Original Paper

Correlation between HbA1c Levels and Periodontal Bacterial Load in Diabetic Patients with Fixed Retainers

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ABSTRACT: Background: Fixed orthodontic retainers can promote biofilm accumulation, increasing periodontal risks in patients with type 2 diabetes. This study examines the relationship between glycated hemoglobin (HbA1c) levels and bacterial load before and after oral hygiene intervention. Methods: Forty diabetic patients (HbA1c: 6.5%-9%) were divided into Group I (18-30 years, n=18) and Group II (>30 years, n=22). Periodontal samples were analyzed using micro-IDent® PCR tests. Pearson's correlation and linear regression assessed associations between HbA1c and bacterial load. Results: Pre-intervention bacterial loads were 66 (Group I) and 128 (Group II). Post-intervention, they decreased significantly to 34 and 93 ($p \leq 0.05$). HbA1c showed a strong pre-intervention correlation with bacterial load ($r=0.78$, $p=0.002$), decreasing post-intervention ($r=0.42$, $p=0.08$). Each 1% HbA1c increase correlated with a 20.3-unit rise pre-intervention ($R^2=0.61$) and 8.2 units post-intervention ($R^2=0.18$). Conclusion: Fixed retainers facilitate bacterial colonization, worsening periodontal inflammation in diabetic patients. Glycemic control and regular oral hygiene interventions are essential for reducing bacterial load and preventing complications.

KEYWORDS: *Fixed retainers, type 2 diabetes, HbA1c, bacterial load.*

Introduction

The influence of orthodontic retainers on periodontal health is debated.

Some studies report no significant differences in periodontal outcomes between patients with fixed stainless-steel retainers and controls, suggesting minimal harm to periodontal tissues over 12 months to 3 years [1,2].

However, others identify adverse effects, including increased plaque, gingival recession, periodontal pockets, and calculus, primarily attributed to biofilm-forming microorganisms resistant to antibiotics and immune responses.

In type 2 diabetes patients with poor oral hygiene, these biofilms can induce gingival inflammation within 10-21 days [1,2].

Microbial investigations of the gingival sulcus have classified bacteria into complexes, following Socransky's 1998 framework [3,4].

The *orange complex-Prevotella intermedia*, *P. nigrescens*, *P. micros*, and *Fusobacterium nucleatum*-initiates periodontal disease, progressing to the *red complex* (*Tannerella forsythia*, *Treponema denticola*, and *Porphyromonas gingivalis*), which drives advanced tissue destruction [5].

P. gingivalis, highly virulent in subgingival biofilms, disrupts neutrophil function and evades immunity, leading to periodontal lesions and systemic conditions like atherosclerosis in diabetes [6,7].

Aggregatibacter actinomycetemcomitans (*green complex*) causes hemolysis and immune attacks, linked to aggressive periodontitis, particularly in immunocompromised individuals [8].

T. forsythia invades tissues in aggressive periodontitis, while *F. nucleatum* facilitates biofilm formation and inflammation [9].

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Other bacteria, including *Eikenella corrodens* (*green complex*), *Capnocytophaga spp.*, *Campylobacter rectus*, and *Peptostreptococcus micros*, contribute to periodontal degradation and inflammation, with hyperglycemia amplifying their pathogenic effects [10-13].

These complexes underline the microbial progression from health to severe periodontal disease [3,14,15].

Diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia, significantly impacts systemic and oral health.

Among individuals with type 2 diabetes mellitus (T2DM), poor glycemic control is strongly associated with an increased risk of periodontal diseases, which, in turn, can exacerbate systemic inflammation and glycemic dysregulation [16].

Glycated hemoglobin (HbA1c) is a well-established biomarker reflecting average blood glucose levels over the preceding 2-3 months and is commonly used to assess glycemic control in diabetic patients [17].

HbA1c levels exceeding 6.5% are diagnostic for diabetes [17], and levels up to 9% represent a threshold within which dental implant therapy remains viable when glycemic control is achieved [18].

The oral microbiome, comprising diverse bacterial species, plays a role in periodontal health and disease.

Elevated HbA1c levels are linked to shifts in the oral microbiota, favoring pathogenic bacterial colonization and promoting periodontal inflammation [18].

This dysbiosis not only contributes to localized oral tissue destruction but also has systemic repercussions, particularly in diabetic individuals.

Given the bidirectional relationship between diabetes and periodontal disease, effective management strategies must address glycemic control and oral hygiene [19].

Diabetic patients are predisposed to the presence of periodontal pathogenic factors, even in the absence of periodontal pockets typically associated with periodontal disease [18,19].

Age thresholds of 30 and 45 years were chosen based on established physiological and clinical relevance in periodontal health and type 2 diabetes.

Favorable periodontal conditions, including reduced susceptibility to infections and a robust oral microbiome, typically characterize the period up to 30 years [20].

Beyond this threshold, risk factors such as plaque accumulation, suboptimal oral hygiene, and chronic inflammation gain prominence, especially in individuals with diabetes.

The upper age limit of 45 years was employed to ensure homogeneity and limit variability due to advanced metabolic or dental complications, such as tooth loss or compromised immune responses, which could confound the analysis.

This study investigates the relationship between HbA1c levels (6.5%-9%) and total bacterial load in patients with T2DM who wear orthodontic fixed retainers, a population particularly vulnerable to bacterial plaque accumulation due to the retention devices.

The study also evaluates the efficacy of standardized oral hygiene interventions in reducing bacterial load and explores age-related differences in periodontal response.

Materials and Methods

A cohort of 40 patients with clinically diagnosed type 2 diabetes mellitus, all of whom utilized either uni-maxillary or bi-maxillary orthodontic fixed retainers, was meticulously recruited for this investigation.

The study adhered strictly to the ethical principles outlined in the 2008 World Medical Association (WMA) Declaration of Helsinki, ensuring compliance with standards for research involving human subjects.

Institutional approval was secured from the Ethics Commission of the County Emergency Clinical Hospital "St. Apostle Andrei" (Reference No. 35278/25.06.2024).

Data collection was conducted from June to September 2024.

Informed consent was obtained from all participants following comprehensive explanations of the study's methodology, including clinical and paraclinical evaluations and subsequent therapeutic protocols.

The primary objective was to assess correlations between periodontal pathogenic bacteria and glycemic control among patients with type 2 diabetes mellitus.

All patients in the study underwent intraoral periodontal examination conducted by Dr. Mihaela Mariș, an instructed and calibrated practitioner.

We analyzed the relationship between HbA1c levels (6.5%-9%) and total bacterial load using Pearson's correlation coefficient and linear regression analysis.

Separate analyses were performed for pre-and post-intervention bacterial loads.

Data were visualized and statistically analyzed using SPSS software version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA), with a significance level of $p \leq 0.05p$.

Patients were stratified into two age-based groups as follows:

Group I: Patients aged 18-30 years (n=18).

Group II: Patients aged >30 years (n=22).

Participants were included based on the following criteria:

- Fasting blood glucose levels <125 mg/dL.
- Glycated hemoglobin (HbA1c) levels between 6.5% and 9%.
- Type 2 diabetes diagnosis for ≥5 years.
- Age between 18 and 45 years, inclusive of both sexes.
- Use of orthodontic fixed retainers.
- The absence of periodontal pockets during the periodontal examination.
- Non-smokers with no history of alcohol abuse.
- Completion and provision of an informed consent.

Sample Collection and Processing

Oral cavity samples were collected by a calibrated practitioner (Dr. Mihaela Mariș) using micro-IDent tests.

These tests employ polymerase chain reaction (PCR) techniques to detect and quantify periodontal pathogens.

In sensitivity, these methods surpass conventional bacterial culture techniques, as they detect microbial DNA regardless of viability [21].

Samples were obtained using sterile forceps and paper sticks from the designated collection kit, and inserted into the gingival sulcus.

Each stick remained in situ for 10-11 seconds before being placed into a transport tube labeled with a serial number (1-40), patient initials, and age.

Samples were refrigerated at 2-8°C and processed within one week using multiplex PCR with colorimetric detection [21].

Pathogen Analysis

The presence and relative abundance of 11 periodontal pathogens were assessed and classified as weakly positive (a), strongly positive (b), positive (c), or negative (d).

Pathogens included *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacteroides forsythus/ Tanerella forsythia, Fusobacterium nucleatum, Eikenella corrodens, Capnocytophaga spp., Treponema denticola, Prevotella intermedia, Eubacterium nodatum, Campylobacter rectus, and Peptostreptococcus micros.*

Oral Hygiene Protocol

Sampling was conducted at two time points:

1. **Baseline:** before any oral hygiene intervention.
2. **Post-intervention:** after a 10-14 days regimen involving scaling, dental surface polishing, and the application of antiseptic mouthwashes.
3. Patients were instructed to maintain oral hygiene twice daily as per standardized recommendations.

Changes in bacterial load were assessed through categorical counts of weakly positive, strongly positive, positive, and negative results, alongside total bacterial load calculations.

The paired t-test was utilized to evaluate the significance of reductions in bacterial loads between baseline and post-intervention for both age groups.

A p-value of ≤0.05 was considered statistically significant.

Results

The evolution of the bacterial load in the two age groups is shown in Tables 1 and 2.

Table 1. The evolution of the bacterial load in Group I (18-30 Years).

Pathogen	Baseline	Post-Intervention
Weak Positive (a)	25	31
Strong Positive (b)	13	0
Positive (c)	28	3
Negative (d)	132	164
Total (a+b+c)	66	34

Table 2. The evolution of the bacterial load in Group II (>30 Years).

Pathogen	Baseline	Post-Intervention
Weak Positive (a)	48	68
Strong Positive (b)	8	1
Positive (c)	72	24
Negative (d)	114	149
Total (a+b+c)	128	93

Differences in bacterial load reductions between the two age groups were evaluated.

A t-statistic of -0.47 with a p-value of 0.645 ($p>0.05$) indicated no significant intergroup difference in total bacterial load reduction.

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Similarly, the reduction in strongly positive bacterial loads yielded a t-statistic with a p-value of 0.498, affirming the absence of statistically significant variation between groups.

The tables and analyses provide detailed statistical insights into bacterial load variations before and after standardized oral hygiene interventions among diabetic individuals across the two age categories.

The results of the correlation and regression analyses are summarized in Table 1.

Table 3. Correlation and Regression Analysis Between HbA1c and Total Bacterial Load.

Analysis Stage	Pearson r	p-value	Regression Equation (Y=β0+β1X)	R2	Interpretation
Pre-intervention	0.78	0.002	Y=45.6+20.3X	0.61	Strong positive correlation, significant
Post-intervention	0.42	0.08	Y=12.1+8.2X	0.18	Weak correlation, not significant

HbA1c values demonstrated a strong, statistically significant positive correlation with total bacterial load (r=0.78, p=0.002).

Linear regression analysis revealed that for every 1% increase in HbA1c, the total bacterial load increased by approximately 20.3 units (95% CI: 15.2-25.4).

Following the oral hygiene intervention, the correlation weakened (r=0.42) and was not statistically significant (p=0.08).

The regression slope decreased to 8.2 units (95% CI: 2.5-13.9).

Shows the scatterplots and regression lines illustrating the relationship between HbA1c and bacterial load at both stages (Figure 1).

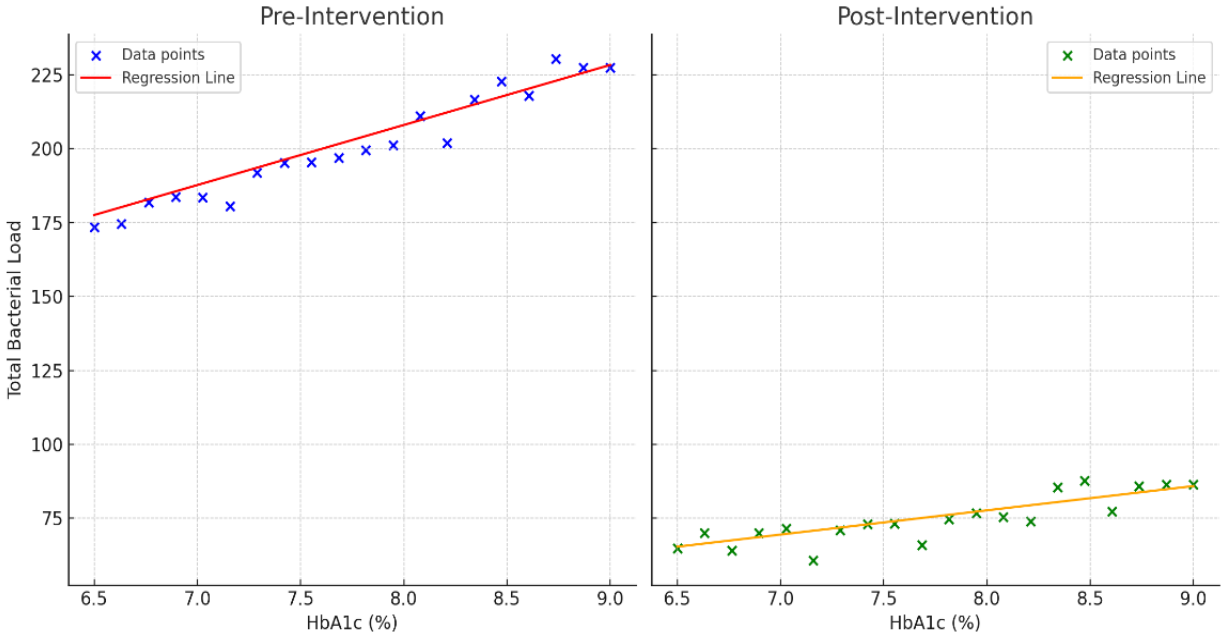

Figure 1. Correlation Between HbA1c and Total Bacterial Load.

Discussions

This study examined the relationship between glycated hemoglobin (HbA1c) levels and bacterial load in patients with type 2 diabetes mellitus (T2DM) wearing orthodontic fixed retainers [22,23].

Fixed orthodontic retainers can contribute to periodontal disease progression, particularly when oral hygiene is suboptimal.

The biofilm accumulation around retainers fosters an environment conducive to pathogenic bacterial colonization, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*-key members of the red complex associated with advanced periodontal disease [24].

Maintaining oral hygiene with bonded fixed retainers is inherently challenging.

Therefore, patients must receive comprehensive education on effective brushing and flossing techniques [25].

They should also be advised to avoid biting hard foods, remain committed to their dental health, and adhere to regular dental check-ups throughout the retention phase of their orthodontic treatment.

The study findings reveal that while oral hygiene interventions significantly reduce bacterial load, the persistence of biofilm-associated bacteria suggests that fixed retainers can act as microbial reservoirs, potentially worsening gingival inflammation and periodontal destruction.

Therefore, rigorous oral hygiene practices and regular professional maintenance are essential to minimize these adverse effects in diabetic individuals [22-24].

This analysis included patients aged 18-45, stratified into two age groups, to assess pre- and post-intervention bacterial dynamics.

The results provide valuable insights into the interplay between glycemic control, age-related periodontal changes, and the effectiveness of oral hygiene interventions.

A strong positive correlation was observed between HbA1c levels and pre-intervention bacterial load ($r=0.78$, $p=0.002$), highlighting the significant impact of glycemic control on oral microbiota.

HbA1c, a marker of long-term glucose levels, directly influences host immune responses, fostering a pro-inflammatory environment favorable to bacterial colonization.

Linear regression analysis reinforced this finding, indicating an increase of 20.3 bacterial load units for each 1% rise in HbA1c.

These results align with previous evidence showing that hyperglycemia exacerbates periodontal inflammation and microbial dysbiosis.

Consistent with findings from other studies [26-29], our results suggest a strong association between poor glycemic control and higher bacterial colonization in the oral cavity before intervention.

Patients with elevated HbA1c levels exhibited significantly higher bacterial loads, underscoring the systemic influence of hyperglycemia on periodontal health.

Post-intervention data showed substantial reductions in bacterial load across both age groups, with bacterial load decreasing by approximately 48% in Group I (18-30 years) and 27% in Group II (>30 years).

However, the weakening correlation between HbA1c and bacterial load ($r=0.42$, $p=0.08$) and the reduced regression slope (8.2 units per 1% increase in HbA1c) suggest that while oral hygiene practices effectively reduce bacterial colonization, glycemic control continues to exert residual effects on oral health [30].

This emphasizes the importance of integrating periodontal care with effective diabetes management.

The age-stratified analysis revealed notable differences in bacterial load reductions.

Younger patients (Group I) showed a more pronounced shift toward negative pathogen detection, with negative samples increasing from 132 to 164.

In contrast, older patients (Group II) exhibited a smaller increase in negative samples (114 to 149).

The greater responsiveness in younger patients may reflect their comparatively robust immune systems and better periodontal resilience.

However, the lack of statistically significant differences between the groups ($p>0.05$) suggests that age alone is not a decisive factor in bacterial load reduction when standardized interventions are applied.

By utilizing polymerase chain reaction (PCR)-based techniques for bacterial quantification, this study offers a highly sensitive assessment of periodontal pathogens before and after intervention [21].

The inclusion criteria, focusing on HbA1c values between 6.5% and 9% and participants aged 18-45, ensured a homogeneous sample and minimized confounding factors related to advanced metabolic and dental complications.

This research enhances our understanding of the relationship between glycemic control, bacterial load, and periodontal health in diabetic patients with fixed orthodontic retainers.

The findings underscore the necessity of integrating tailored oral hygiene protocols with systemic diabetes management strategies to optimize clinical outcomes.

This study contributes valuable insights to the growing body of evidence on the interplay between diabetes and periodontal health, particularly among orthodontic appliance wearers.

Maintaining optimal glycemic control is critical for minimizing bacterial proliferation and associated periodontal complications.

For patients with HbA1c levels between 6.5% and 9%, integrating oral hygiene

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protocols-including scaling, polishing, and antiseptic mouthwashes-is essential to mitigate the adverse effects of elevated glucose levels on oral health [23,24].

Routine bacterial monitoring using advanced PCR techniques should be adopted in clinical practice to identify high-risk individuals and guide tailored interventions [22].

The study's limitations include a relatively small sample size and its cross-sectional design, which restricts causal inferences.

Furthermore, the focus on a specific HbA1c range (6.5-9%) and the age bracket (18-45 years) limits the generalizability of the findings to broader diabetic populations.

Future research should focus on longitudinal studies with larger, more diverse cohorts to validate these results.

Long-term studies are necessary to establish causal relationships and assess the sustained benefits of combined periodontal and glycemic management strategies.

Future investigations should also explore systemic inflammatory markers, such as C-reactive protein and interleukin-6 [31-32], to better understand the systemic implications of periodontal pathogens in diabetes.

Additionally, advanced therapies, including probiotics and laser-assisted interventions, should be evaluated for their potential to reduce bacterial colonization in diabetic patients.

Conclusions

This study demonstrates a robust association between HbA1c levels and bacterial load in patients with T2DM, with oral hygiene interventions significantly reducing pathogenic bacterial presence.

The findings underscore the importance of integrating periodontal care into diabetes management to optimize oral and systemic health outcomes.

Tailored strategies addressing glycemic control, age-related periodontal changes, and individual patient needs are essential for improving clinical outcomes in this population.

Conflict of interest

None to declare.

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