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Systemic Inflammatory and Th17-Related Immune Responses in Patients with Oral Candidiasis and Type 2 Diabetes Mellitus: A Case-Control Study from Iraq

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Abstract

Background: Oral candidiasis is common in type 2 diabetes mellitus (T2DM), and the host immune response may determine whether oral Candida colonization becomes disease. The systemic inflammatory and Th17-related cytokine profile of diabetic patients with oral candidiasis is unclear. **Methods:** In this case-control study in Iraq, 180 adults were enrolled in three groups of 60: T2DM with clinically diagnosed and mycologically confirmed oral candidiasis (Group I), T2DM without clinical candidiasis (Group II) and healthy controls (Group III). Serum IL-17A (primary outcome), IL-6, TNF-α and IL-10 were measured by sandwich ELISA, and HbA1c and local oral risk factors were recorded. **Results:** Median serum IL-17A was 37.4, 24.3 and 15.7 pg/mL in Groups I, II and III (Kruskal–Wallis $p<0.001$; Group I vs II, Bonferroni-adjusted $p=0.008$), but the Group I vs II difference was no longer significant after covariate adjustment (geometric mean ratio 1.33, 95% CI 0.97–1.82). Adjusted differences remained significant for IL-6 (1.48, 95% CI 1.06–2.07) and IL-10 (1.84, 95% CI 1.45–2.33). Each 1% increase in HbA1c was associated with 2.45-fold higher odds of oral candidiasis (95% CI 1.63–3.68). **Conclusions:** In this study, poor glycemic control was the main host factor linked to oral candidiasis in T2DM. IL-6 and IL-10 stayed higher in infected patients after adjustment, whereas the IL-17A difference between diabetic groups was largely explained by glycemic and local oral factors.

Keywords: oral candidiasis; type 2 diabetes mellitus; IL-17A; IL-6; TNF-α; IL-10; Iraq.

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Introduction

Candidiasis is a common opportunistic infection within the oral cavity caused by an overgrowth of candida species; most commonly Candida albicans [1]. Diabetic patients are more susceptible to oral candidiasis due to many risk factors such as age, gender, diet, oral hygiene, smoking, dentures, saliva pH and dry mouth

[2]. The immune system of the host determines if this infection occurs. The Th17 cell predominant cytokine IL-17A plays a key role in protecting mucosal surfaces against fungal infections [3]. IL-6 and TNF-α reflect pro-inflammatory activation, which is already raised in T2DM, while IL-10 limits excessive inflammation. How these markers differ between diabetic patients with and without oral candidiasis is still debated.

Previous work has examined serum IL-10 and IFN-γ in diabetic patients with oral candidiasis [4] and IL-17A gene polymorphism in Iraqi diabetic patients [5]. Few studies, however, compare Th17-related, pro-inflammatory and regulatory cytokines across diabetic patients with oral candidiasis, diabetic patients without it and healthy controls, while accounting for HbA1c and diabetes duration.

We therefore investigated systemic inflammatory and immunological biomarkers in patients with T2DM and oral candidiasis. Our objectives were to compare serum IL-17A, IL-6, TNF-α and IL-10 across the three groups, and examine the relationship of HbA1c and local oral factors with oral candidiasis,

Materials and Methods

Study design and participants

This analytical case-control study was conducted in Iraq, during the period from 16th of January 2024 to the 2nd of December 2024. Adults aged 18–65 years were enrolled in three groups: Group I, T2DM with clinically diagnosed and mycologically confirmed oral candidiasis; Group II, T2DM without clinical oral candidiasis; and Group III, non-diabetic individuals without oral candidiasis or significant immunosuppression. Clinical diagnosis was made by a dentist or trained clinician, and microscopy and/or culture were interpreted together with the clinical findings, because culture positivity alone does not establish disease.

Exclusion criteria were Type 1 Diabetes, active malignancy or chemotherapy, current systemic immunosuppressive therapy, recent systemic antifungal or (where appropriate) antibiotic treatment, other active oral infections and severe systemic inflammatory disease.

Sample size

Sample size was based on the primary outcome, serum IL-17A, using one-way ANOVA with Cohen's f=0.25, α=0.05, 80% power and three groups (G*Power [6]), which gives 159 analyzable participants (53 per group). Allowing for 10% incomplete data, recruitment was set at 180 (60 per group).

Clinical data

A structured questionnaire and examination form recorded age, sex, residence, BMI, smoking, diabetes duration, antidiabetic treatment, complications, denture use, xerostomia and oral hygiene. Lesions were classified as pseudomembranous, erythematous, denture-associated or angular cheilitis. Fasting blood glucose, HbA1c, CBC and C-reactive protein (CRP) were measured as covariates.

Specimen collection and diagnosis of oral candidiasis

In Group I, swabs were taken from the active lesion; in Groups II and III, standardized swabs were taken from the tongue and buccal mucosa to confirm the absence of candidiasis. Participants avoided eating, drinking, smoking and oral hygiene for about one hour beforehand were practical. Direct Gram and 10% KOH preparations were examined for budding yeasts, pseudohyphae and hyphae.

Specimens were cultured on Sabouraud dextrose agar at 35–37 °C and examined for yeast growth at 24–48 hours. Culture results were interpreted together with the clinical findings, because Candida is a normal oral commensal.

Blood collection and ELISA

Venous blood (5–7 mL) was collected before antifungal treatment in Group I, allowed to clot, centrifuged, aliquoted and stored at −80 °C, avoiding repeated freeze-thaw cycles. Serum IL-17A, IL-6, TNF-α and IL-10 were measured with human-specific sandwich ELISA kits from one manufacturer, strictly following the

manufacturer's protocol, with absorbance read at 450 nm. Samples were duplicate with standards and controls on every plate. Intra- and inter-assay coefficients of variation were recorded, over-range samples were re-run after dilution, and laboratory staff were blinded to groups where feasible.

Statistical analysis

Data was analyzed in SPSS (v26). Normality was assessed with the Shapiro–Wilk test. Continuous data are given as mean ± SD or median (IQR) and categorical data as n (%). Three-group comparisons used one-way ANOVA or Kruskal–Wallis tests, with pairwise Mann–Whitney tests and Bonferroni correction as post hoc analysis; two-group comparisons used the t-test or Mann–Whitney U test; categorical data used chi-square or Fisher's exact tests. IL-6, TNF-α and IL-10 were secondary outcomes, and their p values were adjusted for false discovery rate (Benjamini–Hochberg [7]). Correlations between HbA1c and cytokines were analyzed among the diabetic participants.

Results

A total of 180 participants were analyzed (60 per group). Diabetic patients with oral candidiasis had higher HbA1c and fasting glucose, longer diabetes duration, and more denture use, xerostomia and poor oral hygiene than the other groups. Group I was also slightly older (p=0.002), so age was included in adjusted models (Table 1).

Table 1. Characteristics of the study groups

Characteristic	Group I: T2DM + OC (n=60)	Group II: T2DM (n=60)	Group III: Healthy (n=60)	p
Age, years	55.9 ± 6.8	51.4 ± 8.1	52.0 ± 6.9	0.002
Male sex, n (%)	21 (35.0)	23 (38.3)	22 (36.7)	0.931
BMI, kg/m²	29.4 ± 4.6	29.9 ± 4.2	27.1 ± 3.6	<0.001
Diabetes duration, years	9.9 ± 4.3	7.3 ± 3.5	—	<0.001
HbA1c, %	9.6 ± 1.7	7.9 ± 1.3	5.4 ± 0.4	<0.001
Fasting glucose, mg/dL	227.6 ± 55.5	179.0 ± 40.4	92.8 ± 9.4	<0.001
HbA1c ≥ 8%, n (%)	49 (81.7)	30 (50.0)	0	<0.001
Current smokers, n (%)	16 (26.7)	5 (8.3)	12 (20.0)	0.032
Denture use, n (%)	25 (41.7)	12 (20.0)	8 (13.3)	<0.001
Xerostomia, n (%)	37 (61.7)	17 (28.3)	2 (3.3)	<0.001
Poor oral hygiene, n (%)	34 (56.7)	13 (21.7)	6 (10.0)	<0.001
CRP, mg/L, median (IQR)	5.7 (4.0–8.5)	3.3 (2.4–4.6)	2.0 (1.2–2.5)	<0.001

Values are mean ± SD unless stated. Three-group p values: ANOVA, chi-square or Kruskal–Wallis. Diabetes duration and HbA1c ≥ 8% were compared between Groups I and II only. OC, oral candidiasis.

All 60 Group I patients had clinically diagnosed, culture-confirmed oral candidiasis. The most frequent clinical form was pseudomembranous (35.0%), followed by denture stomatitis (28.3%), erythematous (23.3%) and angular cheilitis (13.3%) (Table 2).

Table 2. Clinical forms of oral candidiasis in Group I (n=60)

Clinical form	n	%
Pseudomembranous	21	35.0
Denture stomatitis	17	28.3
Erythematous	14	23.3
Angular cheilitis	8	13.3

All four cytokines differed across groups (Table 3). The primary outcome, IL-17A, was highest in Group I and remained significantly higher than in Group II after Bonferroni correction (p=0.008). IL-6, TNF-α and IL-10 showed larger separations (FDR-adjusted p<0.001 for all three). Serum IL-17A had only modest discrimination between Groups I and II (AUC 0.66; best cut-off 27.5 pg/mL, sensitivity 68%, specificity 58%).

Table 3. Serum cytokines, pg/mL, median (IQR)

Cytokine	Group I	Group II	Group III	Kruskal–Wallis p	I vs II	I vs III	II vs III
IL-17A	37.4 (24.1–59.5)	24.3 (16.4–41.7)	15.7 (11.4–25.2)	<0.001	0.008	<0.001	<0.001
IL-6	17.3 (11.9–30.7)	9.8 (6.6–14.0)	4.7 (3.6–7.2)	<0.001	<0.001	<0.001	<0.001
TNF-α	33.1 (22.3–41.8)	19.6 (14.2–29.4)	11.3 (9.1–17.8)	<0.001	<0.001	<0.001	<0.001
IL-10	30.1 (22.2–37.1)	18.6 (14.0–23.9)	14.9 (10.5–18.9)	<0.001	<0.001	<0.001	0.013

Post hoc p values are Bonferroni-adjusted Mann–Whitney tests.

After adjustment for HbA1c, diabetes duration, age, sex, BMI, smoking, denture use, xerostomia and oral hygiene, the Group I vs II difference remained significant for IL-6 and IL-10 but not for IL-17A or TNF-α (Table 4). This suggests that part of the unadjusted IL-17A difference reflects the worse glycemic control and local oral risk factors of the infected group.

Table 4. Adjusted geometric mean ratio, Group I vs Group II

Cytokine	GMR (95% CI)	p
IL-17A	1.33 (0.97–1.82)	0.078
IL-6	1.48 (1.06–2.07)	0.022
TNF-α	1.21 (0.94–1.56)	0.131
IL-10	1.84 (1.45–2.33)	<0.001

Among the 120 diabetic patients, HbA1c correlated with IL-6 (ρ=0.43, p<0.001) and TNF-α (ρ=0.36, p<0.001) but not significantly with IL-17A (ρ=0.13, p=0.170) or IL-10 (ρ=0.15, p=0.092). Patients with HbA1c ≥ 8% were over four times as likely to be in the oral candidiasis group (OR 4.45; 49 of 79 vs 11 of

41; p<0.001). In (Table 5), higher HbA1c, longer diabetes duration, xerostomia and poor oral hygiene were independently associated with oral candidiasis; smoking showed a borderline association.

Table 5. Factors associated with oral candidiasis among diabetic patients (n=120)

Factor	Adjusted OR (95% CI)	p
HbA1c, per 1%	2.45 (1.63–3.68)	<0.001
Diabetes duration, per year	1.18 (1.01–1.37)	0.036
Age, per year	1.03 (0.96–1.11)	0.349
Male sex	0.90 (0.28–2.91)	0.857
BMI, per kg/m²	0.96 (0.84–1.10)	0.546
Current smoking	4.08 (0.94–17.60)	0.060
Denture use	1.57 (0.43–5.75)	0.499
Xerostomia	4.00 (1.35–11.89)	0.013
Poor oral hygiene	5.89 (1.71–20.34)	0.005

Discussion

T2DM subjects were more likely to suffer poor glycaemic control, dry mouth, poor oral hygiene and increased serum cytokines to cause oral candidiasis. The strong association between HbA1c and oral candidiasis (OR= 2.45 per 1%, OR= 4.45 for HbA1c ≥8%) agrees with a pooled OR of 2.94 for poor control [8] and the increase of carriage rates with increasing levels of poor control [9]. However, in contrast to this study, there was no association observed between oral candidiasis and HbA1c or fasting glucose in a case-control study conducted in 2022 [4]. This could be due differences in sample size and definition of poor control, alongside local oral risk factors. Xerostomia and poor oral hygiene were found to be independent factors associated with oral candidiasis in addition to HbA1c. This supports the idea that oral candidiasis occurs partly due to poor glycaemic control. However, in this study wearing dentures was not significant compared to a pooled OR of 2.22 [8]. This is likely due to dentures overlapping with age, oral hygiene and xerostomia.

IL-17A levels were highest in the infected group, consistent with its role in Th17 mediated protection against Candida at mucosal sites [3]. However, there was little difference between Group I and II and this lost significance when adjusting data, suggesting that in diabetes serum IL-17A will also be partially related to the patient's metabolic status and risk factors at the site. IL-6 and IL-10 were both raised. This is consistent with the 2022 study showing an increased likelihood of developing oral candidiasis with increased IL-10 in T2DM patients [4]. However, a systematic review of diabetes and oral disease showed most studies had reduced IL-10 [10]. Therefore, it appears that IL-10 levels are dependent on the oral condition and assay being used. Overall, this shows a pro-inflammatory profile, consistent with previous research showing differences in TNF- α levels based on the glycemic status of diabetic patients with oral candidiasis [11]. Hba1c levels correlated with increased IL-6 and TNF-α levels but not IL-17A, suggesting that there is partial separation between metabolic inflammatory markers and Th17.

There were increased odds of oral candidiasis for every 1% increase in Hba1c, also xerostomia and poor oral hygiene. Oral Candida isolates from diabetic patients have shown high resistance to fluconazole [9],

therefore it would be beneficial to perform mycological confirmation and susceptibility testing on any persistent or recurrent lesions.

Conclusion

This case control study identified an association between oral candidiasis in patients with T2DM and poor glycaemic control, xerostomia and poor oral hygiene. In addition, increased levels of IL-6 and IL-10 were observed independently of covariates. Differences in IL-17A levels between diabetic groups were largely explained by glycaemic and local oral factors. Further prospective studies of larger size including salivary immune biomarkers are required to further validate these results and establish the nature of the associations.

Declarations

Ethics statement: All participants provided consent.

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Conflict of interest: The author declares no conflict of interest

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