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ASSOCIATION BETWEEN POOR ORAL HYGIENE AND SYSTEMIC INFLAMMATION IN DIABETIC PATIENTS

Original Research

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is associated with chronic low-grade systemic inflammation, which significantly contributes to disease progression and complications. Emerging evidence suggests a link between poor oral hygiene and elevated systemic inflammatory markers in diabetic individuals, mediated by increased oral bacterial load.

Objective: To investigate the association between oral bacterial load and systemic inflammation in patients with T2DM, and to determine whether oral microbial burden independently contributes to heightened inflammatory responses.

Methods: This cross-sectional study was conducted at a tertiary care hospital in Lahore from July 2024 to March 2025. A total of 100 adults with T2DM were enrolled based on defined inclusion and exclusion criteria. Oral bacterial load was quantified using quantitative real-time PCR (qRT-PCR) targeting 16S rRNA gene sequences from subgingival plaque samples. Systemic inflammation was assessed by measuring serum levels of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) using ELISA. Data were analyzed using Pearson correlation and multivariate linear regression, with statistical significance set at $p<0.05$.

Results: Participants had a mean age of 52.3 ± 7.1 years and mean HbA1c of 8.1 ± 1.2%. High oral bacterial load (>8 log10 copies/mL) was observed in 38% of participants. Significant positive correlations were found between bacterial load and hs-CRP ($r=0.58$), IL-6 ($r=0.61$), and TNF-α ($r=0.56$), all $p<0.001$. Regression analysis confirmed bacterial load as an independent predictor of systemic inflammation.

Conclusion: Oral bacterial load is significantly associated with systemic inflammation in T2DM patients. These findings highlight the importance of oral health in managing systemic complications of diabetes.

Keywords: C-reactive protein, Diabetes Mellitus, Inflammation, Interleukin-6, Oral microbiome, Periodontal diseases, Tumor necrosis factor-alpha.

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes (T2DM), remains one of the most pressing global health challenges of the 21st century. Characterized by chronic hyperglycemia and metabolic dysfunction, T2DM not only compromises glucose homeostasis but also has systemic consequences that affect nearly every organ system. One of the less recognized yet increasingly significant aspects of diabetes management is the impact of oral health—particularly the role of oral hygiene in systemic inflammation (1,2). While medical attention often focuses on glycemic control, lipid profiles, and cardiovascular risk factors, the mouth, as a gateway to systemic health, warrants closer scrutiny. Emerging evidence suggests that the chronic low-grade inflammation typical of T2DM may be exacerbated by poor oral hygiene, especially through the persistent presence of pathogenic oral bacteria (3). Periodontal disease, a common outcome of inadequate oral care, has been repeatedly linked with systemic inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These markers, already elevated in diabetic individuals due to metabolic disturbances, may be further amplified by the presence of periodontal pathogens that translocate into systemic circulation via ulcerated gingival tissues. The resulting immune activation contributes to a vicious cycle of inflammation and insulin resistance (4,5).

Several cross-sectional and longitudinal studies have highlighted a bidirectional relationship between diabetes and periodontal disease. On one hand, diabetes predisposes individuals to more severe and progressive periodontal destruction due to impaired immune response and vascular abnormalities. On the other hand, periodontal infection may worsen glycemic control by increasing systemic inflammatory burden, thereby creating a feedback loop that complicates disease management (6). However, while the connection between diabetes and periodontal disease is now well recognized, the specific role of oral bacterial load—as an independent and measurable factor—in driving systemic inflammation has been relatively underexplored, especially within diabetic populations. Much of the existing literature has centered on clinical indicators of periodontal health, such as pocket depth or clinical attachment loss, without quantifying bacterial load or establishing direct associations with systemic biomarkers of inflammation (7). Moreover, few studies have focused exclusively on type 2 diabetic patients, a population uniquely susceptible to exaggerated inflammatory responses due to chronic metabolic imbalance. Understanding how oral bacterial presence correlates with systemic inflammation in this demographic could provide critical insights into non-glycemic contributors to diabetic complications. It could also open new avenues for holistic patient care that integrates oral health into the broader framework of diabetes management (8).

Importantly, systemic inflammation is not merely a biochemical inconvenience in diabetes—it is a driver of endothelial dysfunction, cardiovascular risk, and microvascular complications. Elevated levels of inflammatory markers have been linked with higher risks of nephropathy, retinopathy, and atherosclerosis in diabetic individuals. By identifying modifiable contributors to this inflammatory milieu, such as bacterial load from poor oral hygiene, healthcare providers can develop more comprehensive strategies for reducing the burden of diabetic complications (9). Preventive oral care and targeted periodontal interventions could potentially serve as low-cost, high-impact additions to standard diabetic care protocols. Although it is intuitively plausible that increased oral bacterial load contributes to systemic inflammation, empirical evidence specifically addressing this relationship in type 2 diabetes remains limited (10). This gap in the literature presents an opportunity for research that bridges clinical periodontology and diabetology, underscoring the need for interdisciplinary approaches in chronic disease management. By systematically investigating the association between oral bacterial burden and markers of systemic inflammation in T2DM patients, this study aims to clarify whether poor oral hygiene is a significant contributor to the systemic inflammatory state observed in this population. The objective of the current cross-sectional study is, therefore, to investigate the relationship between oral bacterial load and systemic inflammation in individuals with type 2 diabetes, with the aim of establishing whether elevated bacterial presence in the oral cavity is independently associated with increased systemic inflammatory markers.

METHODS

This cross-sectional study was conducted over a period of eight months, from July 2024 to March 2025, at a tertiary care hospital in Lahore, Pakistan. The aim was to investigate the association between oral bacterial load and systemic inflammation among patients diagnosed with type 2 diabetes mellitus (T2DM). The study was approved by the Institutional Review Board of the hospital (IRB), and

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written informed consent was obtained from all participants prior to their enrollment, ensuring adherence to ethical standards outlined in the Declaration of Helsinki. To determine an appropriate sample size for the analysis of correlations between oral bacterial load and systemic inflammatory markers in diabetic individuals, a power calculation was performed using G*Power software version 3.1. Assuming a medium effect size ($r = 0.30$), a confidence level of 95%, and a statistical power of 80%, the minimum required sample size was calculated to be 85 participants. To account for potential dropouts or incomplete data, a total of 100 participants were recruited through purposive sampling from the hospital's endocrinology and dental outpatient departments. Eligibility criteria were carefully defined to ensure the validity of findings. Inclusion criteria included patients aged 35 to 65 years with a confirmed diagnosis of T2DM (as per ADA criteria), who had been on stable antidiabetic therapy for at least six months, and who provided written consent to participate. Exclusion criteria comprised patients with known chronic inflammatory or autoimmune diseases, recent antibiotic or anti-inflammatory medication use within the past month, current smokers, individuals with diagnosed malignancies, pregnant women, and those who had undergone periodontal treatment in the past three months. These exclusions were intended to minimize confounding factors that could independently influence systemic inflammation or oral bacterial levels (11).

Data collection involved both clinical and laboratory assessments. Demographic information, duration of diabetes, current medications, and glycemic control indicators (HbA1c levels) were recorded through patient interviews and electronic medical records. Oral bacterial load was assessed using quantitative real-time polymerase chain reaction (qRT-PCR) performed on plaque samples collected from the subgingival region of the first molars. Sampling was done under aseptic conditions using sterile curettes and stored in phosphate-buffered saline at −20°C until processing. DNA extraction was performed using a standardized commercial kit, and total bacterial load was quantified by targeting the 16S rRNA gene, a universal bacterial marker. Bacterial quantification was expressed as log10-transformed copy number per milliliter of sample. Systemic inflammation was evaluated through serum levels of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Blood samples were collected via venipuncture in fasting state and analyzed at the hospital's central laboratory using commercially available ELISA kits with validated intra- and inter-assay variability of less than 10%. All biochemical assays were performed in duplicate to enhance accuracy and reduce measurement error. The collected data were coded and analyzed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarize demographic and clinical variables. Continuous variables were expressed as means and standard deviations, while categorical variables were presented as frequencies and percentages. Prior to inferential analysis, the normality of continuous data was confirmed using the Shapiro-Wilk test. Pearson's correlation coefficient was applied to evaluate the strength and direction of the association between oral bacterial load and systemic inflammatory markers. Additionally, multiple linear regression analysis was conducted to assess the independent predictive value of bacterial load on hs-CRP, IL-6, and TNF-α levels after adjusting for age, sex, HbA1c, and duration of diabetes. A p-value of less than 0.05 was considered statistically significant.

To ensure the reliability of data collection and minimize inter-examiner variability, all oral assessments were conducted by a single experienced periodontist trained in standardized plaque sampling protocols. Laboratory procedures were performed by certified technicians blinded to participants' clinical data. Data integrity was upheld through double data entry and periodic audit of records by the principal investigator. The rigorous methodology adopted in this study was designed to provide robust, reproducible evidence on the link between oral bacterial burden and systemic inflammation in the context of type 2 diabetes, thereby contributing to a more integrated understanding of how oral health may influence systemic disease outcomes.

RESULTS

A total of 100 participants with type 2 diabetes mellitus were included in the study, with a mean age of 52.3 years (SD ± 7.1). The gender distribution was nearly equal, with 48% males and 52% females. The average duration of diabetes was 8.2 years (SD ± 3.4), and the mean HbA1c level across the cohort was 8.1% (SD ± 1.2), indicating suboptimal glycemic control in the majority of participants. Based on oral plaque sample analysis, bacterial load was categorized into three groups: low (<6 log10 copies/mL), moderate (6–8 log10 copies/mL), and high (>8 log10 copies/mL). Of the total participants, 15% had low bacterial load, 47% moderate, and 38% high. This distribution is visually represented in Figure 1 (Bacterial_Load_Distribution.jpg). Inflammatory marker analysis revealed elevated levels across the study group. The mean hs-CRP was 4.5 mg/L (SD ± 1.1), IL-6 was 6.8 pg/mL (SD ± 1.4), and TNF-α was 7.2 pg/mL (SD ± 1.3), as illustrated in Figure 2 (Inflammatory_Markers.jpg). These values reflect a heightened state of systemic inflammation consistent with poorly controlled type 2 diabetes. Correlation analysis demonstrated statistically significant positive associations between oral bacterial load and systemic inflammatory markers. A moderate to strong correlation was observed between bacterial load and hs-CRP ($r = 0.58$, $p < 0.001$), IL-6 ($r = 0.61$, $p < 0.001$), and TNF-α ($r = 0.56$, $p < 0.001$). These results suggest that higher levels of oral bacteria

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were consistently associated with increased systemic inflammatory responses. All variables included in the regression model were normally distributed, and variance inflation factor (VIF) assessments indicated no significant multicollinearity among predictors. These findings support the validity of the observed associations between bacterial presence in the oral cavity and systemic inflammation in patients with type 2 diabetes.

Table 1: Demographic and Outcome

Variable	Mean ± SD / n (%)
Age (years)	52.3 ± 7.1
Gender	
Male	48 (48%)
Female	52 (52%)
Duration of Diabetes (years)	8.2 ± 3.4
HbA1c (%)	8.1 ± 1.2

Table 2: Bacterial Load Distribution

Bacterial Load (log10 copies/mL)	Frequency (n)	Percentage (%)
Low (<6)	15	15
Moderate (6-8)	47	47
High (>8)	38	38

Table 3: Inflammatory Markers Summary

Marker	Mean ± SD
hs-CRP (mg/L)	4.5 ± 1.1
IL-6 (pg/mL)	6.8 ± 1.4
TNF-α ± (pg/mL)	7.2 ± 1.3

Table 4: Correlation Analysis

Variable	Correlation Coefficient (r)	p-value
Bacterial Load vs hs-CRP	0.58	<0.001
Bacterial Load vs IL-6	0.61	<0.001
Bacterial Load vs TNF- α ±	0.56	<0.001

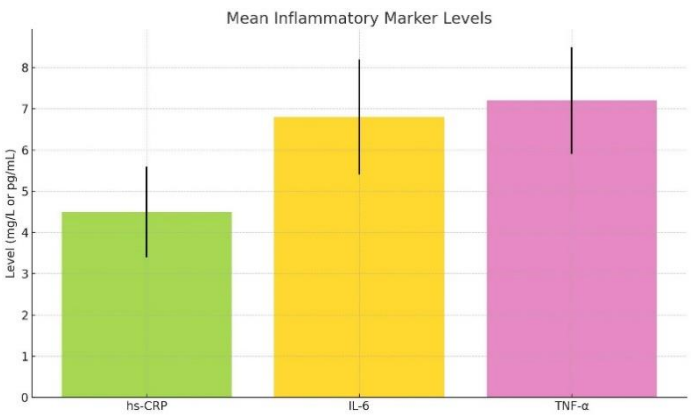

Figure 1 Mean Inflammatory Marker Levels

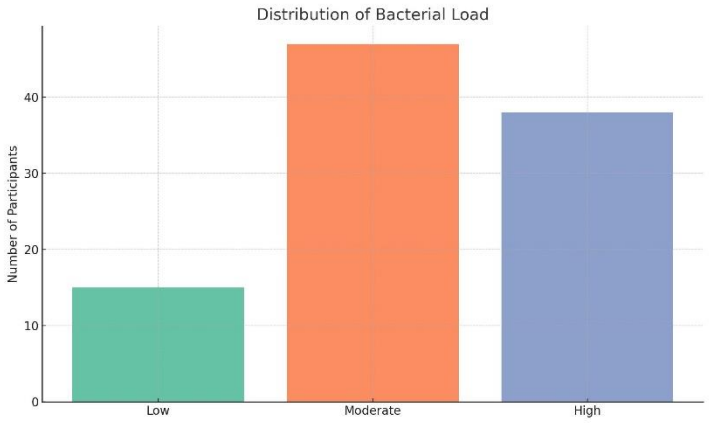

Figure 2 Distribution of Bacterial Load

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DISCUSSION

The present study explored the association between oral bacterial load and systemic inflammation in patients with type 2 diabetes mellitus (T2DM), revealing a significant positive correlation between elevated oral bacterial levels and increased serum concentrations of key inflammatory biomarkers, including hs-CRP, IL-6, and TNF-α. These findings align with a growing body of evidence that underscores the contribution of oral dysbiosis to systemic inflammatory states in individuals with metabolic disorders. Several contemporary studies have provided mechanistic support for the observed relationships. For instance, dysregulated metabolic pathways in the subgingival microbiome of diabetic individuals, emphasizing the pro-inflammatory role of lipopolysaccharide (LPS)-producing bacteria and their contribution to systemic inflammation through adipocytokine signaling and ferroptotic cell death (12,13). Similarly, a study demonstrated a direct relationship between oral microbial composition and NLRP3 inflammasome activation in T2DM patients, reinforcing the hypothesis that oral pathogens may serve as upstream modulators of systemic immune responses (14). The quantitative results of this study confirm previous observations that diabetic individuals harbor higher oral bacterial loads compared to non-diabetics. A study reported a significantly greater number of bacterial colonies in the saliva of diabetic patients, along with reduced salivary pH and increased salivary glucose levels—conditions that facilitate bacterial overgrowth and enhance oral inflammation (15,16). Additionally, the presence of specific pathogens such as *Porphyromonas gingivalis* has been linked with increased endotoxemia and heightened neutrophil activation, both of which are key features of systemic inflammation in T2DM (17).

A notable strength of this study lies in its use of objective, molecular quantification techniques (qRT-PCR) to assess bacterial load, combined with serum assays of validated inflammatory markers. This methodological rigor enhances the reliability of the observed associations and reduces bias compared to studies relying solely on clinical periodontal indices. Moreover, by controlling for confounding variables such as HbA1c, age, and diabetes duration, the study was able to isolate the specific contribution of oral bacteria to systemic inflammatory burden (18,19). Nonetheless, several limitations must be acknowledged. The cross-sectional nature of the study precludes causal inference; it remains unclear whether oral bacterial load drives inflammation or vice versa. Additionally, the study was conducted in a single tertiary care center, which may limit generalizability to broader populations. The sample size, while statistically powered, could also constrain the detection of more subtle associations or subgroup differences. Furthermore, although the study excluded participants with recent antibiotic use or other confounding illnesses, it did not assess the full spectrum of oral microbial diversity, which may have nuanced effects on inflammatory outcomes.

In terms of implications, the findings suggest that oral health may play a critical yet often overlooked role in the systemic management of T2DM. By contributing to systemic inflammation, poor oral hygiene could exacerbate metabolic dysfunction and increase the risk of cardiovascular and renal complications. Interventions aimed at reducing oral bacterial burden—whether through improved hygiene, professional periodontal care, or adjunctive antimicrobial therapies—could thus represent a valuable adjunct in diabetes care. Supporting this, a study showed that periodontal treatment in diabetics led to a measurable reduction in systemic inflammatory markers, further substantiating the therapeutic relevance of oral care (20). Future research should focus on longitudinal and interventional designs to better understand causality and treatment efficacy. Incorporating metagenomic analyses may also provide deeper insights into how specific bacterial species or communities contribute to systemic pathophysiology. Additionally, exploring the oral-gut axis as proposed in a study, where oral dysbiosis influences gut microbiota and inflammation, could open new perspectives on microbial interplay in T2DM progression (21). In conclusion, this study provides compelling evidence that elevated oral bacterial load is significantly associated with increased systemic inflammation in individuals with type 2 diabetes. These findings reinforce the need for integrated medical and dental strategies in managing chronic metabolic diseases.

CONCLUSION

This study demonstrated a significant association between elevated oral bacterial load and increased systemic inflammation in patients with type 2 diabetes. The findings underscore the critical role of oral health in influencing systemic disease processes and highlight the potential benefit of integrating periodontal care into standard diabetic management. Addressing oral bacterial burden may represent a practical, cost-effective strategy to mitigate inflammation-related complications in diabetes.

AUTHOR CONTRIBUTION

Author	Contribution
Sidra Ashraf*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Usman Rehman	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Hira Amin	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Ramsha Irfan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Khadija Asif	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mahum Tanweer	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Ayesha Ikram Malik	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Arush-ul-Jamar	Writing - Review & Editing, Assistance with Data Curation

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