

RESEARCH ARTICLE

Glycemic control and denture hygiene behaviors influence fungal colonization in older adult diabetic patients

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ARTICLE INFO

Article history:

Received 26 September 2025

Received in revised form 14 November 2025

Accepted 29 November 2025

Available online 30 November 2025

Keywords:

Denture contamination

Diabetes mellitus

Glycemic control

Oral hygiene

Candida spp.

Older adult

Denture stomatitis

ABSTRACT

Objective: Denture-associated infections are common among older adult diabetic patients, particularly those with poor glycemic control. Chronic hyperglycemia promotes fungal adhesion, biofilm formation, and impaired oral immunity, while few studies have investigated how blood glucose levels and hygiene behaviors jointly influence microbial colonization on dentures. This study aimed to investigate the association between glycemic control, denture hygiene behaviors, and fungal colonization among older adult diabetic denture wearers in Nakhon Si Thammarat, Thailand.

Methods: In this cross-sectional study of 80 diabetic denture wearers, we stratified participants by HbA1c status ($\leq 7.0\%$ vs $> 7.0\%$) and quantified denture fungal burden alongside hygiene practices. Denture swabs were cultured on Sabouraud dextrose agar for quantitative fungal enumeration and species identification using VITEK MS PRIME.

Results: Patients with poor glycemic control exhibited significantly higher fungal loads ($P < 0.001$). HbA1c emerged as the independent predictor in multiple regression analysis ($\beta = 0.35$, $P = 0.041$). Species level profiling revealed *Candida albicans* as dominant in uncontrolled patients followed by *C. glabrata*, *C. tropicalis*, *Trichosporon asahii*, and *C. nivariensis*. Conversely, *C. dubliniensis* and *C. pelliculosa* were uniquely identified in patients with good glycemic control. Hygiene practices revealed that brushing combined with chemical cleansing significantly reduced fungal concentration compared with rinsing with water alone ($P = 0.013$). Participants who cleaned their dentures after every meal also exhibited significantly lower fungal counts than those who cleaned only once daily ($P = 0.05$).

Conclusion: Poor glycemic control is strongly associated with denture mycobiome dysbiosis and increased fungal diversity in diabetic patients. The findings underscore HbA1c as a potential microbial risk indicator and emphasize the need for integrated diabetes management and targeted oral hygiene interventions to prevent denture-related fungal infections among older adults.

1. Introduction

Diabetes mellitus (DM) is a major global health problem, particularly among aging populations, and has been widely linked to oral opportunistic infections. Globally, more than 540 million adults are affected by DM, and projections estimate a 46 % increase by 2045, posing substantial challenges for health systems and healthy aging initiatives.¹ Previous studies have demonstrated that chronic hyperglycemia alters salivary composition, impairs innate immunity, and promotes fungal adhesion and biofilm formation on oral surfaces.^{2–4} As populations age

worldwide, denture use is increasing in parallel with diabetes prevalence, especially in low- and middle-income countries where access to oral care is limited. In Thailand, the prevalence of DM has steadily increased among adults aged 60 years or older, who also constitute the majority of denture wearers.^{5,6} Denture-associated infections, particularly denture stomatitis, are common in DM patients and can negatively impact nutrition, quality of life, and systemic health.^{7–9} Importantly, denture surfaces provide a reservoir for microorganisms, including opportunistic fungi, that may become pathogenic under conditions of poor glycemic control.^{10–17} While poor glycemic control is a key contributor

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to fungal overgrowth, denture-related stomatitis (DRS) is a multifactorial condition. Recent evidence shows that DRS can develop rapidly, even in new denture wearers, and that continuous denture use combined with inadequate cleaning substantially accelerates its onset.¹⁸ Beyond metabolic factors, behavioral practices such as sleeping with dentures and infrequent cleaning create favorable conditions for mixed *Candida* biofilm formation and mucosal inflammation.

Mechanistically, sustained hyperglycemia elevates glucose in saliva and crevicular fluid, providing fermentable substrates that enhance microbial adhesion, proliferation and biofilm maturation.^{19–21} Diabetes-related defects in neutrophil function and mucosal barriers hinder biofilm clearance, collectively fostering the development of robust, treatment-refractory biofilms on prosthetic surfaces.^{19,22–25} Importantly, hyperglycemia has been shown to enhance *Candida* adhesion, biofilm formation, and antifungal resistance, suggesting that glycemic control is a critical determinant of oral fungal ecology in diabetic patients.^{26,27} Hyperglycemia also augments *C. albicans* virulence by promoting adhesion to acrylic resin, yeast-to-hypha transition, and thicker, structurally stable biofilms, thereby increasing the likelihood of denture stomatitis and mucosal injury.^{26–29}

Several studies confirm that diabetic denture wearers exhibit significantly higher fungal counts and greater species diversity compared with non-diabetics.^{30–32} Meta-analytic evidences indicated that individuals with diabetes are at increased risk of oral *Candida* infection, and that denture wearing further doubles this risk, highlighting the acrylic–mucosal interface as a particularly susceptible niche for dysbiosis in this population.^{33–37} Notably, patients with uncontrolled diabetes consistently exhibit both higher fungal counts and greater species diversity than those well-controlled counterparts, reflecting the profound ecological shift induced by sustained hyperglycemia.^{2,38} While *Candida albicans* is classically dominant within denture biofilms, non-*albicans Candida* (NAC) and other opportunistic yeasts are increasingly recognized, especially among individuals with poor glycemic control.^{4,10,31,35,39,40} Consistent with recent clinical observations, *C. albicans* continues to be the most frequently identified species in denture-related stomatitis, followed by *C. glabrata*, which often coexists within mixed biofilms and contributes to disease persistence in poorly maintained prostheses.¹⁸ Beyond these two species, emerging opportunistic fungi have been documented more often in poorly controlled diabetes, raising concern for invasive disease in immunocompromised hosts.^{41–44} Uncontrolled glycemic patients often exhibit higher prevalence of opportunistic fungi, including *C. glabrata*, *C. tropicalis*, and emerging species (*Trichosporon asahii* and *Candida nivariensis*).^{41,42,45} These organisms are not only more difficult to eradicate due to their biofilm-forming capacity but also raise concern for systemic dissemination in immunocompromised hosts. Therefore, denture wearers with diabetes constitute a uniquely vulnerable group, as prosthetic surfaces provide a favorable niche for fungal colonization and biofilm development, further aggravated under hyperglycemic conditions.^{19,35,46} From a global health perspective, these findings are concerning because fungal infections contribute to the hidden burden of disease in aging and diabetic populations, yet they remain largely neglected in international oral health and noncommunicable disease (NCD) surveillance frameworks.

Although global literature has extensively addressed oral candidiasis and denture care in diabetes, few studies have simultaneously linked objective hemoglobin A1c (HbA1c) status, denture hygiene behaviors, and species-level fungal profiling within a single framework.^{38,47} Preliminary evidence suggests that higher HbA1c correlates with greater fungal load and that specific opportunistic pathogens may be over-represented in poorly controlled diabetes.^{48–50} Elevated glucose levels not only favor the proliferation of *Candida* spp. but can also enhance antifungal resistance and pathogenic virulence. However, most existing studies have focused on systemic or mucosal infections and on *Candida albicans* alone, with limited attention to how glycemic control (HbA1c) and denture hygiene behaviors influence the burden and diversity of oral fungi in older diabetic populations. The species mapping points to distinguish

mycobiome signatures by glycemic status, with certain opportunists appearing uniquely in uncontrolled diabetes. Moreover, little is known about whether modifiable denture care practices, such as brushing or soaking, can mitigate the microbial burden in this high-risk population. Recent evidence also indicates that non-brushing of the tongue and sleeping with dentures significantly increase oral microbial accumulation and halitosis, even among new complete denture wearers.⁵¹ Addressing these gaps aligns with global health priorities to promote oral health equity, reduce NCD comorbidities, and support healthy aging as outlined in the WHO Global Oral Health Action Plan (2023–2030). In Thailand, previous studies have primarily examined xerostomia, cariogenic bacteria, or general *Candida* carriage, with little emphasis on the ecological interplay between systemic glycemic control and denture-associated.^{52–54} This lack of integrative data is particularly concerning given Thailand's rapidly aging population and the increasing number of older adult individuals living with both diabetes and prosthetic devices.

To address these gaps, this study aimed to investigate the relationship between glycemic control, denture hygiene behaviors, and fungal colonization among older adult diabetic denture wearers in Nakhon Si Thammarat, Thailand. By combining quantitative measures of fungal concentration with species-level identification. The study hypothesized that poor glycemic control and inadequate denture hygiene behaviors are significantly associated with higher fungal concentration and greater species diversity on dentures among older adult diabetic patients. This study provides a comprehensive assessment of fungal concentration and diversity in relation to HbA1c among diabetic denture wearers, offering clinically relevant evidence to guide interdisciplinary care between dental practitioners and diabetes teams. Ultimately, these findings may inform preventive protocols that reduce denture-related morbidity and mitigate systemic risks linked to opportunistic fungal infections.

2. Material and methods

2.1. Study design and participants

This cross-sectional study was conducted between May to September 2025 at Sichol Hospital, Nakhon Si Thammarat, Thailand. The objectives and other important information in this study were explained and informed. All participants provided written informed consent prior to enrollment.

Participants were identified through the hospital's diabetes outpatient clinic records and prosthodontic service database. Consecutive eligible patients attending routine diabetes follow-up or denture maintenance appointments were invited to participate to minimize selection bias. The study population therefore represents older adult diabetic denture wearers regularly receiving care in secondary healthcare.

This study compares the prevalence of fungal infection between two patient groups with poor glycemic control and those with good glycemic control. Sample size was estimated based on differences in fungal prevalence between controlled and uncontrolled diabetes reported by,⁵⁵ using the formula for comparing two proportions.

$$n = \frac{\left(Z_{\frac{\alpha}{2}} + Z_{\beta}\right)^2 \left[p_1(1-p_1) + p_2(1-p_2)\right]}{(p_1 - p_2)^2}$$

Where n is required sample size per group; $Z_{\frac{\alpha}{2}}$ is Z-value for the chosen significance level (α), for $\alpha = 0.05$, $Z_{\frac{\alpha}{2}} \approx 1.96$; Z_{β} = Z-value corresponding to the desired power ($1-\beta$), for 80 % power, $Z_{\beta} \approx 0.84$; $(p_1 - p_2)$ is the minimum detectable difference in proportions (effect size); p_1 is 0.79 (79.4 % prevalence among poorly controlled diabetic denture wearers); p_2 is 0.50 (50 % prevalence among well-controlled diabetic denture wearers).

A total of 84 older adult patients with type 2 DM who were regular denture wearers were recruited. Inclusion criteria were: (1) age ≥ 50 years; (2) wearing removable complete or partial dentures for ≥ 3 months; and (3) confirmed diagnosis of DM. Exclusion criteria included:

(1) systemic immunosuppressive conditions (cancer, HIV infection); (2) use of antifungal or broad-spectrum antibiotic therapy within the preceding 3 months; (3) other oral diseases that may affect fungal infections; and (4) unwillingness to participate. Patients were stratified according to glycated hemoglobin (HbA1c) levels: controlled DM (HbA1c $\leq$ 7.0 %) and uncontrolled DM (HbA1c > 7.0 %).

2.2. Data collection

Demographic and clinical information was collected using structured questionnaires and medical records, including age, sex, educational attainment, occupation, HbA1c level, DM duration, medication, and comorbidities. Denture-related behaviors were assessed, covering type of denture, duration of use, removal habits, cleaning frequency, cleaning methods, and adherence to dental hygiene instructions. Lifestyle variables (smoking, alcohol intake, diet, xerostomia) were also recorded.

2.3. Ethics approval and consent to participate

The study protocol was reviewed and approved by the Human Research Ethics Committee of Nakhon Si Thammarat Provincial Public Health Office (study code: 103/2568) on 29 April 2025. Written informed consents were obtained from participants included in the study.

2.4. Denture sampling and microbial isolation

All procedures were conducted using standardized aseptic techniques and reproducible protocols, with clinical sample collection adapted from previously described methods.^{56–60} Denture biofilm samples were obtained immediately after denture removal in the clinic. Each denture was swabbed and placed in a sterile container containing 5 mL phosphate-buffered saline (PBS; pH 7.2; Merck; Darmstadt, Germany). Samples were placed in sterile PBS and subjected to ultrasonic bath sonication for 40 kHz, 5 min (Elma; Singen, Germany), followed by vortexing for 60 s using Vortex mixer (Vortex-Genie; Scientific Industries, Inc.; Bohemia, NY, USA) to detach adherent biofilm cells. A 1-mL aliquot of the suspension was serially diluted in 9 mL sterile PBS to prepare 10-fold dilutions. Aliquots of 100 μ L from each serial dilutions were spread-plated in duplicate onto Sabouraud Dextrose Agar (SDA; Oxoid; Basingstoke, UK) supplemented with chloramphenicol, 50 mg/L (Sigma-Aldrich; St. Louis, MO, USA) to inhibit bacterial growth. Plates were incubated aerobically at 25 $^{\circ}$ C for 48–72 h. Colony-forming units (CFU) were enumerated and expressed as CFU per denture. All procedures were performed under aseptic conditions.

2.5. Fungal identification

Representative colonies with distinct morphologies were subcultured for purification. Preliminary identification was based on colony morphology, and microscopic characteristics. For fungal species identification, a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system (MALDI-TOF MS; VITEK MS PRIME; bioMérieux; Marcy-l'Étoile, France) was used according to the manufacturer instructions. Quality control was ensured using reference strain *coli* ATCC 8739.

2.6. Statistical analysis

All data were analyzed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Shapiro–Wilk test and presented as mean $\pm$ standard deviation (SD). Comparisons between controlled and uncontrolled DM groups were performed using independent *t*-tests or Mann–Whitney U tests for continuous variables, and χ^2 or Fisher's exact tests for categorical variables. The association between HbA1c levels and fungal CFU counts was assessed using Pearson's correlation and linear regression analysis. Multivariable models were constructed to adjust for potential confounders,

Table 1
Demographic characteristics and factors associated with diabetes control among denture-wearing patients [*n* (%)].

Characteristics	Total	Controlled DM	Uncontrolled DM	<i>P</i>
Age				0.116
50– < 60 years	10 (12.50)	0 (0)	10 (19.23)	–
60– < 70 years	18 (22.50)	4 (14.29)	14 (26.92)	–
$\geq$ 70 years	52 (65.00)	24 (85.71)	28 (53.85)	–
Sex				0.718
Male	20 (25.00)	8 (28.57)	12 (23.08)	–
Female	60 (75.00)	20 (71.43)	40 (76.92)	–
Education				0.063
Primary school	66 (82.50)	18 (64.29)	48 (92.31)	–
High school	12 (15.00)	8 (28.57)	4 (7.69)	–
Diploma degree	2 (2.50)	2 (7.14)	0 (0)	–
Occupation				0.866
Unemployed	36 (45.00)	14 (50.00)	22 (42.31)	–
Business owner	10 (12.50)	4 (14.29)	6 (11.54)	–
Farmer	30 (37.50)	10 (35.71)	20 (38.46)	–
General employee	2 (2.50)	0 (0)	2 (3.85)	–
Aquatic culture	2 (2.50)	0 (0)	2 (3.85)	–
DM duration				0.225
< 1 year	6 (7.50)	4 (14.29)	2 (3.85)	–
1– < 6 years	16 (20.00)	2 (7.14)	14 (26.92)	–
6– < 10 years	10 (12.50)	6 (21.43)	4 (7.69)	–
$\geq$ 10 years	48 (60.00)	16 (57.14)	32 (61.54)	–
Insulin				0.602
No	62 (77.50)	4 (14.29)	4 (7.69)	–
Yes	18 (22.50)	24 (85.71)	48 (92.31)	–
Oral antidiabetic drugs				0.453
No	8 (10.00)	24 (85.71)	38 (73.08)	–
Yes	72 (90.00)	4 (14.29)	14 (26.92)	–
Dietary control				0.602
No	72 (90.00)	24 (85.71)	48 (92.31)	–
Yes	8 (10.00)	4 (14.29)	4 (7.69)	–

DM: Diabetes mellitus; –: Not applicable.

including age, sex, denture duration, and hygiene behaviors. Statistical significance was defined as *P* < 0.05.

3. Results

3.1. Demographic characteristics of denture-wearing patients

Eighty of the 84 participants (95.24 %) successfully completed the questionnaire. In this study, most participants were aged over 70 years (65.0 %), particularly in the controlled diabetes group (85.71 %) as shown in Table 1. Most participants were female (75.0 %), with no significant sex differences between two groups (*P* = 0.718). Primary education was predominant (82.5 %), especially among those with uncontrolled diabetes (92.31 %), whereas higher education levels were slightly more common in the controlled group, with no statistical significance (*P* = 0.063). The most common occupations were unemployment (45.0 %) and farming (37.5 %), with similar distributions across both groups (*P* = 0.866). Most had lived with diabetes for over 10 years (60.0 %), with no significant difference between two groups (*P* = 0.225). Insulin use was relatively low overall (22.5 %) but slightly higher in the uncontrolled group (92.31 %) than in the controlled group (85.71 %) (*P* = 0.602). Use of oral antidiabetic drugs was common (90.0 %), with no significant group differences (*P* = 0.453). Dietary control was rarely practiced (10.0 %) and similarly uncommon in both groups (*P* = 0.602).

3.2. Underlying diseases and association with glycemic control

A high proportion of participants (82.50 %) had at least one underlying disease, with comparable prevalence in the controlled (85.71 %) and uncontrolled DM groups (80.77 %, *P* = 0.529). Hypertension was the most frequently reported comorbidity (77.5 %), slightly more prevalent in the controlled group (85.71 %) than the uncontrolled group

(73.08 %), though not statistically significant ($P = 0.453$). Hyperlipidemia was present in 52.5 % of participants, with a higher proportion in the controlled group (64.29 %) compared to the uncontrolled group (46.15 %) ($P = 0.333$). Furthermore, other underlying diseases including heart disease, thyroid disorder, gout, heart valve regurgitation, and kidney disease, were infrequent (ranging from 2.50 % to 7.50 %), with no significant differences between two groups ($P > 0.05$).

3.3. Denture use and maintenance behaviors

Most participants used full dentures (87.50 %), with a comparable distribution between those with controlled diabetes (85.71 %) and uncontrolled diabetes (88.46 %). The majority had worn dentures for 1–5 years (47.50 %), followed by more than 5 years (27.50 %) and less than 1 year (25.00 %). Most participants reported removing their dentures before bedtime (90.00 %), particularly in the uncontrolled diabetes group (96.15 %). Only 20.00 % regularly cleaned their dentures, and a similarly small proportion (20.00 %) reported sleeping without wearing dentures, which was more common among the controlled group (35.71 %) than the uncontrolled group (11.54 %). Most participants were edentulous (80.00 %), and only 20.00 % reported brushing their remaining teeth more than twice per day. Tongue cleaning was performed regularly by 65.00 % of participants, occasionally by 17.50 %, and never by 17.50 %. Mouthwash use was uncommon (10.00 %), with most participants (90.00 %) reporting they never used it. In addition, 70.00 % of participants received denture cleaning instructions and followed them, whereas 22.50 % had received guidance but did not follow it, and 7.50 % had never received any instruction. Furthermore, most participants reported using brushing alone (50.00 %), only 27.50 % used a combination of brushing and chemical cleansers. The use of denture cleansing tablets (5.00 %) and water alone (17.50 %) was relatively low.

3.4. Health risk behaviors and oral conditions

Most participants were non-smokers (82.50 %), with only a small proportion currently smoking (12.50 %) or having stopped for more than five years (5.00 %). Regarding alcohol consumption, the majority (92.50 %) reported never drinking, while only 7.50 % consumed alcohol 1–3 times per month. In terms of dietary behavior, nearly half of the participants (47.50 %) followed dietary recommendations for diabetic patients, whereas approximately one-third (32.50 %) consumed a general diet, and 20.00 % reported irregular dietary control. For sweet food consumption, over half (52.50 %) reported never consuming sugary foods, while 30.00 % consumed them 2–3 times per week, and 17.50 % once per week. Xerostomia (dry mouth) was infrequently reported; 75.00 % of participants never experienced it, 22.50 % reported occasional symptoms, and only 2.50 % experienced it frequently. In addition, soreness under the denture base was reported by only a few participants (5.00 %), while the vast majority (95.00 %) reported no such symptoms.

3.5. Glycemic control and fungal concentration on dentures in diabetic patients

The fungal concentration (CFU) on dentures was significantly higher in patients with uncontrolled diabetes ($4.86 \pm 5.23 \times 10^5$ CFU) compared to those with controlled diabetes ($5.01 \pm 8.13 \times 10^4$ CFU), with a statistically significant difference ($P = 0.004$) (Fig. 1A). In addition, the glycated hemoglobin (HbA1c) level was significantly higher in the uncontrolled group (8.47 ± 1.06 %) than in the controlled group (6.11 ± 0.67 %, $P < 0.0001$) (Fig. 1B).

3.6. Demographic factors associated with glycemic control in diabetic patients

The correlation analysis revealed a statistically significant positive association between age and glycemic control ($r = 0.339$, $P = 0.032$),

indicating that older individuals tended to have better glycemic control than younger individuals. In addition, a significant positive correlation was found between education level and glycemic control ($r = 0.358$, $P = 0.023$), suggesting that individuals with higher educational attainment were more likely to achieve better blood glucose control. However, no other demographic factors were significantly associated with glycemic control.

3.7. Association between blood glucose levels and denture fungal load

Pearson correlation analysis revealed that HbA1c levels were significantly and positively correlated with fungal concentration on dentures ($r = 0.352$, $P = 0.026$), whereas no other demographic, behavioral, or clinical variables showed significant correlations. Consistently, multiple regression analysis identified HbA1c as the only independent predictor of denture fungal burden ($B = 1.12 \times 10^5$, $\beta = 0.35$, $P = 0.041$). Although the statistical association is moderate, each 1 % increase in HbA1c corresponds to an estimated rise of 1.12×10^5 CFU per denture, suggesting that a 2 %–3 % HbA1c elevation could approximately double the fungal load relative to well-controlled diabetic patients. Other variables, including diabetes mellitus duration, denture cleaning method, duration of denture use, cleaning frequency, and xerostomia did not reach statistical significance. Overall, the model explained 29.5 % of the variance in fungal counts ($R^2 = 0.295$), although the adjusted R^2 was modest (0.114).

3.8. Factors associated with fungal concentration on denture

The average fungal concentration on dentures was significantly influenced by hygiene behaviors (Fig. 2). Participants who used a combination of denture brushing and chemical cleansers exhibited a significantly lower mean CFU count (2.13×10^5 ; 95 % CI: 2.62×10^4 to 4.52×10^5 ; $P = 0.013$) compared to those who used brushing alone (2.45×10^5 ; 95 % CI: 3.27×10^4 to 4.58×10^5), cleanser tablets without brushing (8.80×10^5 ; 95 % CI: 1.92×10^4 to 3.68×10^6), and water rinsing alone (6.20×10^5 ; 95 % CI: 1.04×10^4 to 1.14×10^6). Cleaning frequency also showed a significant impact on fungal concentration, with participants who cleaned their dentures after every meal exhibiting a lower mean CFU count (2.71×10^5 ; 95 % CI: 1.10×10^5 to 4.31×10^5) compared to those who cleaned only once daily (5.87×10^5 ; 95 % CI: 1.52×10^5 to 1.02×10^6 ; $P = 0.05$).

3.9. Fungal species isolated from dentures among diabetic patients

Based on the analysis of yeast species and their proportions isolated from dentures of diabetic patients, categorized by glycemic control status, a total of 11 yeast species were identified, as shown in Fig. 3. The most prevalent species was *Candida albicans*, accounting for 81.25 % of isolates in the uncontrolled diabetes group and 18.75 % in the controlled group. *Candida glabrata* was also frequently detected, found in 75.00 % of the uncontrolled group and 25.00 % of the controlled group. *Candida tropicalis* was present in both groups, with a higher prevalence in the uncontrolled group (62.50 %) compared to the controlled group (37.50 %). In addition, certain yeast species were exclusively found in the uncontrolled group, including *Candida guilliermondii*, *Candida nivariensis*, *Kloeckera apis*, and *Trichosporon asahii*, each representing 100 % of detections within that group. In contrast, *Candida dubliniensis* and *Candida pelliculosa* were found only in the controlled group (100 %).

A bipartite network analysis revealed distinct patterns of fungal distribution between diabetic patients with good glycemic control (Control) and those with poor control (Uncontrolled) as shown in Fig. 4. Among fungi, *Candida albicans*, *C. glabrata*, *C. tropicalis*, *Saccharomyces cerevisiae*, and *Rhodotorula mucilaginosa* were detected in both groups, whereas *C. guilliermondii*, *C. nivariensis*, *Kloeckera apis*, and *Trichosporon asahii* were exclusively associated with the uncontrolled group. *C. dubliniensis* and *C. pelliculosa* were only found in the control group.

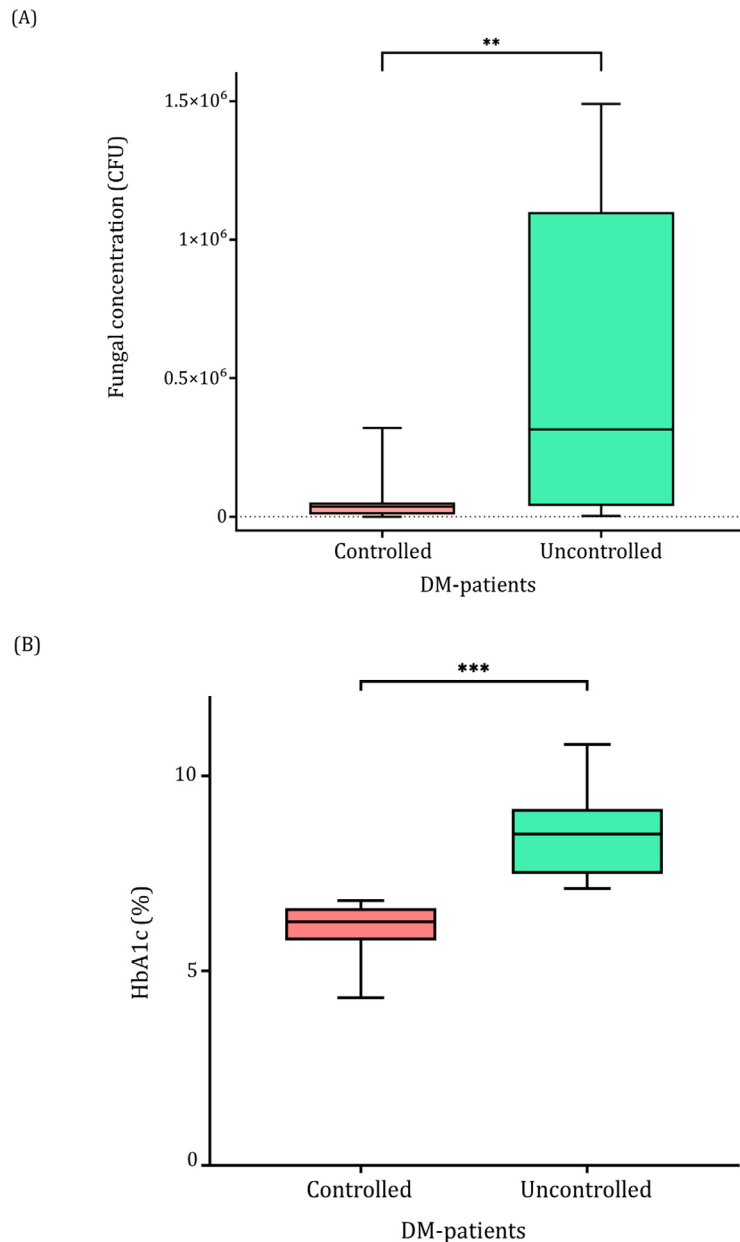


Fig. 1. Fungal concentration on dentures (A) and blood glucose levels (B) between controlled and uncontrolled diabetes groups. ** $P < 0.05$; *** $P < 0.001$. DM: Diabetes mellitus; CFU: Colony forming unit; HbA1c: Hemoglobin A1c.

4. Discussion

The study hypothesized that poor glycemic control and inadequate denture hygiene behaviors are significantly associated with higher fungal concentration and greater species diversity on dentures among older adult diabetic patients. The present findings support this hypothesis, demonstrating that participants with poor glycemic control exhibited significantly higher fungal loads and broader species diversity than those with good glycemic control. HbA1c emerged as the only independent predictor of denture fungal burden, highlighting the strong link between metabolic control and oral microbial ecology. In addition, inadequate denture hygiene practices, particularly limited mechanical cleaning and infrequent postprandial care, were associated with elevated fungal counts, reinforcing the role of behavioral factors in modulating oral fungal colonization.

This study provides a comprehensive analysis of factors influencing denture-associated microbial colonization in diabetic patients, integrat-

ing host demographic variables, glycemic status, and hygiene behaviors. For demographic perspective, age and educational attainment emerged as significant predictors of glycemic control, with older patients and those possessing higher education levels demonstrating lower HbA1c values.

In this cross-sectional study of older adults with diabetes wearing dentures, glycemic status emerged as the primary correlation of denture fungal burden. Patients with uncontrolled diabetes exhibited significantly higher fungal concentrations, averaging $(4.86 \pm 5.23) \times 10^5$ CFU more than controlled patients $[(5.01 \pm 8.13) \times 10^4, P = 0.004]$. The previous meta-analysis reports threefold higher odds of oral *Candida* in diabetes with poor glycemic control and twofold with denture use, aligning with our higher CFU in uncontrolled patients.⁴ Our findings reinforce the concept that hyperglycemia was associated with an ecological imbalance favoring greater microbial proliferation within denture biofilms. In multivariable regression, each 1 % increase in HbA1c was associated with approximately 1.1×10^5 additional CFU per den-

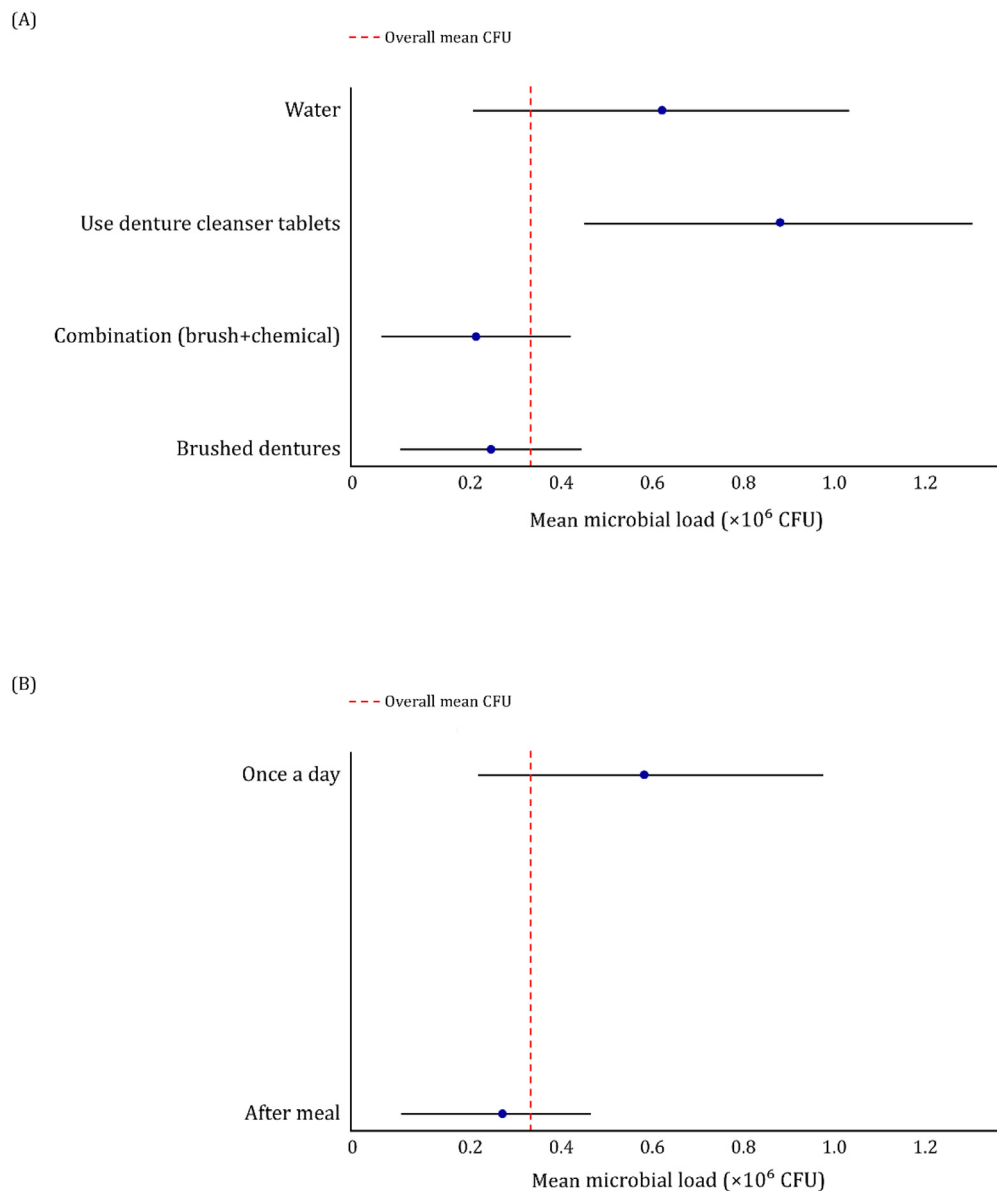


Fig. 2. Forest plot of association between denture cleaning methods (A) and cleaning frequency (B) with fungal concentration. CFU: Colony forming unit.

ture ($B = 1.12 \times 10^5$, $\beta = 0.35$, $P = 0.041$), underscoring glycemic control was the principal factor associated with variations in microbial burden. This association is biologically plausible, as elevated salivary glucose supplies a nutrient source for microbial proliferation, while hyperglycemia simultaneously compromises innate defenses including neutrophil activity, mucosal integrity, and salivary secretion, thereby limiting biofilm clearance and promoting persistence on prosthetic surfaces.^{2,3,19,24,44,46,61–64} In addition, elevated HbA1c levels were correlated with higher salivary glucose concentrations, which may facilitate microbial adhesion, proliferation, and biofilm formation of *Candida spp.*^{20,21,26,28,65} In contrast, patients with well-controlled HbA1c likely maintain more stable salivary composition and immune surveillance, attenuating denture biofilm accumulation.^{19,66–68}

Our findings emphasize HbA1c as the principal determinant of denture fungal colonization in diabetic patients. Both correlation and regression analyses consistently identified glycemic status as the only significant predictor of fungal burden, with each incremental rise in HbA1c associated with a substantial increase in CFU counts. Although behavioral variables such as cleaning method and frequency, duration of denture use, and xerostomia did not reach statistical significance in this

model, their clinical importance should not be discounted, as they may exert effects not captured in this cross-sectional design. The modest adjusted R^2 further indicating that most variation remains attributable to unmeasured host, prosthetic, or behavioral determinants. Evidence from previous studies demonstrated that continuous denture wearing, poor cleaning, and non-brushing of the tongue substantially increase microbial accumulation, halitosis, and risk of denture-related stomatitis, underscoring the importance of comprehensive oral hygiene practices for diabetic denture wearers.^{18,51} Collectively, these results highlight the central role of long-term glycemic control in managing oral microbial risk among denture wearers with diabetes. Our finding align with evidence that sustained hyperglycemia promotes biofilm maturation and virulence expression in oral microorganisms, particularly *Candida* species.^{3,27,38,40,69,70} While glycemic control remains a primary determinant, other contributors such as denture hygiene, salivary flow, prosthesis age, and antimicrobial use likely modulate microbial levels.^{39,71–74}

Denture wearers are particularly prone to oral candidiasis due to the conducive environment for *Candida* colonization provided by the denture surfaces.¹⁰ Denture surfaces constitute a reservoir for *Candida*

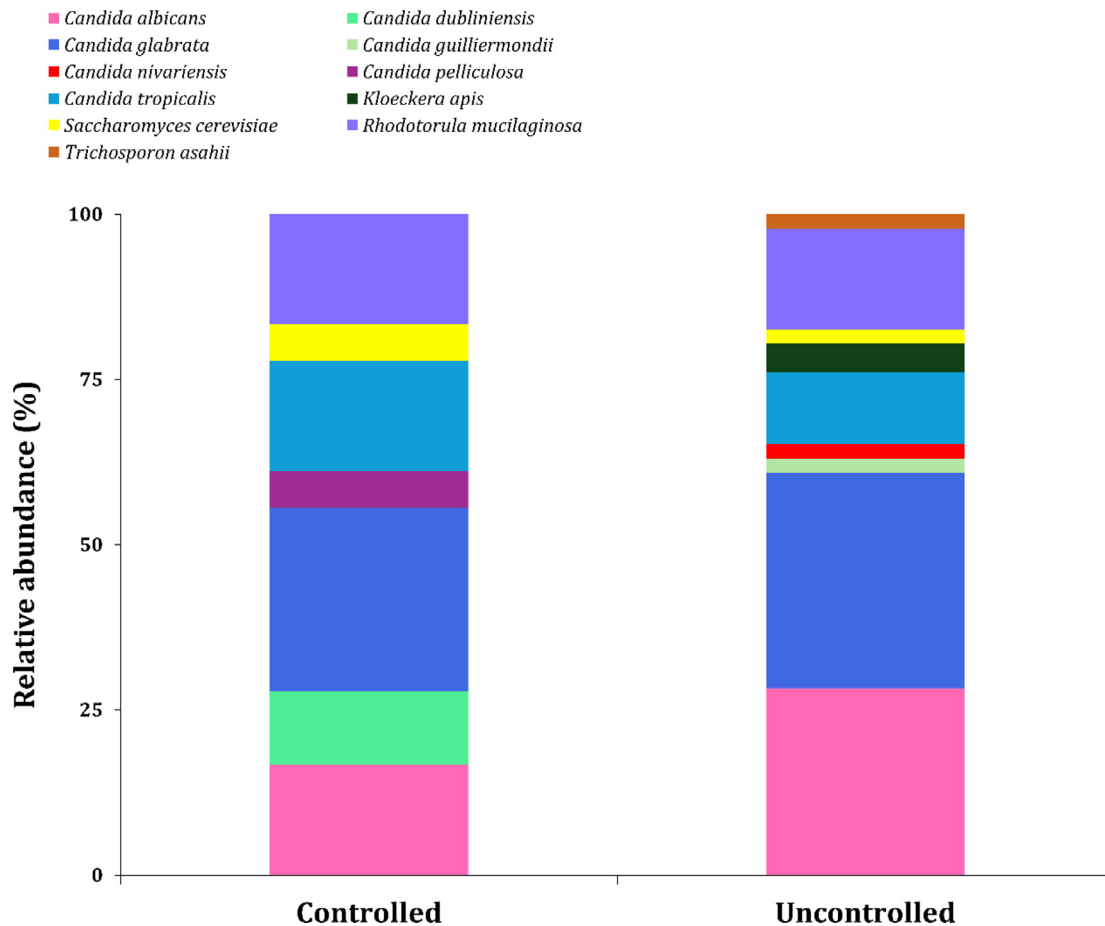


Fig. 3. Proportions of fungal species isolated from dentures among diabetic patients.

species and other opportunistic organisms, whose biofilm-forming potential is exacerbated under elevated glucose conditions, resulting in enhanced adhesion, accelerated biofilm development, biofilm density, and virulence factor.^{11–17} The present study demonstrates that denture hygiene behaviors had a substantial impact on microbial colonization levels. Brushing denture was associated with significantly lower CFU counts compared to not brushing. This finding is consistent with established evidence that mechanical disruption of denture biofilms is a critical step in reducing microbial biomass, including pathogenic *Candida* species, and in preventing denture stomatitis.^{71,75–78} Mechanical cleaning is particularly effective at removing mature biofilm layers that chemical rinses alone may not adequately disrupt.

Interestingly, denture cleaning using only water or cleanser tablets was associated with higher microbial loads. This suggests that, although perceived as adequate hygiene practices, these methods are insufficient for effectively removing adherent biofilms. Moreover, reliance on such approaches may give patients a false sense of cleanliness, potentially leading to the neglect of more effective mechanical or combined cleaning strategies.^{79,80} Effective denture cleaning requires the use of chemical solutions such as sodium hypochlorite, chlorhexidine, or effervescent tablets, often in combination with mechanical cleaning methods to ensure thorough removal of biofilms and reduction of microbial load.^{79,81–83} Integrating brushing with adjunctive cleaning methods, such as soaking in sodium hypochlorite or using denture cleansers, further enhances the reduction of microorganisms. Notably, brushing with denture cleansers was more effective than brushing alone in reducing viable biofilm.^{76,78,81,84} In addition, cleaning frequency was also influential, with cleaning after every meal yielding lower microbial counts than once-daily cleaning, supporting previous findings that frequent cleaning

disrupts biofilm formation cycles.^{78,85,86} Regular brushing of dentures, along with other hygiene protocols, has been shown to significantly reduce the degree of DRS and associated inflammation.^{77,87} This is critical as DRS is often linked to the presence of biofilms containing pathogenic microorganisms, such as *Candida albicans*.⁸⁸

This study identified a variety of yeast species colonizing dentures of diabetic patients, with distinct distribution patterns between individuals with controlled and uncontrolled glycemia. *Candida glabrata* was the most frequently isolated fungal species (31.25 %) from the dentures of diabetic patients, identified in uncontrolled group higher than controlled group. The prevalence of *C. glabrata* observed in this study contrasts with findings from previous studies, where it was reported less frequently than *C. albicans* and *C. tropicalis*.^{18,30,32,47,51} *Candida glabrata* has been reported at relatively low prevalence in diabetic populations, with detection rates of 6.1 % among diabetic patients⁸⁹ and 4.7 % among those with periodontitis.³¹ However, diabetes is recognized as a major risk factor for oral *Candida glabrata* colonization, with studies showing a higher prevalence of *C. glabrata* among diabetic patients compared to non-diabetic individuals.^{90–92} In addition, *C. glabrata* was frequently isolated from diabetic patients who wore dentures, suggesting that denture use may contribute to its colonization.^{45,93}

Candida albicans emerged as the dominant species, representing 81.25 % of isolates in the uncontrolled group compared to 18.75 % in the controlled group. This finding aligns with its well-documented role as the most prevalent fungal pathogen in the oral cavity and its heightened pathogenicity in immunocompromised hosts, including those with poorly controlled diabetes.^{10,94,95} The previous studies indicated that *C. albicans* is the most frequently isolated species in cases of oral candidiasis, with high colonization rates observed in both immunocompromised

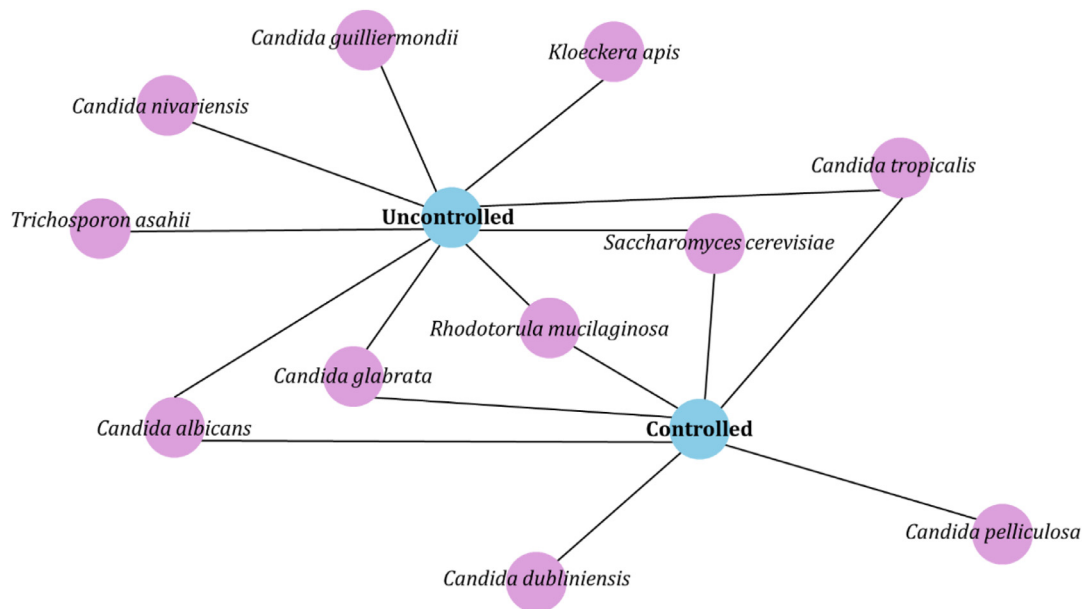


Fig. 4. Bipartite network analysis of fungal distribution on dentures in diabetic patients with controlled and uncontrolled Glycemic groups.

patients and denture wearers.^{95,96} Hyperglycemia has been shown to enhance *C. albicans* adhesion, hyphal transformation, increased adhesion to acrylic surfaces and elevated biofilm-forming capacity under glucose-rich conditions.^{26,28,65}

In addition, *Candida tropicalis* and *Rhodotorula mucilaginosa* were also isolated from the dentures of diabetic patients, with both species showing a higher prevalence among those with uncontrolled diabetes. *Candida tropicalis* is frequently isolated from diabetic patients, though it is less common than *Candida albicans*.^{31,89} Higher prevalence of *Candida* species, including *C. tropicalis*, is noted in patients with poor glycemic control.^{31,32,97} Denture-related factors such as poor maintenance and denture plaque contribute to increased colonization of *C. tropicalis*.⁹⁸ *Rhodotorula mucilaginosa* is an emerging opportunistic pathogen, particularly in immunocompromised patients. It is less commonly isolated compared to *Candida* species but has been identified in various clinical settings.^{45,99,100}

Importantly, *Candida guilliermondii*, *Candida nivariensis*, *Kloeckera apis*, and *Trichosporon asahii* were found exclusively in the uncontrolled group. Although less commonly encountered, these yeast species may represent opportunistic colonizers favored by hyperglycemia-induced alterations in oral immunity, salivary composition, and mucosal surface integrity.^{2,43,44,101} Their exclusive presence in the uncontrolled group may signify that chronic hyperglycemia facilitates colonization by a broader spectrum of opportunists, thereby potentially elevating the risk for systemic mycoses or denture stomatitis. Conversely, *Candida dubliniensis* and *Candida pelliculosa* were uniquely identified in the controlled group, while in lower numbers compared to more virulent fungal species.^{45,102,103}

This study demonstrates a clear association between poor glycemic control and elevated microbial colonization on dentures, with *Candida albicans* as the predominant species and an increased presence of non-*albicans* *Candida*. The clinical implications are clear that denture wearers with poor glycemic control are at increased risk of high microbial loads, which in turn may predispose them to denture stomatitis, angular cheilitis, and other opportunistic infections. For prosthodontists and clinicians, this underscores the necessity of incorporating glycemic monitoring into routine dental care for denture wearers, as oral fungal colonization is closely linked to systemic metabolic status. Dentures should be regarded not only as prosthetic devices but also as potential reservoirs of clinically significant pathogens in diabetic patients. From a clinical

management perspective, prevention and treatment must extend beyond pharmacological interventions. A hygiene protocol involving brushing the palate and dentures significantly reduced the microbial load of *Candida* spp. Gram-negative bacteria, *Staphylococcus* spp. and *Streptococcus mutans* on dentures and the palate.⁷⁷ Combining brushing with other cleaning methods, such as soaking in sodium hypochlorite or using denture cleansers, further enhances the reduction of microorganisms. For instance, brushing combined with microwave irradiation and denture cleansers effectively disinfected dentures and removed biofilm.¹⁰⁴ Similarly, brushing with denture cleansers was more effective than brushing alone in reducing viable biofilm.⁷⁶ Water rinsing alone is ineffective and may exacerbate microbial load, highlighting the need for structured patient education. For patients with denture stomatitis, adjunctive antifungal therapy should be guided by species identification and susceptibility testing. Additional preventive strategies include removal of dentures at night,^{18,51} routine recall appointments for evaluation of mucosal health and denture fit, and periodic relining or replacement of aged prostheses. Ultimately, these findings highlight the importance of an interdisciplinary approach by collaboration between dentists, physicians, and diabetes care providers is essential to optimize both oral and systemic outcomes in this high-risk population.

The main strength of this study lies in its comprehensive and integrative approach, combining quantitative microbiological assessment with clinical and behavioral data in an older diabetic population. Unlike many previous reports, this study stratified participants by objective glycemic status (HbA1c) and employed species-level fungal identification using MALDI-TOF MS, enabling accurate characterization of the denture mycobiome. The use of standardized sampling and aseptic procedures enhances reproducibility, while multivariable modeling allowed adjustment for potential confounders. Furthermore, the study provides clinically relevant evidence from a real-world community setting, reflecting the typical denture-wearing diabetic population seen in secondary healthcare.

This study has several limitations that should be acknowledged. First, its cross-sectional design limits the ability to establish temporal or causal relationships between glycemic control and fungal colonization. Second, the study population was relatively homogeneous and of modest size, which may have reduced the statistical power to detect weaker associations between demographic or behavioral variables and microbial outcomes. Third, microbial quantification was based on culture-dependent

CFU enumeration, which may underestimate the total microbial diversity and fail to capture fastidious or non-culturable species. Future studies employing molecular techniques such as next-generation sequencing or quantitative PCR would provide more comprehensive insights into denture-associated mycobiome and bacteriome profiles. Finally, anti-fungal susceptibility testing was not performed, which would have been valuable in assessing the clinical relevance of non-albicans *Candida* and emerging opportunistic fungi identified in patients with poor glycemic control.

Furthermore, the findings of this study have direct relevance for public health and health policy. Integrating oral health assessment into diabetes management programs could provide an effective, community-based approach to reduce fungal infections and improve overall health outcomes among older adults with diabetes. Regular screening for denture-related fungal colonization and reinforcing hygiene behaviors during diabetes follow-up visits could be implemented through primary care or community health centers. Interprofessional collaboration linking dentists, physicians, nurses, and diabetes educators should be promoted under existing NCD prevention frameworks. Such integration aligns with the WHO Global Oral Health Action Plan (2023–2030), which emphasizes oral health inclusion in chronic disease management and supports healthy aging initiatives within community settings.

Nonetheless, the findings underscore the importance of targeted interventions aimed at older adult diabetic patients. Tailored diabetes self-management education, culturally appropriate health communication, and strategies to improve patient engagement in these groups may help bridge disparities in glycemic outcomes. Prevention and management strategies, including strict glycemic control, regular screening, hygiene practices, and patient education, are emphasized to mitigate the risk of fungal infections.³ Although most participants reported receiving denture care instructions, adherence to recommended hygiene behaviors remained suboptimal, particularly among those with uncontrolled diabetes. This finding highlights the need to strengthen patient support and follow-up rather than relying solely on one-time educational sessions. Practical interventions, including simplified hygiene protocols, reminder systems, and reinforcement during clinical visits, are recommended to improve compliance. Integrating oral health counseling into diabetes care programs may further enhance both oral and systemic health outcomes.

5. Conclusion

This study confirms that glycemic control and denture hygiene behaviors jointly influence fungal colonization among older adults with diabetes. Poor metabolic control was associated with a higher fungal burden and greater species diversity, underscoring the systemic effect of hyperglycemia on oral microbial ecology. Effective denture hygiene, particularly brushing combined with chemical disinfection was linked to reduced fungal load, highlighting the importance of consistent oral care practices. These findings emphasize that maintaining optimal HbA1c levels, together with evidence-based denture cleaning, is critical to preventing fungal overgrowth and promoting oral health in diabetic denture wearers.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Human Research Ethics Committee of Nakhon Si Thammarat Provincial Public Health Office (study code: 103/2568) on 29 April 2025. Written informed consents were obtained from participants included in the study.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

The use of generative AI and AI-assisted technologies in scientific writing

During the preparation of this work, the authors used AI to improve readability and language of some parts of the paper. The use of these technologies complied with the terms of use of the relevant tool or technology of KeAi. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Chutikorn Tanjapatkul: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Montira Channarong:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis, Data curation. **Narisara Kaewchutima:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Siti Zulaikha Zakariah:** Writing – review & editing, Validation, Investigation. **Nopadol Precha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Acknowledgements

The authors would like to express their sincere gratitude to all participants who generously took part in this study. We extend our appreciation to the staff and clinicians of the Denture Department, Sichon Hospital, Nakhon Si Thammarat, for their valuable assistance in patient recruitment and clinical coordination. We also thank the Laboratory, Walailak University, for providing technical support and laboratory facilities essential to this research.

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