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RESEARCH ARTICLE **OPEN ACCESS**

Salivary Biomarkers and *Pseudomonas aeruginosa* Levels in Type 1 Diabetes with Periodontitis: An Uncontrolled State May Exacerbate Oral Inflammation

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Abstract:

Introduction: Periodontitis is a chronic inflammatory disease known to be more severe in individuals with diabetes. However, the specific impact of glycemic control in Type 1 Diabetes (T1D) on a comprehensive panel of oral inflammatory and stress biomarkers, alongside opportunistic pathogens like *Pseudomonas aeruginosa*, requires further elucidation. This study aimed to investigate the associations between glycemic control status in T1D patients with periodontitis and levels of salivary stress (cortisol, Chromogranin A [CgA], Salivary Alpha-Amylase [sAA]) and inflammatory (C-reactive protein [CRP], albumin) biomarkers, subgingival *P. aeruginosa* counts, and clinical periodontal parameters.

Methods: This cross-sectional study enrolled 116 adults who were divided into four equal groups (n=29 each): Uncontrolled T1D with Periodontitis (UC-T1D; HbA1c ≥ 6.5%), controlled T1D with Periodontitis (C-T1D; HbA1c < 6.5%), Non-Diabetics with Periodontitis (NP), and Non-Diabetics with Healthy Periodontium (NH). Salivary biomarkers were quantified using commercial ELISA kits. Subgingival *P. aeruginosa* bacterial loads were determined by real-time Polymerase Chain Reaction (PCR) targeting the *oprL* gene. Clinical periodontal parameters (PPD, CAL, PI, BOP) were recorded by a single calibrated examiner. Non-parametric tests (Kruskal-Wallis, Mann-Whitney U) were used for group comparisons, and Spearman's correlation was used to assess relationships.

Results: The UC-T1D group exhibited significantly higher salivary cortisol, CgA, sAA, CRP, and albumin levels, along with greater subgingival *P. aeruginosa* counts, compared to all other groups (all $p<0.001$). This group also presented with the most severe periodontal parameters (highest mean PPD, CAL, PI, and BOP%; $p<0.001$). Subgingival *P. aeruginosa* counts were significantly and positively correlated with salivary cortisol ($r=0.52$, $p<0.01$), CgA ($r=0.48$, $p<0.01$), CRP ($r=0.67$, $p<0.001$), and albumin ($r=0.62$, $p<0.001$).

Discussion: The findings indicated that an uncontrolled glycemic state in T1D is strongly associated with a heightened oral inflammatory and stress environment. The elevation of both HPA and SAM axis markers (cortisol, CgA, sAA) suggests that systemic physiological stress in uncontrolled T1D may contribute to oral inflammation. The parallel increase in salivary CRP and albumin confirms a state of local and systemic inflammation with compromised gingival vascular integrity. The higher burden of the opportunistic pathogen *P. aeruginosa* in the UC-T1D group suggests that poor glycemic control may create a favorable niche for its colonization, potentially exacerbating periodontal destruction. Study limitations include the cross-sectional design and focus on a single opportunistic pathogen.

Conclusion: Uncontrolled T1D is associated with significantly elevated salivary stress and inflammatory biomarkers, increased subgingival *P. aeruginosa* colonization, and more severe periodontitis. These findings underscore the critical importance of diligent glycemic management in T1D patients to mitigate oral inflammation and alter microbial profiles that contribute to the development of periodontal disease. Longitudinal studies are warranted to confirm these associations and explore causality.

Keywords: Periodontitis, Type 1 diabetes, Salivary biomarkers, *Pseudomonas aeruginosa*, Oxidative stress.

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1. INTRODUCTION

Periodontitis, a chronic inflammatory condition affecting the tissues supporting the teeth, remains a significant global health issue. Characterized by the progressive destruction of periodontal ligaments and alveolar bone, it can lead to tooth mobility, discomfort, and ultimately, tooth loss [1], thereby impacting chewing function, aesthetics, and the overall quality of life [2]. The disease often follows a pattern of exacerbations and remissions, sometimes resolving spontaneously but frequently causing irreversible damage [3]. Crucially, emerging evidence highlights intricate bidirectional links between periodontitis and various systemic conditions, underscoring the importance of oral health in overall wellness and the need for a holistic diagnostic and therapeutic approach [4].

Among the systemic conditions strongly associated with periodontitis is diabetes mellitus. Individuals managing diabetes, particularly those experiencing challenges with glycemic control, face an elevated risk of developing periodontitis and suffering more severe consequences [5]. Type 1 Diabetes (T1D), characterized by autoimmune destruction of pancreatic beta cells leading to absolute insulin deficiency, often presents unique challenges due to its typical onset in younger individuals and the lifelong dependence on exogenous insulin for managing blood glucose levels [6]. This dependency can lead to significant glycemic variability, which may further complicate inflammatory responses. While the link between diabetes (broadly) and periodontitis is well-established, the specific impact of varying glycemic control within the T1D population on the oral inflammatory environment, including specific microbial and biomarker profiles, warrants more focused investigation compared to the more extensively studied Type 2 Diabetes. The hyperglycemic state common in poorly controlled diabetes disrupts immune cell function (*e.g.*, impaired neutrophil activity), promotes a pro-inflammatory cytokine profile, and impairs wound healing. This collectively exacerbates periodontal tissue destruction and creates a detrimental cycle of persistent inflammation and delayed repair, accelerating disease progression [7-9].

The oral cavity hosts a complex polymicrobial community. While classical pathogens, such as *Porphyromonas gingivalis*, are strongly implicated in periodontitis, the role of opportunistic pathogens in disease modulation, especially under conditions of systemic compromise like diabetes, is increasingly recognized. *Pseudomonas aeruginosa*, a Gram-negative bacillus known for its virulence factors and ability to thrive in diverse environments, is typically considered an opportunistic pathogen [10]. While not traditionally classified as a primary periodontal pathogen, its presence has been noted in subgingival plaque, and it possesses mechanisms that can induce inflammation and contribute to tissue damage [11]. Notably, *P. aeruginosa* infections are frequently associated with immunocompromised states. Given that poorly controlled diabetes impairs immune function [7, 8], investigating the levels of *P. aeruginosa* in the subgingival environment of T1D patients with varying glycemic control may provide insight into whether this opportunistic bacterium flourishes and contributes to the exacerbated periodontal destruction observed in this population. Therefore, this study specifically quantified *P. aeruginosa* not as the sole causative agent, but as a potential indicator or contributor to the altered microbial ecology and inflammatory state associated with poor glycemic control in T1D.

Traditional periodontal assessments, including clinical examinations and probing depths, provide valuable information about existing tissue destruction [12], but may not fully capture the underlying biological activity or the influence of systemic factors, such as glycemic control and stress responses [13]. Saliva-based biomarkers offer a non-invasive window into the local oral and systemic physiological state, reflecting levels of stress, inflammation, and potentially microbial activity [14]. Analyzing specific salivary markers in individuals with T1D and periodontitis could reveal crucial details about disease severity, the impact of glycemic management, and potential therapeutic targets [15]. For this study, a panel of biomarkers representing key physiological axes was selected. Salivary cortisol, the primary Glucocorticoid Hormone, Was Chosen As A Classical Indicator Of Hypothalamic-Pituitary-Adrenal (HPA) axis activation and chronic stress, which can modulate immune responses [16, 17]. CgA and sAA were selected as markers of the Sympathetic Nervous System (SNS) and Sympathoadrenal Medullary (SAM) axis activity [18]. CgA is co-released with catecholamines [19], while sAA activity reflects rapid SNS responses to stressors [16, 20]. To assess inflammation, the C-Reactive Protein (CRP), a systemic acute-phase reactant, was measured in saliva, as it can reflect both systemic inflammation common in diabetes and potentially local gingival inflammation spillover [21, 22]. Finally, salivary albumin, typically present at low levels, was chosen because its elevation indicates increased gingival crevicular fluid leakage due to compromised vascular integrity and local inflammation within the periodontal tissues [23, 24]. Evaluating this combination of

stress and inflammatory markers provides a more comprehensive understanding of the complex interplay between systemic factors, such as diabetes and stress, and local periodontal factors [25].

Therefore, while the link between diabetes and periodontitis is established, this study is among the first in our specific population (Iraqi T1D patients) to simultaneously investigate the interplay between glycemic control status, a comprehensive panel of selected salivary stress and inflammatory biomarkers, the quantitative levels of the opportunistic pathogen *P. aeruginosa*, and clinical periodontal parameters. This multi-faceted approach aimed to provide a more nuanced understanding of how poor glycemic control in T1D might distinctly shape the oral microenvironment.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

This cross-sectional clinical study was conducted at the Diabetic Department of Al-Sader Hospital in Najaf, Iraq, between January 2022 and July 2023. A total of 116 adult participants, aged 18 years or older, were recruited and categorized into four groups based on their diabetic status and periodontal health:

2.1.1. Uncontrolled Type 1 Diabetic with Periodontitis (UC-T1D)

Participants with type 1 diabetes mellitus and an HbA1c level ≥ 6.5% who exhibit at least two teeth with a Pocket Depth (PD) of 4 mm and a positive Bleeding On Probing (BOP) response. (n=29).

2.1.2. Controlled Type 1 Diabetic with Periodontitis (C-T1D)

Participants with type 1 diabetes mellitus and an HbA1c level < 6.5%, exhibiting at least two teeth with a PD of 4 mm and a positive BOP. (n=29).

2.1.3. Non-diabetic with Periodontitis (NP)

Participants without diabetes mellitus, exhibiting at least two teeth with a PD of 4 mm and a positive BOP. (n=29).

2.1.4. Non-diabetic with Healthy Periodontium (NH)

Participants without diabetes mellitus, exhibiting a clinical attachment loss (CAL) ≤ 2 mm. (n=29).

2.2. Sample Size

The sample size was determined using a power analysis (G*Power software) with a desired power of 80%, an alpha level of 0.05, and an estimated effect size of 0.5 (considered a medium effect size) based on previous research [13]. This analysis indicated that a minimum sample size of 29 per group was required to detect a statistically significant difference in mean pocket depth between the groups.

2.3. Inclusion and Exclusion Criteria

2.3.1. Inclusion Criteria

Adult patients aged 18 years or older, at least 20 teeth present in the mouth, ability and willingness to provide informed consent in writing, no intake of antibiotics or anti-inflammatory drugs within the 6 months preceding the baseline examination.

2.3.2. Exclusion Criteria

Pregnant or nursing, Use of steroid medication, Organ complications/failure, inability to provide saliva, Uncooperative patients, Smoking.

2.4. Periodontal Assessment

Periodontal disease status was evaluated at four sites per tooth (mesiobuccal, labial/buccal, distobuccal, lingual/palatal) using a UNC-15 probe (Hu-Friedy's, USA). All periodontal examinations were performed by a single calibrated examiner **(S.M.I.).** The following parameters were measured:

2.4.1. Pocket Depth (PD)

Measured as the distance from the gingival margin to the base of the periodontal pocket in millimeters.

2.4.2. Clinical Attachment Loss (CAL)

Measured as the distance from the cementoenamel junction to the base of the periodontal pocket in millimeters.

2.4.3. Plaque Index (PI)

Assessed using the Silness and Löe plaque index [26], which remains a foundational and widely utilized method for quantifying supragingival plaque.

2.4.4. Bleeding on Probing (BOP)

Recorded as present or absent, indicating bleeding upon gentle probing of the gingival sulcus.

2.5. Saliva Collection and Analysis

Unstimulated whole saliva samples were collected in the morning between 8:00 am and 11:00 am from all participants using the spitting method. Participants were instructed to rinse their mouths with water to remove food debris, sit still for six minutes, and avoid chewing or speaking until the saliva sample was collected. The samples were stored in sterile, plain bottles and immediately transported to the laboratory in an ice pack at 4°C, then frozen at or below -20°C [27].

According to the manufacturer's instructions, the following salivary markers were analyzed using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Monobind Inc., Lake Forest, CA, USA). These kits are validated for use with saliva samples; their use with saliva is supported by literature, and appropriate sample processing protocols were followed as per manufacturer guidelines:

2.5.1. Salivary Cortisol

Cortisol levels were measured using a Ruthenium complex-labeled cortisol derivative and a cortisol-specific biotinylated antibody [28].

2.5.2. Salivary Chromogranin A (CgA)

CgA levels were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) technique [29].

2.5.3. Salivary Alpha-amylase (sAA)

sAA was extracted from oral fluid using an amylase-removing device, eluted from a potato starch matrix, and analyzed using a standard assay [30].

2.5.4. Salivary C-Reactive Protein (CRP)

CRP levels were measured using a standard ELISA kit [31].

2.5.5. Salivary Albumin

Albumin levels were estimated using the Bromocresol Green (BCG) dye method [32].

2.6. Subgingival Plaque Sampling and *P. Aeruginosa* Quantification

Subgingival plaque samples were collected from the deepest pocket in each quadrant using sterile paper points. Each paper point was inserted into the base of the pocket for 20 seconds. The four paper points from each participant were pooled into a single vial containing Reduced Transport Fluid (RTF). Samples were processed for DNA extraction and real-time PCR within one hour of collection [1, 9].

2.7. Real-time PCR for *P. Aeruginosa* Quantification

Bacterial chromosomal DNA was extracted from the pooled subgingival plaque samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for Gram-negative bacteria.

The quantification of P. aeruginosa was performed using a commercial real-time PCR kit (Microis Co., Ltd., Seoul, Korea) on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Standard PCR laboratory practices were implemented to prevent cross-contamination, including using separate pre- and post-PCR work areas, dedicated micropipettes with aerosol-resistant filter tips, and regular decontamination of surfaces. Each PCR run included No-Template Controls (NTCs) to monitor for contamination and positive controls (derived from the provided DNA standard) to ensure the validity of the amplification process. The kit utilized specific primers and a probe targeting the *oprL* gene, which is highly specific to *P. aeruginosa*. Amplification was performed according to the manufacturer's recommended cycling conditions, typically consisting of an initial polymerase activation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and combined annealing and extension at 60°C for 60 seconds. Fluorescence data were collected during the annealing and extension steps.

Quantification was based on the Cycle threshold (Ct) values obtained. Absolute quantification was performed using a standard curve method. The standard curve was generated using serial dilutions (*e.g.*, 10^7 to 10^1 copies/reaction) of a provided DNA standard containing a known concentration of the *P. aeruginosa* oprL target sequence, which was run in parallel with the clinical samples. The bacterial load was determined by comparing the sample Ct values to the standard curve using the PCR instrument's software (*e.g.*, Bio-Rad CFX Maestro Software). Results were calculated based on the initial sample processing volumes and DNA elution volume. Finally, they expressed the number of *P. aeruginosa* cells per microliter (log10 cells/µL) of the original sample, assuming a single copy of the oprL gene per *P. aeruginosa* cell, as per standard conversion practices referenced by the kit manufacturer [33]. An example of an amplification plot is shown in Fig. (**1**).

2.8. Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 for Windows. Descriptive statistics were calculated for all variables. Non-parametric tests, including the Kruskal-Wallis test and the Mann-Whitney U test, were used to compare salivary marker levels, *P. Aeruginosa* counts, and periodontal parameters among the four groups. Spearman's rank correlation coefficient was used to assess the relationships between salivary markers and *P. Aeruginosa* counts. Statistical significance was set at $p < 0.05$.

2.9. Ethical Considerations

This study was approved by the Ethical Committee of the College of Dentistry, University of Kufa (Reference number 4367, dated June 24, 2022). Informed consent was obtained from all participants prior to their enrollment.

3. RESULT

3.1. Participant Characteristics and Baseline Periodontal Status

The demographic characteristics, including age and gender distribution, were comparable across the four groups, with no statistically significant differences observed ($p = 0.345$ for age, $p = 0.789$ for gender; Table **1**, Fig. **2**). As expected based on group definitions, HbA1c levels were significantly higher in the UC-T1D group (Mean 8.1%, SD 1.2) compared to the C-T1D group (Mean 5.8%, SD 0.8) ($p<0.001$; Table **1**).

Baseline periodontal parameters showed significant differences among the groups, reflecting their defined health status (Table **2**). The UC-T1D group exhibited significantly worse periodontal health compared to all other groups, demonstrating the highest mean Pocket Depth (PD) (5.2 ± 1.3 mm), Clinical Attachment Loss (CAL) (4.8 ± 1.5 mm), Plaque Index (PI) (2.3 ± 0.6), and percentage of sites with Bleeding on Probing (BOP) (83%) (all $p<0.001$). The C-T1D group also exhibited significantly worse periodontal parameters than the NP and NH groups, although generally less severe than those of the UC-T1D group (Table **2**). The NH group displayed parameters consistent with periodontal health, serving as the baseline control (all $p<0.001$ compared to the periodontitis groups).

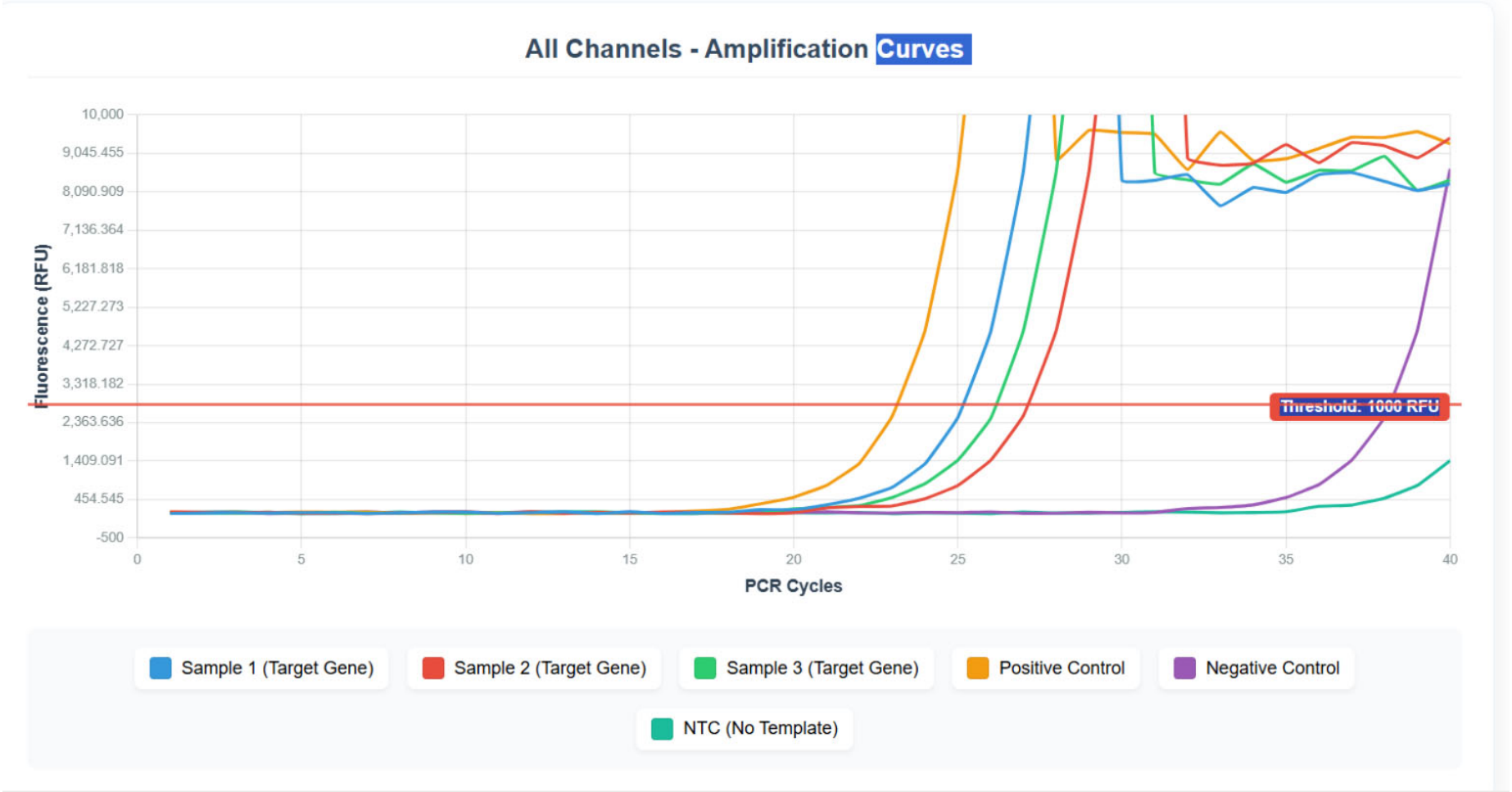

Fig. (1). Real-time PCR amplification plot.

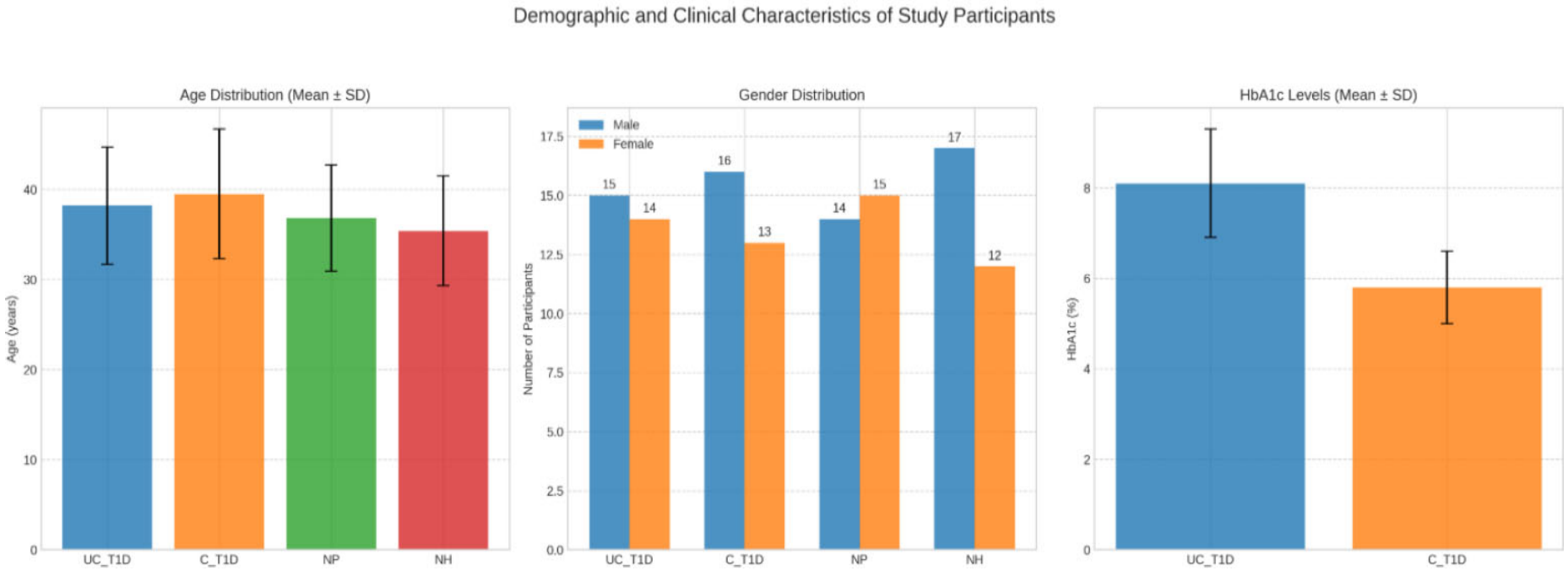

Fig. (2). Demographic and clinical characteristics of study participants across different groups.

Table 1. Demographic and clinical characteristics of study participants.

Variable	UC-T1D (n=29) Mean (SD)	C-T1D (n=29) Mean (SD)	NP (n=29) Mean (SD)	NH (n=29) Mean (SD)	*p*-value
Age (years)	38.2 (6.5)	39.5 (7.2)	36.8 (5.9)	35.4 (6.1)	0.345
Gender (Male/Female)	15/14	16/13	14/15	17/12	0.789
HbA1c (%)	8.1 (1.2)	5.8 (0.8)	N/A	N/A	<0.001*

Abbreviations: UC-T1D: Uncontrolled Type 1 Diabetic with Periodontitis; C-T1D: Controlled Type 1 Diabetic with Periodontitis; NP: Non-Diabetic with Periodontitis; NH: Non-Diabetic with Healthy Periodontium; SD: Standard Deviation; HbA1c: Glycated Hemoglobin; N/A: Not Applicable. *Indicates statistical significance ($p < 0.05$).

Table 2. Periodontal parameters in study groups.

Parameter	UC-T1D (n=29) Mean (SD)	C-T1D (n=29) Mean (SD)	NP (n=29) Mean (SD)	NH (n=29) Mean (SD)	*p*-value
PD (mm)	5.2 (1.3)	4.6 (1.1)	3.9 (0.9)	2.1 (0.5)	<0.001*
CAL (mm)	4.8 (1.5)	4.1 (1.2)	3.4 (0.8)	1.2 (0.4)	<0.001*
PI	2.3 (0.6)	2.0 (0.5)	1.7 (0.4)	0.8 (0.3)	<0.001*
BOP%	83%	72%	62%	24%	<0.001*

Abbreviations: UC-T1D: Uncontrolled Type 1 Diabetic with Periodontitis; C-T1D: Controlled Type 1 Diabetic with Periodontitis; NP: Non-Diabetic with Periodontitis; NH: Non-Diabetic with Healthy Periodontium; SD: Standard Deviation; PD: Pocket Depth; CAL: Clinical Attachment Loss; PI: Plaque Index; BOP: Bleeding on Probing. *Indicates statistical significance ($p < 0.05$).

Table 3. Descriptive statistics of salivary markers in study groups.

Variable	UC-T1D (n=29) Mean (SD)	C-T1D (n=29) Mean (SD)	NP (n=29) Mean (SD)	NH (n=29) Mean (SD)	*p*-value
Cortisol (ng/mL)	15.2 (3.8)	12.1 (2.9)	10.5 (2.1)	8.7 (1.5)	<0.001*
CgA (ng/mL)	10.8 (2.7)	8.5 (1.9)	7.2 (1.6)	5.9 (1.2)	<0.001*
sAA (U/mL)	125 (35)	108 (28)	95 (20)	82 (15)	<0.001*
CRP (ng/mL)	12.3 (3.1)	9.7 (2.5)	8.1 (1.8)	6.4 (1.3)	<0.001*
Albumin (mg/dL)	18.5 (4.2)	15.6 (3.7)	13.2 (2.9)	10.8 (2.2)	<0.001*

Abbreviations: UC-T1D: Uncontrolled Type 1 Diabetic with Periodontitis; C-T1D: Controlled Type 1 Diabetic with Periodontitis; NP: Non-Diabetic with Periodontitis; NH: Non-Diabetic with Healthy Periodontium; SD: Standard Deviation; CgA: Chromogranin A; sAA: Salivary Alpha-Amylase; CRP: C-Reactive Protein. *Indicates statistical significance ($p < 0.05$).

Table 4. Descriptive statistics of *P. Aeruginosa* count in study groups.

Variable	UC-T1D (n=29) Mean (SD)	C-T1D (n=29) Mean (SD)	NP (n=29) Mean (SD)	NH (n=29) Mean (SD)	*p*-value
P.aeruginosa Count (log10 cells/µL)	6.8 (1.2)	5.9 (1.0)	5.2 (0.8)	4.1 (0.6)	<0.001*

Abbreviations: UC-T1D: Uncontrolled Type 1 Diabetic with Periodontitis; C-T1D: Controlled Type 1 Diabetic with Periodontitis; NP: Non-Diabetic with Periodontitis; NH: Non-Diabetic with Healthy Periodontium; SD: Standard Deviation; *P. aeruginosa: Pseudomonas aeruginosa*. *Indicates statistical significance ($p < 0.05$).

3.2. Salivary Biomarker Levels

Analysis of salivary biomarkers revealed significantly different levels across the study groups (Table 3, Fig. 3). The UC-T1D group consistently demonstrated the highest levels for all measured stress and inflammatory markers. Specifically, mean salivary cortisol (15.2 ± 3.8 ng/mL), chromogranin A (10.8 ± 2.7 ng/mL), alpha-amylase (125 ± 35 U/mL), C-reactive protein (CRP) (12.3 ± 3.1 ng/mL), and albumin (18.5 ± 4.2 mg/dL) were significantly elevated in the UC-T1D group compared to the C-T1D, NP, and NH groups (Kruskal-Wallis test, $p<0.001$ for all biomarkers). Post-hoc analyses confirmed significant differences between most group pairings, generally showing a trend of UC-T1D > C-T1D > NP > NH (Table 3).

3.3. Subgingival *Pseudomonas aeruginosa* Levels

Quantification of *Pseudomonas aeruginosa* in subgingival plaque samples also revealed significant differences between the groups (Table 4, Fig. 4). The highest mean bacterial load was observed in the UC-T1D group (6.8 ± 1.2 log10 cells/µL). This level was significantly higher than that found in the C-T1D group (5.9 ± 1.0 log10 cells/µL), the NP group (5.2 ± 0.8 log10 cells/µL), and the NH group (4.1 ± 0.6 log10 cells/µL) (Kruskal-Wallis test, $p<0.001$). Post-hoc analyses indicated significant differences between all group comparisons (Table 4).

3.4. Correlations between Salivary Markers, *P. aeruginosa* Count, and Periodontal Parameters

To examine the associations between measured variables in all participants, Spearman rank correlation analyses were performed (Tables 5 and 6). Significant positive correlations were found between subgingival *P. aeruginosa* values and all measured salivary biomarkers (Table 5). In particular, moderate positive correlations were found between *P. aeruginosa* levels and the stress markers salivary cortisol ($r = 0.52$, $p < 0.01$) and chromogranin A ($r = 0.48$, $p < 0.01$). Stronger positive correlations were found between *P. aeruginosa* levels and salivary inflammatory markers CRP ($r = 0.67$, $p < 0.001$) and albumin ($r = 0.62$, $p < 0.001$). A significant moderate positive correlation was also found between *P. aeruginosa* and salivary α-amylase ($r = 0.39$, $p < 0.05$).

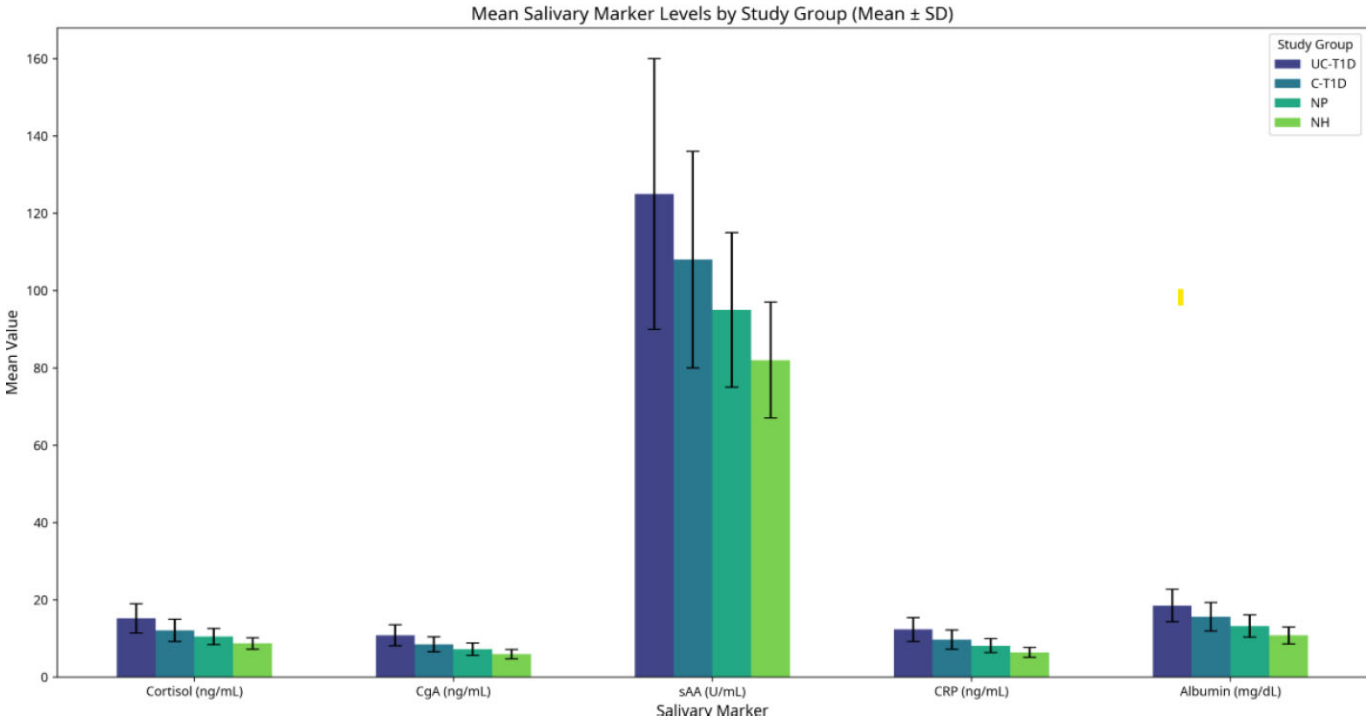

Fig. (3). Salivary markers across different study groups.

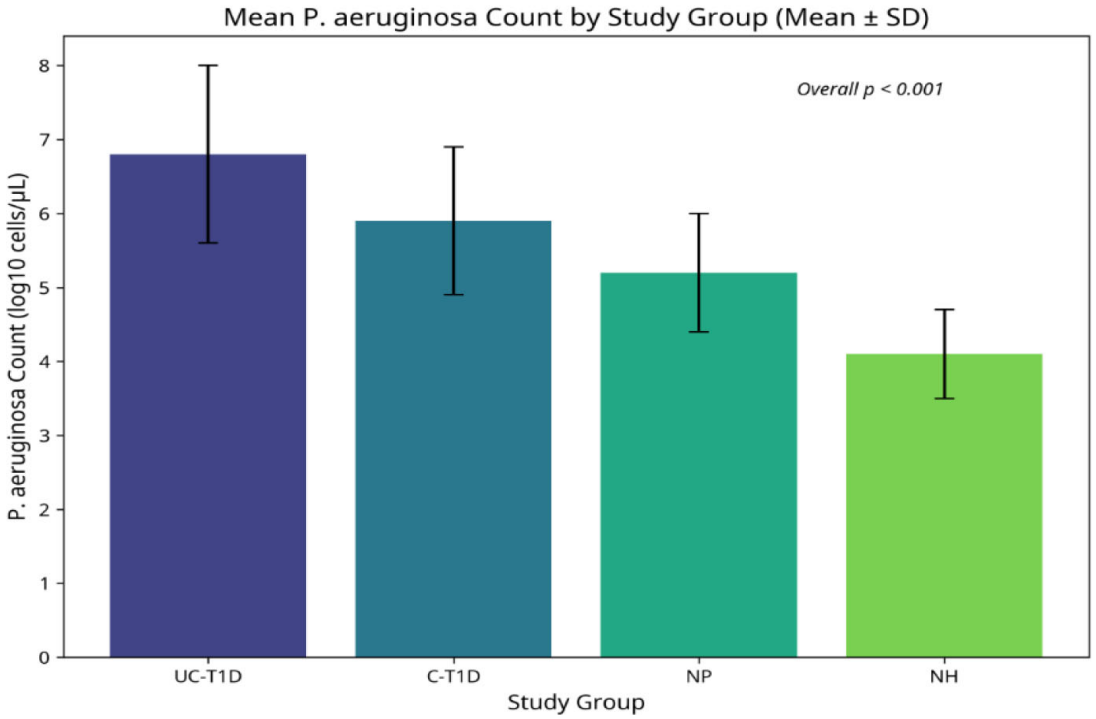

Fig. (4). *Pseudomonas aeruginosa* count in study groups with different diabetic status and periodontal health.

Table 5. Correlation between salivary markers and *P. Aeruginosa* count.

Salivary Marker	Correlation Coefficient (*r*)	*p*-value
Cortisol	0.52	<0.01**
CgA	0.48	<0.01**
sAA	0.39	<0.05*
CRP	0.67	<0.001***
Albumin	0.62	<0.001***

Abbreviations: *r*: Spearman's Rank Correlation Coefficient; CgA: Chromogranin A; sAA: Salivary Alpha-Amylase; CRP: C-Reactive Protein. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 6. Correlations between salivary markers and periodontal parameters.

Salivary Marker	(PD) r (*p*-value)	(CAL) r (*p*-value)	(PI) r (*p*-value)	(BOP) r (*p*-value)
Cortisol	0.54*** ($p < 0.001$)	0.51*** ($p < 0.001$)	0.42*** ($p < 0.001$)	0.61*** ($p < 0.001$)
CgA	0.48*** ($p < 0.001$)	0.45*** ($p < 0.001$)	0.36*** ($p < 0.001$)	0.57*** ($p < 0.001$)
sAA	0.39** ($p < 0.01$)	0.35** ($p < 0.01$)	0.29* ($p = 0.002$)	0.45*** ($p < 0.001$)
CRP	0.67*** ($p < 0.001$)	0.65*** ($p < 0.001$)	0.58*** ($p < 0.001$)	0.72*** ($p < 0.001$)
Albumin	0.62*** ($p < 0.001$)	0.60*** ($p < 0.001$)	0.52*** ($p < 0.001$)	0.69*** ($p < 0.001$)

Abbreviations: *r*: Correlation Coefficient; CgA: Chromogranin A; sAA: Salivary Alpha-Amylase; CRP: C-Reactive Protein; PD: Pocket Depth; CAL: Clinical Attachment Loss; PI: Plaque Index; BOP: Bleeding on Probing. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Furthermore, significant positive correlations were observed between all salivary biomarkers and the severity of periodontal parameters, indicating that higher biomarker levels were associated with worse periodontal health (Table **6**). Salivary CRP and albumin demonstrated strong positive correlations with Pocket Depth (PD) (r=0.67 and r=0.62, respectively; p<0.001), Clinical Attachment Loss (CAL) (r=0.65 and r=0.60, respectively; p<0.001), Plaque Index (PI) (r=0.58 and r=0.52, respectively; p<0.001), and Bleeding on Probing (BOP) (r=0.72 and r=0.69, respectively; p<0.001). Salivary cortisol and chromogranin A also exhibited moderate-to-strong positive correlations with all four periodontal parameters, with r values ranging from 0.36 to 0.61 and all p values < 0.001. Salivary alpha-amylase showed statistically significant positive correlations with all periodontal parameters, although generally weaker than the other biomarkers, PD (r=0.39, p<0.01), CAL (r=0.35, p<0.01), PI (r=0.29, p=0.002), and BOP (r=0.45, p<0.001).

Within the biomarkers themselves, a strong positive correlation was observed between salivary CRP and albumin levels (r = 0.78, p < 0.001), consistent with their roles as inflammatory markers.

4. DISCUSSION

This cross-sectional study, investigating the intricate relationship between salivary biomarkers, *P. aeruginosa* levels, and periodontal parameters in patients with type 1 diabetes mellitus, revealed a significant association between uncontrolled diabetes and an exacerbated inflammatory state within the oral environment. The results demonstrated the potential for uncontrolled diabetes to contribute to the development and progression of periodontitis, underscoring the importance of strict glycemic control and early intervention for optimal oral health in diabetic individuals.

The study enrolled 116 participants, carefully distributed across four groups (Table **1**). Importantly, the groups were relatively homogenous in terms of age and gender, minimizing the influence of these confounding factors on our results. The significant difference in HbA1c levels between the uncontrolled and controlled diabetic groups (p<0.001) confirmed the effectiveness of our grouping strategy and allowed us to directly assess the impact of glycemic control on the parameters of interest [34].

The significantly elevated levels of salivary stress and inflammatory biomarkers in the UC-T1D group compared to C-T1D, NP, and NH groups are particularly noteworthy. The concurrent elevation of salivary cortisol, Chromogranin A (CgA), and Salivary Alpha-Amylase (sAA) in individuals with uncontrolled T1D points to heightened activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympathetic Nervous System (SNS)/Sympathoadrenal Medullary (SAM) axis. This indicates increased physiological stress, which is often associated with poor metabolic control and can, in turn, modulate immune responses and exacerbate inflammation [35-38]. Elevated sAA levels, an enzyme involved in starch digestion found in saliva, further support the idea of increased stress and sympathetic activity, hinting at an impact on oral health in uncontrolled diabetes [39]. Simultaneously, the increased salivary C-Reactive Protein (CRP) and albumin levels in this group reflect both systemic inflammation—a hallmark of uncontrolled diabetes—and increased local oral inflammation with gingival crevicular fluid leakage due to compromised vascular integrity within periodontal tissues [40]. This pattern suggests that poor glycemic control in T1D contributes to a pro-inflammatory oral microenvironment, likely aggravated by systemic stress responses [41]. The significantly higher *P. Aeruginosa* counts observed in the uncontrolled diabetic group, compared to all other groups, reinforce the role of this key periodontopathogen in the pathogenesis of perio-

dontitis, particularly in individuals with uncontrolled diabetes. This finding aligned with previous research suggesting that *P. Aeruginosa* is a potent inflammatory agent that can exacerbate periodontal disease [10]. The data indicated that uncontrolled diabetes might create an environment that favors the growth and activity of *P. Aeruginosa*, potentially accelerating periodontal breakdown [42, 43].

The uncontrolled diabetic group showed higher levels of *P. Aeruginosa* compared to other groups, highlighting the important role of this key periodontal bacterium in causing gum disease, especially in individuals with uncontrolled diabetes [44, 45]. Controlling blood glucose levels might reduce the pro-inflammatory environment that favors the growth and activity of *P. Aeruginosa* [46]. Periodontal screening and early intervention are essential for diabetic patients, particularly those with uncontrolled blood sugar [43, 45]. These findings suggested that a panel of salivary biomarkers could, with further validation and standardization, serve as a non-invasive tool for chair-side risk assessment or monitoring of oral inflammatory status in T1D patients. Such tools could help identify individuals requiring more intensive periodontal care or those whose glycemic control might be impacting their oral health, thereby facilitating more personalized management strategies [47]. Antibacterial therapies targeting *P. Aeruginosa* and other periodontal pathogens might be beneficial in managing periodontitis in diabetic patients, helping to control the bacterial load and reduce inflammation [9].

5. LIMITATIONS

It is important to acknowledge the limitations of this study. The study's design makes it challenging to establish a connection between diabetes, salivary markers, *P. Aeruginosa*, and the development of disease. Additionally, the small sample size and reliance on a hospital for participants may hinder the generalizability of these findings. The focus on *P. aeruginosa* alone means that the contributions of other established periodontal pathogens, such as *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticol*a, as well as the broader shift in the subgingival microbiome (dysbiosis), were not assessed. The recruitment of participants from a single hospital center may limit the generalizability of these findings to the broader population of individuals with T1D and periodontitis.

6. FUTURE DIRECTIONS

Future longitudinal studies are crucial to establish causality and to track changes in these biomarkers and microbial profiles in response to periodontal therapy and improved glycemic control. Exploring the utility of serial salivary biomarker measurements could help monitor treatment efficacy or identify T1D patients at higher risk for periodontal disease progression, potentially guiding the tailoring of preventive and therapeutic interventions. Comprehensive microbiome analyses (*e.g.*, 16S rRNA sequencing) would also provide a complete picture of the dysbiotic shifts beyond *P. aeruginosa* alone.

CONCLUSION

This study provided evidence of a connection between managed T1D and higher levels of stress, inflammation, and *P. Aeruginosa* in the oral cavity. These findings emphasized the importance of regulating blood sugar levels and identifying and addressing the disease early in people with diabetes. Further research is needed to investigate the underlying mechanisms and develop targeted treatment strategies for managing the interplay between overall health and oral health.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: S.M.I.: Study conception and design; A.M.K., M.N.H.: Data collection. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

T1D	=	Type 1 Diabetes
PLI	=	Plaque Index
PD	=	Pocket Depth
CAL	=	Clinical Attachment Loss
BOP	=	Bleeding on Probing
CgA	=	Chromogranin A
sAA	=	Salivary Alpha-Amylase
CRP	=	C-Reactive Protein
NTC	=	No-Template Control
Ct	=	Cycle Threshold
HbA1c	=	Glycated Hemoglobin
UC-T1D	=	Uncontrolled Type 1 Diabetic with Periodontitis
C-T1D	=	Controlled Type 1 Diabetic with Periodontitis
NP	=	Non-Diabetic with Periodontitis
NH	=	Non-Diabetic with Healthy Periodontium

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee of the College of Dentistry, University of Kufa (Reference number 4367, dated June 24, 2022).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants prior to their enrollment.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

FUNDING

None.

CONFLICT OF INTEREST

The author(s) declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Ibrahim SM, Al-Mizraqchi AS, Haider J. Metronidazole potentiation by Panax ginseng and *Symphytum officinale*: A new strategy for P. gingivalis infection control. Antibiotics 2023; 12(8): 1288. http://dx.doi.org/10.3390/antibiotics12081288 PMID: 37627708

[2] Coelho Paraguassu E. Basic periodontitis manual: What it is, symptoms and treatments. Braz J Implantol Health Sci 2023; 5(2): 01-3. http://dx.doi.org/10.36557/2674-8169.2023v5n2p01-03

[3] Ibrahim SM, Al-Hmedat SJAZ, Alsunboli MH. Histological study to evaluate the effect of local application of *Myrtus communis* oil on alveolar bone healing in rats. Open Dent J 2024; 18(1): 18742106299510. http://dx.doi.org/10.2174/0118742106299510240404504391

[4] Ibrahim SM, Hmedat SJA-ZA. Role of manual and powered tooth brushes in plaque removal and oral health status (a comparative study). Indian J Public Health Res Dev 2019; 10(8): 2192. http://dx.doi.org/10.5958/0976-5506.2019.02183.1

[5] Bitencourt FV, Nascimento GG, Costa SA, Andersen A, Sandbæk A, Leite FRM. Co-occurrence of periodontitis and diabetes-related complications. J Dent Res 2023; 102(10): 1088-97. http://dx.doi.org/10.1177/00220345231179897 PMID: 37448314

[6] Ng MY, Lin T, Chao SC, Chu PM, Yu CC, Yu CC. Potential therapeutic applications of natural compounds in diabetes-associated periodontitis. J Clin Med 2022; 11(13): 3614. http://dx.doi.org/10.3390/jcm11133614 PMID: 35806899

[7] Santonocito S, Polizzi A, Marchetti E, *et al*. Impact of periodontitis on glycemic control and metabolic status in diabetes patients: Current knowledge on early disease markers and therapeutic perspectives. Mediators Inflamm 2022; 2022: 1-7. http://dx.doi.org/10.1155/2022/4955277 PMID: 35996409

[8] Preshaw PM, Bissett SM. Periodontitis and diabetes. Br Dent J 2019; 227(7): 577-84. http://dx.doi.org/10.1038/s41415-019-0794-5 PMID: 31605062

[9] Ibrahim SM, Al-Mizraqchi AS. Comparison of the antibacterial activity of Panax ginseng and Symphytum officinale with metronidazole against P. gingivalis: An MIC and MBC analysis. Open Dent J 2024; 18(1): 18742106299402. http://dx.doi.org/10.2174/0118742106299402240425053257

[10] Al-Qahtani A, Al-Mubarak MA, Al-Ghamdi SS. Prevalence of *Pseudomonas aeruginosa* colonization in patients with diabetes mellitus: A systematic review and meta-analysis. J Infect Public Health 2015; 8(4): 318-24. http://dx.doi.org/10.1016/j.jiph.2014.11.005

[11] Gasmi A, Benahmed A, Noor S, Gasmi A. *Pseudomonas aeruginosa* in the development of periodontitis: Impact on dysbiosis and inflammation. Arch Razi Inst 2022; 77(1): 339-46. http://dx.doi.org/10.22092/ARI.2021.356596.1875 PMID: 35891722

[12] Ibrahim S, Hussein AS. Role of hexidine, zak and biofresh mouth wash in commemoration deletion and oral health status. (comparative study). Int J Pharm Res 2019; 11(1): 390-4. http://dx.doi.org/10.31838/ijpr/2019.11.01.045

[13] Miller CS, Ding X, Nagarajan R, Dawson DR III, Ebersole JL. Biomarker panel discriminates diabetics with and without periodontitis pre- and POST-THERAPY. J Periodontal Res 2023; 58(3): 493-502. http://dx.doi.org/10.1111/jre.13127 PMID: 37042710

[14] Ji S, Kook JK, Park SN, Lim YK, Choi GH, Jung JS. Characteristics of the salivary microbiota in periodontal diseases and potential roles of individual bacterial species to predict the severity of periodontal disease. Microbiol Spectr 2023; 11(3): e04327-22. http://dx.doi.org/10.1128/spectrum.04327-22 PMID: 37191548

[15] Mirnic J, Djuric M, Veljović T, *et al*. Evaluation of lipid peroxidation in the saliva of diabetes mellitus type 2 patients with periodontal disease. Biomedicines 2022; 10(12): 3147. http://dx.doi.org/10.3390/biomedicines10123147 PMID: 36551903

[16] Ali N, Nater UM. Salivary alpha-amylase and cortisol as biomarkers in stress research: State of the art. Int J Environ Res Public Health 2020; 17(20): 7645. http://dx.doi.org/10.3390/ijerph17207645 PMID: 33092146

[17] Woods DL, Kim H, Yefimova M. State of the science on salivary cortisol measurement: A narrative review. Nurs Res 2020; 69(5): 378-91. http://dx.doi.org/10.1097/NNR.0000000000000441

[18] Engert V, Linz R, Grant JA. Embodied stress: The physiological resonance of psychosocial stress. Psychoneuroendocrinology 2017; 83: 168-78. http://dx.doi.org/10.1016/j.psyneuen.2017.03.020 PMID: 30594324

[19] Nater UM, Rohleder N. Salivary alpha-amylase and cortisol: Assessment of sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axis activity. In: Luecken LJ, Gallo LC, Eds. Handbook of Physiological Research Methods in Health Psychology. SAGE Publications 2008; pp. 129-50.

[20] Thoma MV, Gianferrari E, Frewen P, Nater UM. Salivary alpha-amylase and cortisol responses to different stress tasks: A meta-analysis. Psychoneuroendocrinology 2019; 110: 104420. http://dx.doi.org/10.1016/j.psyneuen.2019.104420

[21] Goyal L, Bey A, Gupta ND, Sharma VK. Salivary C-reactive protein and matrix metalloproteinase-8 in type 2 diabetes mellitus and chronic periodontitis. Oral Dis 2022; 28(3): 700-8. http://dx.doi.org/10.1111/odi.13799

[22] Azzi L, Maurino V, Baj A, Cerrato L, Fazio G, Viganò P, *et al*. Salivary C-reactive protein as a biomarker of periodontal disease: A systematic review and meta-analysis. Int J Environ Res Public Health 2021; 18(11): 5995. http://dx.doi.org/10.3390/ijerph18115995 PMID: 34204928

[23] Korte DL, Gkogkolou P. Salivary albumin as a potential biomarker of periodontal disease status: A systematic review. Int J Dent 2018; 2018: 7178106. http://dx.doi.org/10.1155/2018/7178106

[24] Jaedicke KM, Preshaw PM, Taylor JJ. Salivary biomarkers for the diagnosis and monitoring of periodontal diseases. Periodontol 2000 2020; 83(1): 166-81. http://dx.doi.org/10.1111/prd.12292

[25] Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. J Oral Biol Craniofac Res 2016; 6(1): 67-76. http://dx.doi.org/10.1016/j.jobcr.2015.08.006 PMID: 26937373

[26] Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22(1): 121-35. http://dx.doi.org/10.3109/00016356408993968 PMID: 14158464

[27] Zhang Y, Kang N, Xue F, *et al*. Evaluation of salivary biomarkers for the diagnosis of periodontitis. BMC Oral Health 2021; 21(1): 266. http://dx.doi.org/10.1186/s12903-021-01600-5 PMID: 34001101

[28] Alvi SN, Hammami MM. Measurement of cortisol and cortisone in

human saliva by UPLC-MS/MS. Int J Pharm Pharm Sci 2021; 13(8): 54-8.
http://dx.doi.org/10.22159/ijpps.2021v13i8.41829

[29] Alagendran S, Velayutha Prabhu S, Pushpa N, Ponraj M, Rajasekaran M, Fernandez-Saavedra G, *et al*. Salivary Chromogranin A: A novel diagnostic bio-marker for Alzheimer's disease. Int J Adv Res Sci Commun Technol 2022; 2(1): 110-5.
http://dx.doi.org/10.48175/IJARSCT-2825

[30] Franco-Martínez L, Cerón JJ, Martínez-Subiela S, Tvarijonaviciute A. Effects of filtration and alpha-amylase depletion on salivary biochemical composition measurements. PLoS One 2023; 18(5): 0286092.
http://dx.doi.org/10.1371/journal.pone.0286092 PMID: 37235564

[31] Ibrahim SM, Leka'a MI. Biochemical analysis and periodontal health status in type 1 and type 2 diabetes (comparative study). J Baghdad Coll Dent 2012; (Spec Iss 1)24

[32] van Schrojenstein Lantman M, van de Logt AE, Prudon-Rosmulder E, *et al*. Albumin determined by bromocresol green leads to erroneous results in routine evaluation of patients with chronic kidney disease. Clin Chem Lab Med 2023; 61(12): 2167-77.
http://dx.doi.org/10.1515/cclm-2023-0463 PMID: 37401696

[33] Marín MJ, Figuero E, Herrera D, Sanz M. Quantitative analysis of periodontal pathogens using real-time polymerase chain reaction (PCR). In: Sanz M, Herrera D, van Winkelhoff AJ, Eds. Methods for research in periodontology and implantology. Cham: Springer 2022; pp. 157-77.
http://dx.doi.org/10.1007/978-1-0716-2780-8_10

[34] Mohammed MJ, Al-mizraqchi AS, Ibrahim SM. Oral findings, salivary copper, magnesium, and leptin in type II diabetic patients in relation to oral Candida species. Int J Microbiol 2024; 2024(1): 8177437.
http://dx.doi.org/10.1155/2024/8177437 PMID: 39071038

[35] Tsalamandris S, Antonopoulos AS, Oikonomou E, *et al*. The role of inflammation in diabetes: Current concepts and future perspectives. Eur Cardiol 2019; 14(1): 50-9.
http://dx.doi.org/10.15420/ecr.2018.33.1 PMID: 31131037

[36] Diedisheim M, Carcarino E, Vandiedonck C, Roussel R, Gautier JF, Venteclef N. Regulation of inflammation in diabetes: From genetics to epigenomics evidence. Mol Metab 2020; 41: 101041.
http://dx.doi.org/10.1016/j.molmet.2020.101041 PMID: 32603690

[37] Chen S, Huang D, Zhu L, Jiang Y, Guan Y, Zou S, *et al*. Contribution of diabetes mellitus to periodontal inflammation during orthodontic tooth movement. Oral Dis 2023; 29(6): 2511-22.
http://dx.doi.org/10.1111/odi.14365 PMID: 36050281

[38] Polak D, Sanui T, Nishimura F, Shapira L. Diabetes as a risk factor for periodontal disease—plausible mechanisms. Periodontol 2000 2020; 83(1): 46-58.
http://dx.doi.org/10.1111/prd.12298 PMID: 32385872

[39] Ikeda A, Steptoe A, Brunner EJ, *et al*. Salivary alpha-amylase activity in relation to cardiometabolic status in Japanese adults without history of cardiovascular disease. J Atheroscler Thromb 2021; 28(8): 852-64.
http://dx.doi.org/10.5551/jat.53926 PMID: 33041312

[40] Khatri P, Thakare K, Ganvir MN, Khatri M. Evaluation of the C-reactive protein serum levels in periodontitis patients with or without atherosclerosis – A clinical study. Journal of oral research and review. Medknow 2025; 17(1): 1-8.

[41] Ahmed MAA. Salivary α-amylase and albumin levels in patients with chronic periodontitis and poorly or well controlled type II diabetes mellitus. J Baghdad Coll Dent 2016; 28(1): 114-20.
http://dx.doi.org/10.12816/0024719

[42] Little BB, King C, Gurupur VP, Shakib SH. 1337-P: Association of uncontrolled diabetes with periodontal disease in U.S. adults. Diabetes 2023; 72(Suppl 1): 1337-P.
http://dx.doi.org/10.2337/db23-1337-p

[43] Muthu SE, Aberna RA, Mohan V, *et al*. Phenotypes of isolates of *Pseudomonas aeruginosa* in a diabetes care center. Arch Med Res 2006; 37(1): 95-101.
http://dx.doi.org/10.1016/j.arcmed.2005.04.012 PMID: 16314193

[44] Garg R, Kaur S. The role of *Pseudomonas aeruginosa* in the pathogenesis of periodontal disease. J Clin Diagn Res 2019; 13(1): ZE01-4.
http://dx.doi.org/10.7860/JCDR/2019/38611.12496

[45] Qin H, Li G, Xu X, *et al*. The role of oral microbiome in periodontitis under diabetes mellitus. J Oral Microbiol 2022; 14(1): 2078031.
http://dx.doi.org/10.1080/20002297.2022.2078031 PMID: 35694215

[46] Franco MCM, Moraes MMM, Duarte PM, Napimoga MH, Benatti BB. Glycemic control and the production of cytokines in diabetic patients with chronic periodontal disease. Braz Oral Res 2015; 29: S1806-83242015000100276.
http://dx.doi.org/10.1590/1807-3107BOR-2015.vol29.0063

[47] Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Zamanzadeh V. Salivary biomarkers for detection of periodontal diseases: A meta-analysis of diagnostic test accuracy. BMC Oral Health 2020; 20(1): 266.
http://dx.doi.org/10.1186/s12903-020-01257-4 PMID: 32977794