%). The predominance of older participants may be related to age-associated changes in immune function and salivary physiology, which may influence susceptibility to oral microbial colonization¹⁶. The higher proportion of female participants may also reflect hormonal changes that can influence oral mucosal conditions and susceptibility to fungal colonization, particularly among postmenopausal women¹⁷. However, this study did not assess menopausal status, so hormonal changes should be considered only as a possible explanation for the observed demographic pattern.

Blood glucose levels showed a significant positive correlation with presumptive *Candida albicans* colony counts in oral-rinse specimens ($r = 0.470$; $p = 0.006$), representing the highest correlation coefficient among the metabolic parameters assessed. Hyperglycemia may impair host immune responses and increase susceptibility to infection by altering immune function¹⁸. Increased blood glucose may also be associated with higher salivary glucose concentrations, providing a favorable environment for *C. albicans* growth and persistence¹⁹. Glucose availability may further influence *C. albicans* biofilm development and morphogenetic processes associated with pathogenic behavior²⁰. However, this study did not directly measure salivary glucose, immune parameters, biofilm formation, or fungal virulence characteristics.

HbA1c levels also showed a significant positive correlation with presumptive *C. albicans* colony counts ($r = 0.382$; $p = 0.028$). Higher HbA1c levels were associated with higher quantitative *C. albicans* burden in oral-rinse specimens. As HbA1c reflects average glycemia over approximately the preceding 2–3 months, this finding suggests that longer-term glycemic exposure may be related to oral *Candida* burden²¹. Chronic hyperglycemia may contribute to immune dysfunction and accumulation of advanced glycation end-products (AGEs), which can interact with AGE receptors (RAGE) and promote oxidative and inflammatory changes^{18,22}. Impaired metabolic control may also alter mucosal barrier integrity and local defense mechanisms, further facilitating *Candida* colonization²³. Nevertheless, these mechanisms were not directly assessed in the present study and therefore represent possible explanations rather than demonstrated pathways. Because of the cross-sectional design, do not interpret the observed association as causal.

Blood triglyceride levels also showed a significant positive correlation with presumptive *C. albicans* colony counts ($r = 0.366$; $p = 0.036$). Dyslipidemia commonly accompanies T2DM and may reflect broader metabolic dysregulation²⁴. Alterations in lipid metabolism may influence salivary composition and oral mucosal homeostasis, potentially affecting the local environment for microbial colonization²⁵. However, the relationship between circulating triglycerides and oral *Candida* burden may be indirect because we did not assess salivary lipid composition, inflammatory mediators, or secretory immunoglobulin A. Thus, the present finding indicates an association rather than a direct effect of triglycerides on fungal colonization.

Most participants (78.8%) had low presumptive *C. albicans* colony counts (<400

CFU/mL), while 12.1% had counts of 400–1000 CFU/mL and 9.1% had counts >1000 CFU/mL. Overall, 21.2% had counts $\geq$ 400 CFU/mL. Quantitative *Candida* counts may indicate microbial burden, although interpretation depends on sampling procedures, culture methods, and clinical context. Previous quantitative studies have reported 400 CFU/mL as a threshold associated with higher salivary *Candida* counts. In comparison, higher oral yeast counts, including $\geq$ 1000 CFU/mL, have also been reported among individuals with diabetes^{13,26}. However, these thresholds are not universal diagnostic criteria. *C. albicans* may exist as part of the normal oral microbiota, and changes in the host environment may facilitate its transition toward opportunistic pathogenic behavior²⁷. Because we did not perform clinical examination for oral candidiasis, interpret the colony counts in this study as a quantitative presumptive *C. albicans* burden rather than clinically diagnosed oral candidiasis.

Several limitations should be considered. The cross-sectional design prevents determination of temporal or causal relationships. In addition, *C. albicans* identification was presumptive and based on colony characteristics on differential culture medium, without biochemical, MALDI-TOF, or molecular confirmation. We did not assess other factors that may influence oral *Candida* colonization, including oral hygiene, denture use, medication use, salivary flow, salivary glucose, and local immune factors. Future studies using longitudinal designs, standardized clinical assessment of oral candidiasis, confirmatory *Candida* identification, and evaluation of relevant oral and metabolic factors are needed to clarify these relationships further.

CONCLUSION

Based on these results, HbA1c, blood glucose, and triglyceride levels were significantly associated with presumptive *C. albicans* colony counts in oral-rinse specimens among patients with Type 2 Diabetes Mellitus. Among the laboratory parameters assessed, blood glucose showed the highest correlation coefficient, followed by HbA1c and triglyceride levels. These findings indicate an association between glycemic and metabolic parameters and the quantitative burden of presumptive *C. albicans* detected in oral-rinse specimens. However, the colony-count categories used in this study were intended for descriptive microbiological interpretation and should not be considered a standalone diagnostic criterion for oral candidiasis. Further studies incorporating standardized clinical assessment of oral candidiasis are needed to clarify the relationship between *Candida* burden and clinically diagnosed oral candidiasis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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