Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 28 May 2026
Revised 22 June 2026
Accepted 17 July 2026

Natural Resources for Human Health 2026, Vol. 6(10s):1393–1398

KEYWORDS:
Periodontal disease, gingivitis, saliva, streptococcus mutans, oral bacteria.

Quantitively Detection of Oral Colonization by Streptococcus Mutans in Gingivitis

Zahraa F. Shaker[1], Ahmed Abdul-Hassan Abbas[2], Saif Salahuddin Jasim[3], Fadia Abd Almuhsin Al-khayat, Batool Hassan Al-Ghurabi[4*], Rafal Ali Salman[4]

[1]Department of Basic Science, College of Dentistry, University of Al-Mustansiriyah, 89JG+8W, Baghdad, Iraq. Email: zahraa.fsh@uomustansiriyah.edu.iq.

[2] Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq. Email: ahmed26770@yahoo.com. ORCID ID: https://orcid.org/0000-0002-7886-6782.

[3] College of Dentistry, University of Ashur, Baghdad, Iraq.

[4]Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq.

*Corresponding Author Email: batoolamms@codental.uobaghdad.edu.iq
ORCID ID: https://orcid.org/0000-0001- 9775-4906.

ABSTRACT:

Background: Gingivitis is an inflammatory condition that only affects the mucosal epithelial tissue that covers the alveolar processes and the cervical part of teeth. *Streptococcus mutans* (*S. mutans*) is generally linked to dental caries, although its involvement in gingival inflammation is becoming more well acknowledged. Through glucose transferase, it can produce extracellular polysaccharides and encourage bacterial adhesion and accumulation, which can result in plaque buildup and oral cavity colonization. **Aim of study:** The study's objectives are to measure the amount of *S. mutans* in saliva samples from gingivitis patients and healthy controls and to look into the relationship between *S. mutans* copy numbers and clinical periodontal markers.

Methodology: Case-control was used in this investigation. 60 adults between the ages of 22 and 40 were split into two groups: 20 were in good health and 40 had been diagnosed with gingivitis. The plaque index, and bleeding on probing were the oral parameters evaluated in this investigation. Samples of unstimulated saliva were collected from every participant. Real-time polymerase chain reaction was used to quantify *S. mutans* from extracted DNA.

Results: The gingivitis group had a considerably greater ($p<0.01$) S. *mutans* bacterial load than the control group. Additionally, there was a strong positive link between PLI and BOP in the patient group, but there was no significant correlation between *S. mutans* load and either PLI or BOP.

Conclusion: The increased S. mutans in gingivitis points to its possible involvement in caries as well as the development and onset of gingival inflammation. Therefore, oral hygiene practices that target S. mutans may be beneficial in reducing both gingivitis and caries. In order to better understand this link, greater sizes of samples and the identification of bacterial species are advised in future research.

Natural Resources for Human Health
eISSN: 2583-1194

INTRODUCTION

The periodontium, a collection of immune-mediated, complex, chronic illnesses, is destroyed as a result of periodontal disease (1). Gingivitis is a minor form of periodontal disease characterized by inflammation of the gingival tissue surrounding the teeth. Gingivitis is the initial symptom of the inflammatory reaction to the biofilm. That would become a chronic state, nevertheless, if this condition persisted. Periodontitis can sometimes arise from chronic gingivitis (2). Maintaining oral and general health depends on the oral microbiota, a complex ecosystem made up of bacteria, fungi, viruses, and archaea. Tooth decay, gingivitis, periodontitis, oral candidiasis, and halitosis are just a few of the oral health issues that can arise when this ecosystem's equilibrium is upset (3). The World Health Organization (WHO) estimates that 50% of people worldwide suffer from gum disease, which includes gingivitis, to varied degrees. About 10–15% of adults suffer with acute periodontitis, which is thought to be the primary cause of tooth loss globally. Because of plaque buildup and poor dental hygiene, the problem frequently starts in youth or early adulthood. With 70–80% of people above 65 having some kind of periodontal disease, its frequency rises noticeably with age (4). The ability of *S. mutans* to produce extra-cellular polysaccharides contributes to both colonization and pathogenicity. In fact, compared to people lacking biofilm-forming *S. mutans* strains, adult subjects colonized by these strains have more caries. Whether *S. mutans* enhances colonization during gingivitis is still unknown. On the one hand, research has demonstrated that gingival inflammation does not correlate with elevated *S. mutans* levels (5), and larger recoveries of the bacteria have been observed in individuals with treated periodontal disease after follow-up. Similarly, *S. mutans* is more prevalent in periodontally treated individuals than in untreated controls (6). Given these results, it is still unknown if *S. mutans* colonization and gingivitis are related. Thus, the purpose of this study was to measure the amount of *S. mutans* in gingivitis patients' saliva and look into the relationship between *S. mutans* copy numbers and clinical periodontal markers.

METHODOLOGY

Design of the study

study of case control.

Sample size

With a 95% confidence interval and a 5% error margin, it was computed using the online tool EPITOOLS (https://epitools.ausvet.com.au/case controls). The subjects' sample size was determined to be 60 (20 healthy controls and 40 gingivitis).

Subjects

This study included 40 gingivitis patients. The following were among the inclusion criteria utilized in this study: At least 20 natural teeth, no history of systemic disease, patients with generalized gingivitis and intact periodontium, more than 30% of BOP-affected sites without PPD greater than 3 mm, no CAL, and no periodontal therapy within the previous six months were all requirements for inclusion. In this investigation, the following exclusion criteria were applied: displaying early indications of periodontitis and/or cavities. the last three months' worth of antibiotic and/or anti-inflammatory drug use. past alcohol consumption or smoking. Twenty volunteers who appeared to be in good health made up the control group; their age and sex matched those of the patients.

Oral Assessment

Each participant in the study underwent a clinical evaluation by a specialized dentist. Using William's graduated periodontal probe, the periodontal parameters—PLI and BOP—were used to evaluate the periodontal health of every tooth.

Collection of saliva samples

Two study groups provided three milliliters of non-stimulated saliva samples, which were obtained using a spit technique prior to the oral examination. Following collection, the samples were promptly frozen at -40°C for RTqPCR molecular detection of the bacteria.

S. mutans detection in saliva

Each participant's microbial load of S. mutans in saliva was measured using qRT-PCR technique. Samples of saliva were used to extract genomic DNA. The forward 5`-AGCCATGCGCAATCAACAGGTT-3` and reverse 3`-

CGCAACGCGAACATCTTGATCAG-5` primers were created for the precise identification and measurement of *S. mutans* bacterium. Next, qPCR was used to amplify the target bacterial DNA sequences (16r RNA), and each amplification cycle's fluorescence was tracked in real time. For every sample, the cycle threshold—which is the cycle number at which fluorescence above a predefined threshold—was noted. By comparing the samples' cycle threshold values to a standard curve created from repeated dilutions of known DNA concentrations, quantification was accomplished (7).

Analysis of statistics

GraphPad Prism software (version 9.0) and statistical package for the social sciences (SPSS) software (version 25, IBM, USA) were used for statistical analysis. The Independent Samples T-test was used to compare continuous variables with a normal distribution, which were reported as mean ± SD. The Mann-Whitney U test was used to compare non-normally distributed variables, which were reported as median and interquartile range (IQR). A p-value of less than 0.05 was regarded as significant.

RESULTS

Features of the population and clinical markers

Table 1 shows the demographic characteristics of 60 subjects. There were no significant differences (P>0.05) between the gingivitis group's mean age of 31.30±7.80 years and the control group's mean age of 31.65±5.75 years. Additionally, there was no statistically significant difference in the distribution of sexes between the gingivitis and control groups (P>0.05). However, Table 2 displays the mean values of PLI and BOP, both of which were considerably greater in the gingivitis group than in the healthy controls (p<0.01).

Table-1: Demographic characteristic in in gingivitis and control groups.

Demographic characteristics	Study groups		*P*-value
	Gingivitis patients n=40	Healthy control n=20	
Age (years)			0.780^{NS}
Range	(20-40)	(22-40)	
Mean ± SD	31.30 ± 7.80	31.65 ± 5.75	
Sex			0.360^{NS}
Female (%)	20 (50%)	14 (70%)	
Male (%)	20 (50%)	6 (30%)	

Table-2: PLI and BOP in gingivitis and control groups.

Periodontal parameters	Study groups		*P*-value
	Gingivitis patients n=40	Healthy control n=20	
PLI			0.011*
Rang	(0.50-2.42)	(1.05-1.99)	
Mean ± SD	1.75 ± 0.49	1.47 ± 0.27	
BOP	(0.40-1.00)	(0.42-0.39)	
Mean ± SD	0.78 ± 0.15	0.73 ± 0.15	0.001*

Molecular Detection of *S. mutans*

There was a statistically significant difference between the patient groups and the control group when the median *S. mutans* bacterial load values in the two groups were compared. According to Table 3, the gingivitis group had a considerably greater ($p<0.01$) *S. mutans* bacterial burden than the control group.

Table-3: Bacterial load in gingivitis and control groups

S. mutans	Study groups		*P*-value
	Gingivitis patients n=40	Healthy control n=20	
Rang	(4.50-1605.00)	(3.90-930.00)	0.041*
Median	107.00	38.10	
IQR	(20.70 - 490.00) 415.15	(14.30 - 275.00) 211.85	
Mean ± SD	281.48± 394.39	23615± 363.46	

Correlation between *S. mutans* load and periodontal parameters

Our results showed a non-significant relationship between periodontal parameters (PLI and BOP) with *S. mutans* burden. On the other hand, strong positive correlation was found between PLI and BOP in gingivitis group, as observed in Table 4

Table-4: Correlation between *S. mutans* with PLI and BOP in gingivitis group.

Correlation	Patients group	
	S. mutans	
Spearman correlation	r	p-value
PLI	0.213	0.187
BOP	0.293	0.066
Pearson correlation	PLI	
BOP	0.767	0.001*

DISCUSSION

Saliva may naturally include the well-known aciduric Gram-positive coccus *S. mutans*. Gingival tissue degradation is facilitated by the production of glucans that improve biofilm adherence and can alter the host immune reaction (8). In the current investigation, the gingivitis group's saliva samples had a higher *S. mutans* bacterial load than the controls. This result supports the idea that an increase in total bacterial biomass is linked to gingivitis (9). Our findings are in line with those of Aas et al. (10) and Kumar (11), who noted that patients with gingivitis had increased amount of *S. mutans* and other streptococci and highlighted their function as primary colonizers in biofilm initiation. Marsh (12) and Zijnge et al. (13), on the other hand, reported no discernible variation in the number of S. mutans between gingivitis and healthy locations. However, another study by Koll-Klais et al. revealed high levels of these bacteria in healthy people, suggesting that they support the oral cavity's micro-ecological equilibrium (14). This disparity could result from variations in the patient's oral hygiene state, detecting techniques, or sampling location. Additionally, Takahashi and Nyvad demonstrated that S. mutans's function in the early phases of plaque development leading to gingival inflammation is supported by its presence as a major colonizer that generates glucans and promotes co-aggregation (15). Preianò et al. later discovered that the increased plaque deposition in gingivitis gives primary

colonizers additional retentive surfaces. Additional peptides and heme compounds that promote S. mutans growth and biofilm development are provided by the inflammatory milieu with high GCF. Therefore, an early stage of the transition from a healthy compatible biofilm to a dysbiotic biofilm linked to gingival inflammation may be represented by the rise of S. mutans (16). This supports our study's finding that there is a significant positive association between PLI and BOP in the patient group, indicating that gingival bleeding is a clinical manifestation of increased biofilm mass caused by primary colonizers. Additionally, Dani et al. noted that a high level of S. mutans in gingivitis patients emphasizes the significance of managing early invaders to stop the development of more serious periodontal diseases (17). Furthermore, this result showed that patients had higher levels of PLI and BOP than controls, which is in line with the known fact that dental plaque is the main cause of gingival inflammation. The increased PLI in the gingivitis confirms earlier research showing that plaque buildup is a major factor in the development of gingivitis (18,19). Likewise, BOP is regarded as a trustworthy clinical marker of gingival inflammation and disease activity. Conversely, the researchers detailed the pathophysiological mechanisms that usually take place in inflammatory tissues and the effect of plaque deposition on blood flow, including increased capillary fragility and permeability and how the degree of inflammation affects the severity of bleeding and how simple it is to initiate it (20). The present findings corroborated those of (19, 21), which reported that the gingivitis groups had a higher percentage of bleeding spots than the control group.

The lack of a significant link between S. mutans load and either PLI or BOP in this study indicates that gingival bleeding or plaque buildup in this population are not directly predicted by the amount of S. mutans. However, Wang et al. demonstrated that since not all plaque bacteria are equally harmful, variations in bacterial makeup rather than total count may be responsible for the lack of association between PLI and bacterial load (22). Our findings are in line with those of Aas et al. (10), who found no association between clinical indicators and increased S. mutans levels in gingivitis. The significance of the ecological plaque theory is thus highlighted by the lack of association between S. mutans and clinical indicators in our investigation (12). Similarly, Zijnge et al. found that specific bacterial species and biofilm architecture are more significant indicators of disease than total bacterial load using DNA sequencing. Therefore, if the bacterial makeup stays commensal, a rise in the number of bacteria does not always result in an increase in clinical indices (13).

When interpreting the study's findings, a number of limitations should be taken into account. Initially, qPCR was used to quantify solely S. mutans. Dysbiosis of the entire oral biofilm causes gingivitis, a polymicrobial illness. The interpretation of microbial interactions in this work is limited by the absence of information on additional early invaders and periodontal pathogens. Second, both viable and non-viable bacterial DNA can be found using qPCR. As a result, the metabolically active S. mutans population that is directly involved in biofilm formation and pathogenicity may not be fully reflected by the bacterial counts.Lastly, the results may not be as applicable to other groups with diverse oral health and demographic features because the sample was drawn from a single center.

CONCLUSION

In conclusion, the increased S. mutans in gingivitis points to its possible involvement in both caries and the onset and development of gingival inflammation. Therefore, oral hygiene practices that target S. mutans may be beneficial in reducing both gingivitis and caries. To further clarify this link, greater sample sizes and the identification of bacterial species are advised in future research.

CONSENT TO PARTICIPATE

All patients involved in this study provided their written informed consent.

DISCLOSURE

The authors declare that they have no competing interests.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the first author

REFERENCES

[1] Pihlstrom BL, Michalowicz BS, Johnson NW. (2005). Periodontal diseases. Lancet. 366:1809-20.
[2] Carranza F. (2019). Newman and Carranza's clinical periodontology. Saunders. 1: 944.
[3] Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. (2024). Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. Microorganisms. 12(9): 1797.

[4] Wu L, Zhang SQ, Zhao L, Ren ZH, Hu CY. (2022). Global, regional, and national burden of periodontitis from 1990 to 2019: results from the Global Burden of Disease study 2019. J Periodontol. 93(10):1445-54. doi: 10.1002/JPER.21-0469.

[5] *Ravald N, Hamp SE, Birkhed D.* (1986). *Long-term evaluation of root surface caries in periodontally treated patients. J Clin Periodonto, 13:758–67.*

[6] Dani S, Prabhu A, Chaitra K. (2016). Assessment of *Streptococcus mutans* in healthy versus gingivitis and chronic periodontitis: A clinico-microbiological study. Contemporary Clinical Dentistry. 7(4):529-34.

[7] Al-Kubaisi MW, Al-Ghurabi BH. (2023). Evaluation of Manuka Honey Effects on Dental Plaque and Bacterial Load (Clinical Study). J. Med. Chem. Sci. 6(2): 365-75 https://doi.org/10.26655/JMCHEMSCI.2023.2.17

[8] Nath S, Pulikkotil SJ, Weyrich L, Zilm P, Kapellas K, Jamieson L. (2022). Effect of Periodontal Interventions on Characteristics of the Periodontal Microbial Profile: A Systematic Review and Meta-Analysis. Microorganisms. 10(8):1582. doi: 10.3390/microorganisms10081582.

[9] Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, Abranches J, Brady LJ. (2019). The Biology of *Streptococcus mutans*. Microbiol Spectr. 7(1):10. doi: 10.1128/microbiolspec.GPP3-0051-2018.

[10] Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. (2005). Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 43(11):5721-32.

[11] Kumar PS. (2013). Oral microbiota and systemic disease. Anaerobe. 24:90-93.

[12] Marsh PD. (2006). Dental plaque as a biofilm and a microbial community—implications for health and disease. *BMC Oral Health.* 6(1):14.

[13] Zijnge V, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmür R. (2010). Oral biofilm architecture on natural teeth. PLoS One. 5(2):9321.

[14] *Koll-Klais P, Mandar R, Leibur E, Mikelsaar M. (2005). Oral microbial ecology in chronic periodontitis and periodontal health. Microb Ecol Health Dis.17:146–55. doi: 10.1111/j.1399-302X.2005.*

[15] Takahashi N, Nyvad B. (2011). The role of bacteria in the caries process: ecological perspectives. J Dent Res. 90(3):294-303.

[16] Preianò M, Savino R, Villella C, Pelaia C, Terracciano R. (2020). Gingival Crevicular Fluid Peptidome Profiling in Healthy and in Periodontal Diseases. Int J Mol Sci. 21(15):5270. doi: 10.3390/ijms21155270.

[17] Dani S, Prabhu A, Chaitra KR, Desai NC, Patil SR, Rajeev R. (2016). Assessment of *Streptococcus mutans* in healthy versus gingivitis and chronic periodontitis: A clinico-microbiological study. Contemp Clin Dent. 7(4):529-34. doi: 10.4103/0976-237X.

[18] Al-Ghurabi BH. (2021). The Role of Soluble TLR-2 in the Immunopathogenesis of Gingivitis. Intern. Med. 28(1): 37–39.

[19] Jasim FS, and Al-Ghurabi BH. (2025). Soluble FcγRIIIb (CD16b) and Alpha Defensin as Markers of Neutrophils Activation in Periodontal Disease. *Ibn AL*-Haitham Journal for Pure and Applied Sciences. 38(3): 58–67. doi:https://doi.org/10.30526/38.3.3660.

[20] Greenstein G. (1984). The role of bleeding upon probing in the diagnosis of periodontal disease: a literature review. Journal of Periodontology. 55(12): 684-88.

[21] Bostanci N, Mitsakakis K, Afacan B, Bao K, Johannsen B, Baumgartner D, Müller L, Kotolová H, Emingil G, Karpíšek M. (2021). Validation and verification of predictive salivary biomarkers for oral health. Sci Rep. 11(1):6406. doi: 10.1038/s41598-021-85120-w.

[22] Wang Y, Yang F, Wang Y, Deng S, Zhu R. (2024). Alterations and correlations in dental plaque microbial communities and metabolome characteristics in patients with caries, periodontitis, and comorbid diseases. BMC Oral Health. 24(1):132. doi: 10.1186/s12903-023-03785-3.