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Review article

Differential Association of Salivary Proinflammatory Mediators with Type 2 Diabetes: A Network Meta-Analysis

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Abstract

Background: Salivary compounds can be used as diagnostic markers for changes in the oral cavity that cause oral problems in type 2 diabetes mellitus (T2DM).

Methods: This meta-analysis searched PubMed/Medline, EMBASE, Scopus and Cochrane Library, and the Web of Science until Nov 2023. The observational studies included patients with T2DM and healthy controls aged > 18 yr with no oral health problems or systematic or periodontal diseases. The Quality in Prognostic Studies (QUIPS) tool was used to evaluate the risk of bias. The random-effects model was constructed using standardized mean differences (SMD).

Results: The meta-analysis analyzed 13 observational studies that included 519 patients with T2DM and 356 healthy controls. Non-fasting periods of 30 min to 8 h were used to measure salivary profiles. Overall, salivary proinflammatory mediators favored patients with T2DM (SMD: 1.66; $CI_{95\%}$: 0.42, 2.91, $P<0.01$) compared with healthy subjects. Subgroup analysis revealed that interleukin-6 (SMD: 1.33; $CI_{95\%}$: -0.04, 2.69, $P<0.05$), followed by interleukin-8 (SMD: 0.92; $CI_{95\%}$: -0.71, 2.55, $P<0.13$), was greater in patients with T2DM than in healthy subjects. Among patients with T2DM, network analysis identified salivary factors most closely associated with male sex (i.e., tumor necrosis factor), female sex (i.e., interleukin-8), fasting plasma glucose (i.e., C-reactive protein), HbA1c (i.e., IL-8), and age (i.e., C-reactive protein).

Conclusion: Overall, salivary IL-6 levels were greater in patients with T2DM and might be considered for monitoring oral changes. Moreover, network analysis could identify different salivary components that were most closely associated with patient characteristics.

Keywords: Saliva; Proinflammatory mediators; Type-2 diabetes mellitus; Meta-analysis; Network analysis

Introduction

Type 2 diabetes mellitus (T2DM) has reached epidemic proportions around the world, with this metabolic disease expected to increase to 592 million people (1 in 10 adults) by 2035 (1). The body can suffer from various health problems because of T2DM, a complex metabolic disease (2, 3).

A significant amount of evidence indicates that hyperglycemia results in the oxidation and protein glycation of many body tissues (4). Glucose toxicity is the most important factor in the devel-

opment of diabetic complications, such as high incidence of cerebrovascular disease, cardiovascular disease, and retinopathy (5). In addition, T2DM is believed to be a risk factor for oral complications (6). Diabetic impairment can modulate periodontal tissue damage by causing polymorphonuclear leukocyte dysfunction, altered collagen and glycosaminoglycan production, and deregulated cytokine production (7).

Furthermore, T2DM may be an inflammatory disease that can affect specific parts of the immune system (8). Increased levels of circulating interleukin (IL)-1β, IL-6, and acute-phase proteins, such as C-reactive protein (CRP), in T2DM may be due to the activation of innate immune cells (9, 10). Therefore, T2DM can alter the immune responses, cytokines, chemokines, growth factors, nuclear factors, and immune cells involved in the development and complications of T2DM (5). Because of the many and varied factors contributing to diabetes development and complications, it is important to study routine biomarkers that represent the pathophysiology of diabetes (5). For example, an increase in IL-6 and CRP levels can be indicative of T2DM (11) or a recent study reported sex-specific mRNA expression in peripheral blood mononuclear cells (PBMCs) in patients with T2DM (5).

Saliva as a biological fluid is an alternative to serum for diagnostic purposes because of the great similarities between the proteomes of saliva and serum (12). Saliva may also be utilized as a diagnostic fluid for oral diseases, which is noteworthy (7). For example, the concentrations of interferon gamma (IFNγ), procalcitonin, IL-6, and tumor necrosis factor alpha (TNF-α) in the blood are significantly correlated with their respective concentrations in saliva (13). The prevalence of oral complications and underlying diseases in HIV-infected individuals is linked to elevated levels of salivary IFNγ (14). Identification of cytokine patterns in saliva may be useful in diagnosing tuberculosis (14). The presence of proinflammatory cytokines in saliva may serve as an intravenous agent for measuring inflammation in patients with T2DM (13). Therefore, monitoring proinflammatory cytokine levels in saliva may be useful in assessing the pathophysiology and progression of patients with T2DM (13, 14). Quantification of biomarkers in saliva could serve as a useful tool for predicting susceptibility to periodontitis in humans (15). This provides information on periodontal activity and monitoring of the effectiveness of periodontal treatment.

In this study, we conducted a meta-analysis to estimate which salivary proinflammatory mediators were associated with T2DM. In addition, subnetwork analysis was performed to understand the interrelationships between salivary proinflammatory mediators and patient characteristics, such as fasting plasma glucose (FPG), age, sex, and HbA1c levels.

Methods

Search strategy

The PRISMA statement was followed in this study (Fig. 1). A literature search was performed in the following databases: PubMed/Medline, EMBASE, Scopus and Cochrane Library, and Web of Science to evaluate the salivary profile in adults with T2DM using MeSH and free words: "type 2 diabetes, diabetes mellitus type 2, type II diabetes, diabetes type II, diabetes type 2, or diabetes mellitus type II," "immunoglobulin A, IgA, cytokines, chemokines, inflammatory factors, CRP, C-reactive protein, interleukin, IL-(1 to 18) TNFA, TNF-α, tumor necrosis factor-alpha" and "saliva" or "salivary". Only English publications were searched until Nov 2023. To determine the likelihood of studies being missed, we reviewed the studies in Google Scholar using the aforementioned search terms. In addition, these articles and other systematic reviews were analyzed using reference lists. All texts, tables, and figures in diabetes-related publications were carefully checked for saliva composition.

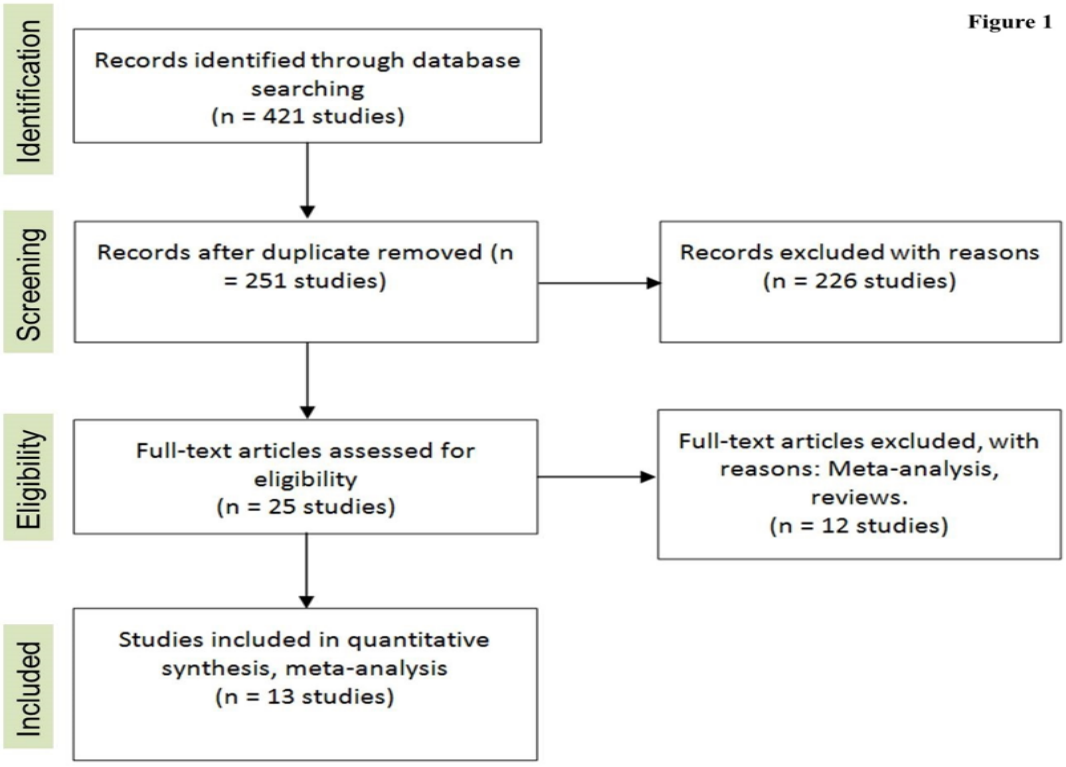


Fig. 1: PRISMA flow chart of the selection of studies

Inclusion/exclusion criteria

Observational studies were included in this meta-analysis if they included two groups (both men and women aged 18 yr or older): T2DM patients (the exposure) and healthy controls (the comparator). In addition, studies that included subjects with no oral health issues or systematic and periodontal diseases were selected. Patients had only T2DM. Reviews, letters to the editor, or case reports were not included in this study. Studies not reporting the mean or median salivary profile with the corresponding standard deviation (SD) or those reporting the salivary components in children (< 18 yr) were excluded.

Data extraction/synthesis

Two authors (Y. Z. and L. L.) searched for studies, checked the title and abstract of each study against the abovementioned criteria, and extracted data. Data obtained for each study included the first author's name, year of publication, country, sample size of patients and controls, power analysis, study type, any treatment, percentage of males or females, mean age and body mass index (BMI), condition of saliva sampling, salivary cytokines/interleukins/inflammatory factors, IgA levels, FPG, and HbA1c levels in patients and controls. These data underwent a second analysis by two authors (Y. Z. and L. L.). Meta-analysis was performed using META-MAR version 2.7.0. Standardized mean differences (SMD) were used to determine the effect sizes for salivary cytokines/interleukins/inflammatory factors and IgA in patients with T2DM or healthy subjects. Due to within- and between-parameter heterogeneity, a random-effects model was used to calculate the weighted mean effect sizes and 95% confidence intervals ($CI_{95\%}$).

Heterogeneity and risk of bias in individual studies

The I^2 and Tau^2 statistics were employed to estimate statistical heterogeneity. According to the Cochrane Handbook of Systematic Reviews of

Interventions, I^2 is used as a measure for the proportion of variance caused by heterogeneity, i.e., 30%-60%, 50%-90% and 75%-100% represent moderate, marked, and severe levels of heterogeneity, respectively (16). In addition, the Quality in Prognostic Studies (QUIPS) tool, an untested tool with space for personal interpretation, was used to evaluate the risk of bias in each study (17). The Cochrane Methods Prognosis Group recommends the QUIPS tool for research prediction because it accounts for all common sources of bias (17). Based on this, the QUIPS methodology was sufficient to determine the risk of bias. Each evaluated study was independently assessed for risk of bias (low, medium, or high risk of bias) by two team members. All disagreements were resolved by consensus. The QUIPS tool includes domains on study participation, study attrition, prognostic factor measurement, outcomes measurement, statistical analysis and reporting, and study confounding (18). Studies with a low risk of bias included a detailed description of the population, design, and measures. They also included a clear explanation of how the intervention was conducted, the type of equipment used, and how the data were interpreted. Studies with a moderate risk of bias had some biases, but not enough to invalidate the data. Although these studies did not meet all the requirements for a low risk of bias ranking, they were more likely to be free of errors that would introduce significant bias. Studies at high risk of bias had significant flaws and exhibited various forms of bias that could invalidate the results. High-risk studies contained one or more major or "fatal" errors in design, analysis, reporting, and large amounts of information were missing. Despite being present in the narrative synthesis, the results of the risk of bias assessment were excluded from the meta-analysis. In addition, publication bias was assessed by funnel plot analysis and Egger's regression test, which showed that publication selection, was likely a source of bias.

Network analysis

We used network analysis to explore the salivary factors most linked to diabetes, age, sex, HbA1c, or FPG levels using interaction network analysis with a maximum cutoff criterion. According to the highest cut-off criteria, no variables were associated with the outcome. A force-directed layout algorithm for network analysis was developed using Spearman's Rho similarity index and the Fruchterman-Reingold algorithm. This approach builds a network based on the frequency of node connections. Network visualization was constructed at the highest cutoff point of the network (i.e., 50%). The clustering and correlation coefficients are represented by the size of the nodes (variables) and edges (interactions between parameters) with a maximum cutoff criterion. None of the variables was associated with the outcome after the highest cutoff criterion. The size of the nodes indicates the degree of connectivity, whereas the thickness of the edges shows the link between the two variables. Network analysis was performed using the PAST tool.

Results

Characteristics of the studies

As shown in Fig. 1, the databases were searched, and 421 studies were found. Following the removal of duplicates, 251 studies were identified for screening. Of these, 226 studies were deemed irrelevant, and 12 more studies were removed for specific reasons from the remaining 25 studies. As a result, 13 studies —11 of which were cross-sectional and two of which were case-control— were included and examined in the current meta-analysis (7, 12, 19-29). Studies were published from 2009 to 2023. Two studies were reported from Nigeria, one from Brazil, one from Romania, four from Iran, two from the United States, one from India, one from Spain, and one from Iraq. Table 1 presents the features of the 13 studies that were part of the meta-analysis, with 875 participants (539 diabetes patients and 336 healthy individuals). In six studies (7, 22, 23, 25, 27, 29), salivary profiles of patients and controls were measured under non-fasting conditions (30 min, 90 min, or 2 h) and in three studies (20,26,28) under fasting conditions (overnight).

Four studies did not specify the fasting period (12, 19, 21, 24). In nine studies, participants with T2DM lacked information about the duration of diabetes (19-22, 24-26, 28).

Table 1: Characteristics of the studies used in this meta-analysis

Study	*Country*	*No. of people (No. diabetes)*	*Age, year (mean)*	*Male, %*	*Sampling time*	*Study type*
Agho 2021 [12]	Nigeria	75 (36)	46.2	57.4	NR	Case control
Costa 2010 [7]	Brazil	42 (20)	48.5	33.3	2 h PP	Cross sectional
Monea 2018 [19]	Romania	40 (20)	55.0	39.0	NR	Cross sectional
Srinivasan 2018 [23]	USA	40 (20)	52.3	55.0	2 h PP	Cross sectional
Srinivasan 2015 [24]	USA	40 (20)	NR	NR	NR	Cross sectional
Tvarijonaviciute 2017 [27]	Spain	65 (31)	49.6	38.5	2 h PP	Cross sectional
Balaji 2017 [29]	India	40 (20)	50.0	NR	2 h PP	Cross sectional
Omamuzo 2021 [20]	Nigeria	216 (176)	45.0	NR	Overnight fasting	Cross sectional
Omer 2021 [21]	Iraq	91 (61)	39.7	59.3	NR	Cross sectional
Sardari 2015 [22]	Iran	38 (25)	55.6	34.2	30 min PP	Cross sectional
Vaziri 2010 [28]	Iran	60 (40)	54.2	50.0	Overnight fasting	Case control
Shirzaiy 2023 [25]	Iran	73 (35)	47.6	41.0	90 min ***PP***	Cross sectional
Tavangar 2017 [26]	Iran	55 (35)	NR	NR	8 h PP	Cross sectional

NR: not reported; PP: p*ostprandial*.

Table 2 shows the subjects' characteristics. The average age of the T2DM and healthy groups was 51.2 and 46.1 yr, respectively. The proportion of males in the T2DM and healthy groups was 44.3% and 45.1%, respectively. The T2DM group had a greater FPG (147.7 versus 86.4 mg/dl) and HbA1c (65.0 versus 31.0 mmol/mol) than the healthy group. BMI was greater in the T2DM group than in the healthy group (27.6 versus 25.5 kg/m^2).

Quantitative data synthesis

The pooled effect estimates for all factors revealed that salivary proinflammatory mediators significantly favored T2DM patients compared with healthy controls (SMD = 1.66; $CI_{95\%}$: 0.42, 2.91; P<0.01; I^2 = 96%; Fig. 2).

Table 2: Baseline characteristics of patients with type 2 diabetes and healthy controls

Parameters	*All subjects (n = 875)*	*Type 2 diabetes group (n = 539)*	*Healthy group (n = 336)*
Age, year, mean [min, max]	48.7 [34.5, 57.0]	51.2 [40.4, 57.0]	46.1 [34.5, 55.5]
Male, %, mean [min, max]	44.8 [23.0, 72.2]	44.3 [31.4, 60.0]	45.1 [23.0, 72.2]
Female, %, mean [min, max]	55.3 [27.8, 77.0]	55.7 [40.0, 68.6]	54.9 [27.8, 77.0]
Hb1Ac, mmol/mol, mean [min, max]	52.0 [22.0, 75.0]	65.0 [56.0, 75.0]	31.0 [22.0, 37.0]
FPG, mg/dl, mean [min, max]	117.1 [76.3, 215.4]	147.7 [81.1, 215.4]	86.4 [76.3, 98.9]
BMI, kg/m^2, mean [min, max]	26.6 [24.8, 28.5]	27.6 [26.4, 28.5]	25.5 [24.8, 26.7]
Salivary items			
IL-6, pg/ml, mean [SD]	89.9 [123.4]	114.4 [126.9]	68.4 [125.7]
IL-8, pg/ml, mean [SD]	494.8 [453.1]	535.0 [503.6]	454.6 [504.8]
TNF-α, pg/ml, mean [SD]	117.33 [67.2]	130.58 [106.7]	104.1 [210.4]
CRP, µg/ml, mean [SD]	4.2 [4.2]	6.2 [5.4]	2.1 [1.8]
IgA, µg/ml, mean [SD]	838.0 [1318.0]	1063.8 [1784.5]	612.1 [1005.8]

CRP: C-reactive protein; IL: interleukin; TNF-α: Tumor necrosis factor alpha; BMI: body mass index.
FPG: fasting plasma glucose; SD: standard deviation

Tests for subgroup differences revealed no statistically significant subgroup effects (P=0.28), indicating that the type of proinflammatory mediator did not alter the overall effect. As shown in Fig. 2, of the 13 studies, 5 investigated TNF-α in the saliva of patients with T2DM and healthy controls (12, 19, 23, 24, 27). Meta-analysis showed that salivary TNF-α levels were not significantly different between the T2DM and healthy groups (SMD 0.63; $CI_{95\%}$: -0.65, 1.91; P = 0.24; and I^2 = 90%). However, 4 of 5 studies reported higher salivary TNF-α levels in patients with T2DM than in healthy subjects. Seven studies compared salivary IL-6 levels between patients with T2DM and healthy controls (7, 12, 19, 23, 24, 27, 29). Salivary IL-6 levels significantly favored patients with T2DM (SMD 1.33; $CI_{95\%}$: -0.04, 2.69; P<0.01) compared with healthy subjects. Heterogeneity was high in this subgroup analysis (I^2 = 89%, P<0.01).

Figure 2

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
subgroup = subgroup1								
Agho et al TNFA	5.39	12.1000	36	1.51	3.6600	39	4.8%	0.44 [-0.02; 0.90]
Monea et al	64.10	52.1000	20	5.20	8.1000	20	4.8%	1.55 [0.83; 2.26]
Srinivasan et al	555.20	95.3000	20	480.00	145.2000	20	4.8%	0.60 [-0.03; 1.24]
Srinivasan et al	19.10	5.2000	20	28.60	12.5000	20	4.8%	-0.97 [-1.63; -0.31]
Tvarijonaviciute et al	9.11	3.2900	31	5.10	1.6300	34	4.8%	1.55 [0.99; 2.11]
Total (95% CI)			127			133	23.9%	0.63 [-0.65; 1.91]
Heterogeneity: Tau^2 = 0.9506; Chi^2 = 40.13, df = 4 (P < 0.01); I^2 = 90%								Test for overall effect: Z: 1.37, P = 0.24
subgroup = subgroup2								
Balaji et al IL6	143.34	42.7600	20	0.01	0.0100	20	4.6%	4.65 [3.41; 5.88]
Monea et al	92.10	69.0000	20	8.10	10.0000	20	4.8%	1.67 [0.94; 2.40]
Tvarijonaviciute et al	35.20	17.5800	31	16.00	4.2100	34	4.8%	1.52 [0.96; 2.07]
Srinivasan et al	69.30	18.6000	20	53.00	20.3000	20	4.8%	0.82 [0.17; 1.47]
Srinivasan et al	382.20	72.5000	20	350.20	50.2000	20	4.8%	0.50 [-0.13; 1.13]
Costa et al	10.12	1.9000	20	9.40	2.7000	22	4.8%	0.30 [-0.31; 0.91]
Agho et al	47.20	18.4900	36	41.94	16.8800	39	4.8%	0.29 [-0.16; 0.75]
Total (95% CI)			167			175	33.3%	1.33 [-0.04; 2.69]
Heterogeneity: Tau^2 = 1.8616; Chi^2 = 56.38, df = 6 (P < 0.01); I^2 = 89%								Test for overall effect: Z: 2.38, P = 0.05
subgroup = subgroup3								
Omamuzo et al CRP	9.42	0.6000	176	2.89	0.1000	40	4.6%	11.96 [10.77; 13.14]
Omer et al	9.20	1.2000	61	3.30	0.6000	30	4.7%	5.61 [4.67; 6.55]
Agho et al	0.05	0.0400	36	0.02	0.0200	39	4.8%	0.95 [0.47; 1.43]
Total (95% CI)			273			109	14.1%	6.15 [-7.57; 19.87]
Heterogeneity: Tau^2 = 30.2777; Chi^2 = 318.08, df = 2 (P < 0.01); I^2 = 99%								Test for overall effect: Z: 1.93, P = 0.19
subgroup = subgroup4								
Omer et al IgA	3124.00	1555.0000	61	1773.00	113.0000	30	4.8%	1.05 [0.58; 1.51]
Sardari et al	67.30	20.6000	25	63.30	15.2000	13	4.8%	0.21 [-0.47; 0.88]
Vaziri et al	0.07	0.2000	40	0.07	0.0300	20	4.8%	0.00 [-0.54; 0.54]
Total (95% CI)			126			63	14.4%	0.44 [-0.97; 1.85]
Heterogeneity: Tau^2 = 0.2578; Chi^2 = 9.42, df = 2 (P < 0.01); I^2 = 79%								Test for overall effect: Z: 1.34, P = 0.31
subgroup = subgroup5								
Tavangar et al IL8	382.35	95.7000	35	254.30	77.0500	20	4.8%	1.41 [0.80; 2.02]
Shirzaiy et al	125.34	38.4100	35	80.63	34.7600	38	4.8%	1.21 [0.71; 1.71]
Tvarijonaviciute et al	1097.30	399.5400	31	1028.75	303.6100	34	4.8%	0.19 [-0.30; 0.68]
Total (95% CI)			101			92	14.4%	0.92 [-0.71; 2.55]
Heterogeneity: Tau^2 = 0.3593; Chi^2 = 12.17, df = 2 (P < 0.01); I^2 = 84%								Test for overall effect: Z: 2.44, P = 0.13
Total (95% CI)			794			572	100.0%	1.66 [0.42; 2.91]
Heterogeneity: Tau^2 = 7.1977; Chi^2 = 555.35, df = 20 (P < 0.01); I^2 = 96%								
Test for subgroup differences: Chi^2 = 5.12, df = 4 (P = 0.28)								Test for overall effect: Z: 2.78, P = 0.01

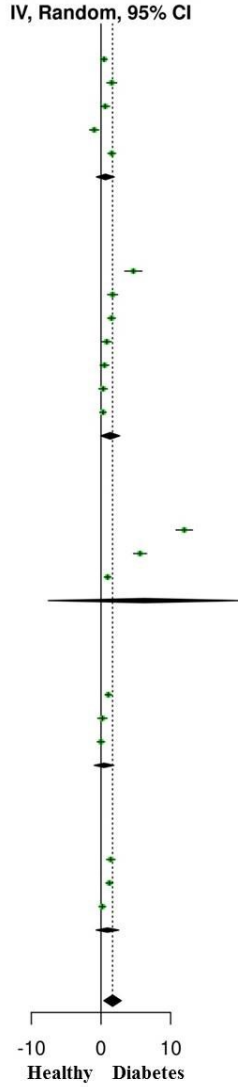
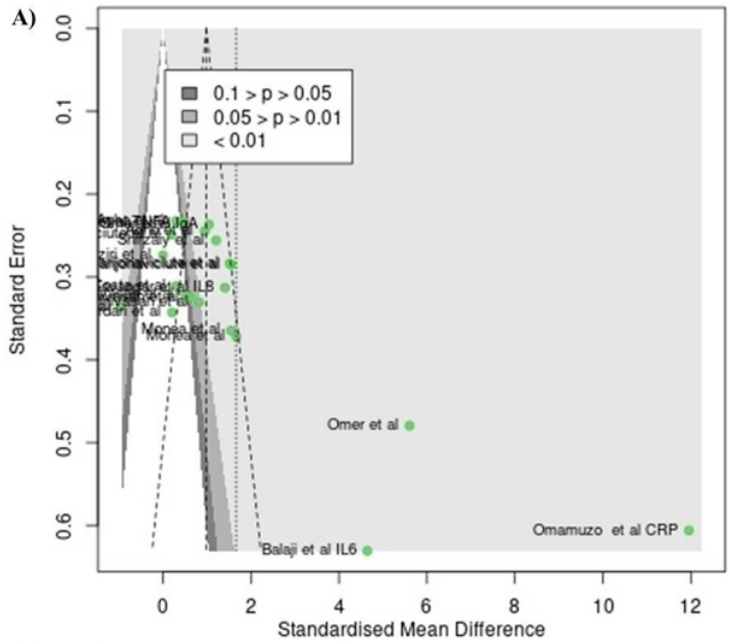


Fig. 2: The forest plot depicts the pooled effect size and subgroup analysis in healthy subjects and patients with type 2 diabetes mellitus. The standardized mean difference (SMD) was estimated by meta-analysis. CRP: C-reactive protein; IL: interleukin; TNFA: tumor necrosis factor alpha

A meta-analysis of 3 studies (12, 20, 21) showed that salivary CRP levels were associated with T2DM (SMD 6.15; $CI_{95\%}$: -7.57, 19.87; P=0.19, I^2 =99%). All three studies included in the meta-analysis reported greater salivary CRP levels in patients with T2DM than in healthy subjects. Three studies reported that salivary levels of IgA (21, 22, 28) (SMD 0.44; $CI_{95\%}$: -0.97, 1.85; P=0.31, I^2 = 79%) and IL-8 (25-27) (SMD 0.92; $CI_{95\%}$: -0.71, 2.55; P=0.13, I^2 = 84%) were higher in patients with T2DM than in healthy subjects.

Figure 3



B)

Publication bias			Heterogeneity	
Egger's Regression Test		Rosenthal's Fail-safe N	Meta regression	
t	p-value	2608	Q	p-value
4.13	0.0006		436.16	0.0001

Fig. 3: (**A**) Funnel plot of 13 studies included in this meta-analysis. (**B**) Risk of publication bias and heterogeneity

Heterogeneity and the risk of bias

The confounding study and statistical analysis were a concern in this meta-analysis, with 11 and 8 studies being at high risk of bias due to confounding or statistical analysis, respectively. In this analysis, most studies (11/13) had a high and moderate risk of bias, according to the QUIPS tool (Table 3). Asymmetry in the funnel plot (Fig. 3A) was assessed using the Egger regression test (Fig. 3B). This revealed a significant publication bias (t: 4.13; P=0.0006; Fig. 3B). Asymmetry in the funnel plot suggests the presence of publication bias and an overestimation of the overall effect size.

A high degree of heterogeneity was evident in all meta-analyses, as evidenced by high I^2 indices (I^2: 79 to 99%; P<0.01; Fig. 2). Meta-regression analysis confirmed significant heterogeneity (P<0.0001) in the outcomes of interest (Fig. 3B). This heterogeneity might be due to patient characteristics, unknown patient conditions (i.e., different diets or physical activities), or heterogeneity in the source of exposure (i.e., race, age, different drug delivery methods or systems, etc.).

Table 3: Risk of publication bias (according to the QUIPS tool)

Study	*Participation*	*Attrition*	*Prognostic factor*	*Outcome*	*Statistical analysis and reporting*	*Study confounding*	*Risk of bias*
Agho 2021 [12]	Low	Low	Moderate	Low	Low	Low	-
Costa 2010 [7]	Moderate	Low	Low	Low	Low	Low	-
Monea 2018 [19]	Moderate	Low	Low	Low	High	High	+/-
Srinivasan 2018 [23]	High	High	High	Moderate	High	High	+
Srinivasan 2015 [24]	High	High	Moderate	Moderate	High	High	+
Tvarijonaviciute 2017 [27]	Moderate	Moderate	Moderate	Moderate	Moderate	High	+/-
Balaji 2017 [29]	Moderate	Low	Moderate	Moderate	High	High	+/-
Omamuzo 2021 [20]	Moderate	Low	Moderate	Moderate	Moderate	High	+/-
Omer 2021 [21]	Moderate	High	Moderate	Low	High	High	+
Sardari 2015 [22]	High	Moderate	Moderate	Moderate	High	High	+
Vaziri 2010 [28]	High	High	Moderate	Moderate	High	High	+
Shirzaiy 2023 [25]	Low	Low	Moderate	Moderate	Moderate	High	+/-
Tavangar 2017 [26]	Moderate	High	Moderate	Moderate	High	High	+
Overall: High risk	4/13	5/13	1/13	0/13	8/13	11/13	6/13
Overall: Moderate risk	7/13	2/13	10/13	9/13	3/13	0/13	5/13
Overall: Low risk	2/13	6/13	2/13	4/13	2/13	2/13	2/13

+: high; **+/−:** moderate; **-** : low

Network analysis

To identify salivary components most closely associated with patient characteristics (i.e., FPG, HbA1c, age, and sex), we performed network analysis using the highest cutoff points for network visualization. To achieve this, the cutoff point was increased stepwise until a point was reached where no association was established between patient characteristics and salivary components.

Network analysis (at the highest cutoff: 67%) revealed that salivary CRP was the mediator most closely associated with FPG levels (Fig. 4A). At the highest network visualization cutoff of 59%, we found that salivary CRP was the factor most closely associated with age (Fig. 4B). At the highest cutoff point of the network visualization, salivary IL-8 was most closely associated with female sex (network cutoff point: 67%, Fig. 4A) or HbA1c (network cutoff point: 59%, Fig. 4C). The highest cutoff point for network visualization revealed that TNF-α was the salivary factor most strongly associated with male sex (network cutoff point: 50%, Fig. 4D).

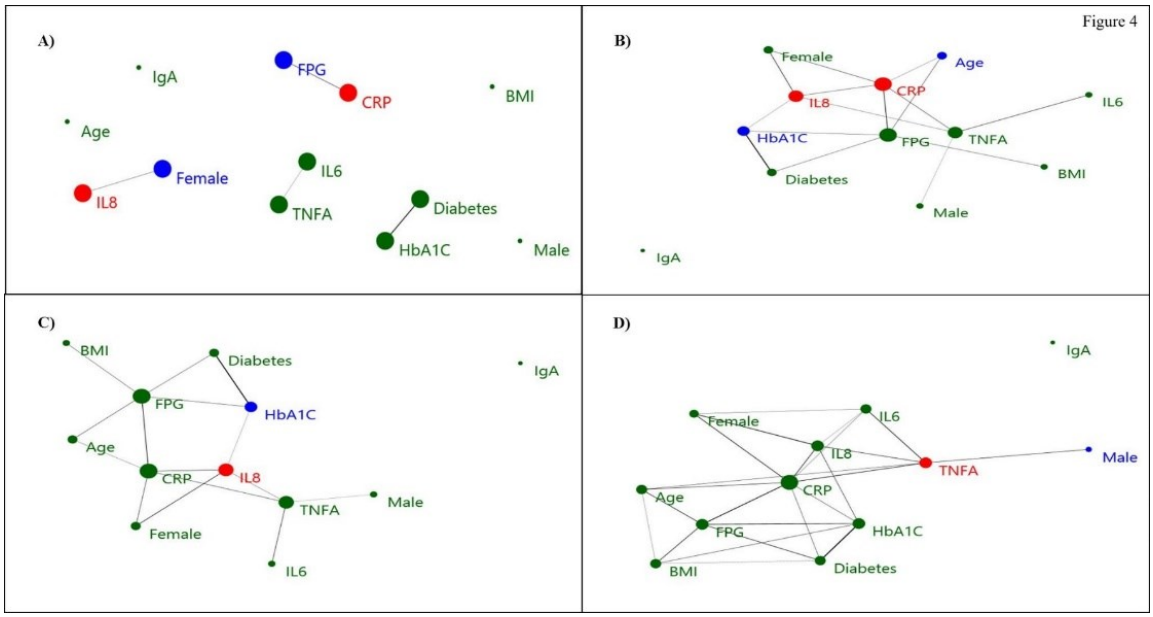


Fig. 4: Sub-network Enrichment analysis to reveal complex links among variables. The salivary components most closely associated with (**A**) fasting plasma glucose (FPG) and female sex; (**B**) age; (**C**) HbA1c; and (**D**) male sex. CRP: C-reactive protein; IL: interleukin; TNFA: tumor necrosis factor alpha; BMI: body mass index. Blue represents patient characteristics. Red represents salivary factors associated with patient characteristics

Discussion

This meta-analysis indicated that T2DM was linked to an increase in salivary inflammatory mediators in patients without oral problems. Interestingly, network analysis revealed sex-specific associations of salivary proinflammatory mediators in patients with T2DM.

According to network analysis, CRP was found to be the salivary mediator most closely linked with diabetes. CRP was also the factor most closely related to FPG levels in patients with T2DM. Furthermore, all studies included in this meta-analysis reported that CRP levels were greater in patients with T2DM than in healthy controls. Previous studies have reported that salivary CRP levels are higher in patients with T2DM than in healthy individuals (30). The association between salivary CRP and FPG levels suggests that inflammation is a key feature of insulin resistance in T2DM (31). Other conditions, such as periodontal disease, heart inflammation, obesity, and kidney dysfunction, may also be associated with elevated CRP levels (32). This issue limits the use of these biomarkers to monitor diabetes (32). In this study, BMI was higher in patients with T2DM than in healthy subjects (27.6 vs. 25.5 kg/m^2). A positive association between BMI and CRP levels has been reported (33). Salivary CRP levels were six times higher in obese subjects than in normal-weight subject (34). Therefore, high BMI and the presence of T2DM may partially explain the high levels of salivary CRP in patients with T2M, even in the absence of oral problems. There are inconsistent results regarding the relationship between serum and salivary CRP levels (35-37). Salivary CRP levels currently do not have a consistent and strong correlation with serum CRP levels and are therefore not suitable for predicting systemic inflammation. The discrepant correlation between serum and salivary CRP levels suggests the possibility of local secretion of CRP in saliva. The development of insulin resistance is mainly associated with low-level tissue-specific inflammatory responses triggered by various mediators of proinflammatory and/or oxidative stress (38). Low-grade local inflammation plays a key role in the

development of insulin resistance and the pathogenesis of T2DM. Therefore, T2DM-induced low-grade local inflammation may predispose patients to comorbidities.

This study showed that salivary IL-6 (significantly) and IL-8 favored T2DM. These cytokines are known to be a risk factor for periodontitis (6). Diabetes can regulate periodontal tissue destruction through vascular changes, alter collagen and glycosaminoglycan synthesis, and regulate cytokine production (39). IL-6 stimulates osteoclast activity and bone resorption in periodontitis (40). In addition, IL-8 acts as an important chemokine in the induction and development of periodontitis (41), a potent chemoattractant cytokine and activator of neutrophils at sites of inflammation (42). Therefore, high levels of IL-6 and IL-8 in saliva can cause oral problems in people with T2DM.

TNF-α is another important inflammatory cytokine closely associated with insulin resistance and plays a role in the regulation of CRP expression associated with insulin resistance (43). In addition, TNF-α contributes to the development of periodontal inflammation, such as periodontitis (44). Increased levels of TNF-α in various periodontal tissue cells are associated with periodontal tissue destruction, including bone resorption (44). The findings of the network analysis showed that males were more likely to have elevated levels of salivary TNF-α. Therefore, high levels of TNF-α in saliva may be a sign of periodontal damage, particularly in men.

Network analysis revealed a sex-specific link between patient characteristics and salivary proinflammatory mediators. Males and females were associated with TNF-α and IL-8 levels, respectively. Gene expression in PBMCs from patients with T2DM was recently reported to be sex-specific (5). Regarding sex effects, IL-12p70, IL-1β, IL-13, IL-2, IL-8, and TNF-α were found to have higher concentrations in males than in females (13). In the present meta-analysis, it was not possible to assess sex differences. However, using network analysis, we found sex-specific associations between patient characteristics and salivary cytokines. However, the exact reason for this discrepancy is unclear and requires further research. Knowledge of sex differences in salivary cytokines may have important implications for their use as disease biomarkers and may be associated with sex differences in diabetic conditions.

Conclusion

The results may contribute to knowledge regarding the development of standardized protocols to use saliva as a diagnostic tool for screening, monitoring, and management of T2DM. In addition, elevated levels of proinflammatory mediators in saliva indicated a differential inflammatory process associated with T2DM in both sexes.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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