Assessment of *Streptococcus mutans* in healthy versus gingivitis and chronic periodontitis: A clinico-microbiological study

Sneha Dani, Ashwin Prabhu, K. R. Chaitra[1], N. C. Desai[2], Sudhir R. Patil, Ranjan Rajeev[3]

Abstract

Background: Dental caries and periodontal disease are most common oral diseases. *Streptococcus mutans* are considered to be the major pathogens in initiation of dental caries. Evidence shows that periodontal disease and caries share a number of contributory factors. Thus in view of these findings it would be worthwhile to examine whether *Streptococcus mutans* persist within the saliva and subgingival environment of the periodontitis patients and to determine whether there is any association between *Streptococcus mutans* colonization, pH of saliva and sub-gingival plaque pH in periodontal diseases before therapy. **Methods:** The study comprises of 75 subjects aged between 20-70 years, reporting to department of Periodontology, KLEs Institute of Dental Sciences, Bangalore. Subjects were divided into 3 groups of 25 each. Group 1 – Healthy controls, Group 2 – Gingivitis Group, 3 – Chronic periodontitis. Unstimulated saliva was collected in sterile container and immediately pH was evaluated. Subgingival plaque samples were collected from four deepest periodontal pockets in chronic periodontitis and from first molars in healthy subjects using 4 sterile paper points. In gingivitis subjects samples were collected from areas showing maximum signs of inflammation. All paper points and saliva samples were cultured on mitis salivarius agar culture media with bacitracin for quantification of the *Streptococcus mutans* colonies. **Results:** Increased colonization of *Streptococcus mutans* was seen in chronic periodontitis subjects both in saliva and sub-gingival plaque samples. There was also a positive correlation seen with the periodontal parameters. **Conclusion:** More severe forms of periodontal disease may create different ecological niches for the proliferation of *Streptococcus mutans*.

Keywords: Chronic periodontitis, pH, saliva, *Streptococcus mutans*

Introduction

Throughout life, all interfaces of the body are exposed to colonization by a wide range of microorganisms. In general, microbiota live in harmony with the host. Constant renewal of these surfaces by shedding prevents the accumulation of large masses of microorganisms. However, teeth provide hard nonshedding surfaces for the development of extensive bacterial deposits. The bacteria in the oral cavity remain within a specialized matrix called dental plaque or bacterial plaque. The accumulation and metabolism of the bacteria on hard oral surfaces is considered as the primary cause of dental caries, gingivitis, periodontitis, peri-implant infections, and stomatitis. Oral bacterial inhabit biofilms that form clusters adhering in layers to surfaces are not easily eliminated by immune responses and are resistant to antimicrobial agents. Dental plaque is one such biofilm.[1]

Periodontitis is a disease of the supporting tissues of the teeth whereas dental caries results from frequent exposure of teeth to low pH. These acidic conditions lead to the inhibition of acid-sensitive species and the selection of organisms with an aciduric physiology, such as *Streptococcus mutans* leading to dissolution of enamel.[2]

Evidence has suggested that both dental caries and periodontal diseases are multifactorial in nature and share a number of contributory factors that link them either directly or indirectly.[3]

There are studies suggesting no association between *S. mutans* count, gingival inflammation, periodontal pocket depth, and alveolar bone loss.[4]

Departments of Periodontics, [1]Microbiology and [2]Biochemistry, KLE Dental College, Bangalore, Karnataka, [3]Department of Periodontics, Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India

Correspondence: Dr. Sneha Dani,
Department of Periodontics, K.L.E. Institute of Dental Sciences and Hospital, No. 20, Yeshwanthpur Suburb, Opposite CMTI, Tumkur Road, Bengaluru - 500 092, Karnataka, India.
E-mail: drsnehadani@gmail.com

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However, recent studies have reported that untreated periodontitis patients have high recovery rates of *S. mutans* from saliva, tongue dorsum, buccal mucosa, and supra- and sub-gingival plaque.[5-7]

Given the high prevalence of root caries in periodontally treated patients with root exposure, it has been hypothesized that high levels of these cariogenic microorganisms may act as a predictor for the onset of carious lesions in these patients. Indeed, more root caries have been observed during the follow-up of periodontally treated patients with elevated counts of *S. mutans* than those with lower number of these microorganisms.[8]

In view of these findings, it remains unclear whether there is any correlation between the colonization of *S. mutans* and chronic periodontitis. Thus, the aim of this study was to determine whether there is any association between *S. mutans*, pH of saliva, subgingival plaque pH, and periodontal parameters in patients with varying degrees of periodontal disease before treatment.

Materials and Methods

Patient selection

The present study included 75 participants (range: 18–70 years) selected from the outpatient department of periodontics. Out of these, 25 cases were healthy individuals, 25 cases were diagnosed with generalized chronic gingivitis, and 25 cases were diagnosed with generalized chronic periodontitis. Healthy individuals were selected based on the absence of frank gingival inflammation, with no demonstrable attachment loss and probing depth >3 mm. Chronic periodontitis patients were selected as per the criteria laid down by the AAP 1999 classification and gingivitis patients selected showed signs of gingival inflammation and bleeding on probing with sulcus depth ≤3mm.[9]

All patients were systemically healthy and had not undergone periodontal therapy in the previous 6 months and antibiotic therapy in the previous 3 months. Smokers, patients on corticosteroids and chemotherapeutic agents, pregnant and lactating woman were excluded from the study. Ethical clearance was obtained from the Institutional Ethics Committee, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. After thorough explanation of the procedure, a written informed consent was obtained from all patients.

Clinical parameters assessed for the study were plaque index (PI),[10] gingival index (GI),[11] probing pocket depth,[12] and clinical attachment level.

Method of collection of saliva and subgingival plaque sample
Saliva sample collection
All individuals were asked to refrain from eating or drinking for 1 h prior to sample collection. Unstimulated saliva samples were collected from each participant for duration of 2 min. The participants were asked to bend their head forward, and unstimulated saliva pooled in the floor of the mouth was collected into sterile-disposable bottles without deliberately expectorating into them. Approximately, 10 μl of the saliva samples was plated on culture plates comprising mitis salivarius agar culture media with bacitracin for quantification of the *S. mutans* colonies.[13]

The rest of the saliva sample was immediately subjected to pH analysis. The pH was analyzed using an electronic digital pH meter.[14]

Subgingival plaque samples
After careful removal of supragingival plaque, subgingival plaque samples in periodontitis patients were taken from four deepest periodontal pockets, one in each quadrant, using 1 sterile paper point per site. In gingivitis patients, sites showing maximum signs of gingival inflammation were selected for sample collection. Plaque samples from healthy participants were taken from the mesial aspect of all the four first molars. The paper points were transported in 2 ml reduced transport fluid media, vortexed for 30 s, and 10 μl of the solution was plated on mitis salivarius agar with bacitracin culture plates. All the culture plates were incubated for 48 h.[6]

The subgingival plaque pH measurement was performed with the new so-called "strip method" based on the usage of pH indicator strips (pH 2–10.5). Briefly, the strips were cut into 2 mm width and inserted subgingivally into the same four sites that were selected for plaque collection. The strips were inserted and kept for 10 s. The pH value of all the four sites was assessed by comparison of the color of the strip with the color index guide supplied by the manufacturer, and an average was considered.[15]

Statistical analysis
The three tests used for analysis of the results were Kruskal–Wallis test, Spearman's correlation test, and Wilcoxon-signed rank test.

Results

The study group comprised a total of 75 participants, categorized into three groups – healthy, gingivitis, and chronic periodontitis. The mean age of healthy individuals was 24.84 years, gingivitis group was 32.40 years, and chronic periodontitis group was 38.52 years [Table 1].

The mean PI of healthy individuals was 0.025, gingivitis patients was 1.380, and 1.781 in periodontitis patients. The mean GI was 0.038, 1.385, and 1.890 in healthy, gingivitis, and periodontitis participants, respectively. The average probing pocket depth and clinical attachment level were 4.263 and 5.962, respectively, in periodontitis patients.

Inter- and intra-group comparison of *Streptococcus mutans* count in saliva and subgingival plaque samples

The highest colonies of *S. mutans* were seen in periodontitis patients (187.50) followed by healthy participants (95) and least seen in gingivitis patients (40). Similar to saliva samples, the highest colonies were seen in periodontitis cases (57) followed by healthy (40) and gingivitis cases (17). However, only the subgingival plaque samples showed statistical significance.

On intragroup comparison between plaque and saliva samples within each group, there was a statistical significant difference seen in gingivitis and periodontitis participants. More colonies of *S. mutans* were detected in saliva samples [Table 2].

Comparison of mean saliva pH between the study groups

The mean saliva pH was 7.278, 7.273, and 7.001 in healthy, gingivitis, and periodontitis participants, respectively. However, the difference was not statistically significant [Table 3].

Comparison of mean subgingival plaque pH between the study groups

The mean subgingival plaque pH was 6.6, 7, and 7.40 in healthy, gingivitis, and periodontitis participants, respectively. Unlike the saliva samples, the plaque pH between the three groups showed statistical significance [Table 4].

Correlation between *Streptococcus mutans* count and pH

When *S. mutans* count in saliva was correlated with saliva pH, only the healthy group showed a strong negative correlation between the two. On comparing the plaque pH with *S. mutans* count in plaque, both gingivitis and healthy groups had a strong positive correlation. All the other correlations were weak or negligible [Table 5].

Table 1: Comparison of age between the three groups

	n	Mean	SD	Minimum	Maximum
Healthy (*n*=25)	25	24.84	5.031	18	40
CGG (*n*=25)	25	32.4	7.848	18	47
CGP (*n*=25)	25	38.52	10.365	23	69

The difference in age group between all the three groups was statistically significant. CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis; SD: Standard deviation

Table 2: Inter- and intra-group comparison of *Streptococcus mutans* count in all three groups

Group	n	Mean	SD	Median	Minimum	Maximum	Z*	P
Healthy								
S. mutans in saliva	5	416.2	751.756	95	50	1760	−1.826	0.068
S. mutans in plaque	10	42.1	31.445	40	3	107		
CGG								
S. mutans in saliva	15	95.07	121.973	40	5	416	−2.386	0.017*
S. mutans in plaque	17	29.12	34.016	17	4	120		
CGP								
S. mutans in saliva	16	281.81	380.733	187.5	10	1520	−2.101	0.036*
S. mutans in plaque	18	106.83	177.177	57	3	780		

Intergroup comparison in saliva *S. mutans* count was not statistically significant. However; subgingival plaque *S. mutans* count was statistically significant. Intragroup comparison in *S. mutans* count in CGG and CGP was statistically significant. *S. mutans*: *Streptococcus mutans*, CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis; SD: Standard deviation. *Statistically significant

Table 3: Comparison of mean saliva pH between the study groups

Group	n	Mean	SD	Median	Minimum	Maximum	x^{2}*	P
Healthy (*n*=25)	25	7.278	0.642	7.37	5.87	8.41	1.668	0.196
CGG (*n*=25)	25	7.273	0.432	7.16	6.36	8		
CGP (*n*=25)	25	7.001	0.727	7.3	5.55	8		

*Chi square value. Intergroup comparison in saliva pH in all the three groups was not statistically significant. CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis; SD: Standard deviation

Table 4: Comparison of mean subgingival plaque pH between the study groups

Group	n	Mean	SD	Median	Minimum	Maximum	x^{2}*	P
Healthy (*n*=25)	25	6.6	0.577	7	6	8	10.286	<0.001**
CGG (*n*=25)	25	7	0.577	7	6	8		
CGP (*n*=25)	25	7.4	0.707	7	6	9		

*Chi square value. Intergroup comparison in subgingival plaque pH in all the three groups was statistically significant. CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis; SD: Standard deviation. **Highly statistically significant

Comparison of periodontal parameters between all the three groups

The mean probing pocket depth in chronic periodontitis group was 4.263 mm and mean clinical attachment loss was approximately 5.962 mm. PI and GI between all three groups were statistically significant [Table 6].

Correlation of *Streptococcus mutans* count with periodontal parameters

S. mutans in saliva elucidated a strong negative correlation in healthy group with gingival and PI and in gingivitis group with GI. However, periodontitis group showed a strong positive correlation with clinical attachment level [Table 7].

On correlating the count in plaque with periodontal parameters, only the healthy group demonstrated a strong negative correlation between *S. mutans* count and PI. All other parameters showed no correlation [Table 7].

Discussion

Dental caries and periodontitis are two of the most common oral diseases. They may be synergistically associated, negatively associated, or completely independent of each other. Positive association may relate to their microbiological etiology and biofilm formation. Furthermore, both diseases share many social and behavioral background factors in common, which have been related to their etiology.[16]

Table 5: Correlation between *S. mutans* count and pH

	Very strong negative	Strong positive	Weak negative	Negligible
Saliva counts versus pH of saliva	Healthy	-	CGP	CGG
Plaque counts versus plaque pH	-	Healthy CGG	-	CGP

CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis

Table 6: Comparison of periodontal parameters between all the three groups

	n	Mean	SD	Minimum	Maximum	*F*	*P*
PI							
Healthy	25	0.025	0.017	0.01	0.09	113.067	<0.001**
CGG	25	1.38	0.475	0.8	2.3		
CGP	25	1.781	0.579	0.75	2.8		
GI							
Healthy	25	0.038	0.042	0.01	0.17	139.085	<0.001**
CGG	25	1.385	0.52	0.7	2.3		
CGP	25	1.89	0.471	1.13	2.9		
PPD							
CGP	25	4.263	0.745	2.59	5.62		
CAL							
CGP	25	5.962	0.674	5.12	7.72		

PI and GI between all the three groups were statistically significant. PI: Plaque index; GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment level; SD: Standard deviation; CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis. *P*<0.001 highly statistically significant. **Highly statistically significant

Table 7: Correlation of *Streptococcus mutans* count with periodontal parameters

	PI	GI	PPD	CAL
Saliva				
Healthy	Strongly negative	Strongly negative	-	-
CGG	Moderately negative	Strongly negative	-	-
CGP	No	No	Weakly positive	Strongly positive
Plaque				
Healthy	Strongly negative	No	-	-
CGG	No	No	-	-
CGP	No	Moderately positive	Moderately positive	Weakly positive

CGG: Chronic generalized gingivitis; CGP: Chronic generalized periodontitis; PI: Plaque index; GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment level

S. mutans is an aciduric Gram-positive coccus which may be naturally found in dental plaque and saliva. In this study, high proportions of *S. mutans* were seen in the saliva and subgingival plaque samples of chronic periodontitis. This is in contradiction to studies by Koll-Klais *et al.* who found high proportions of these species in healthy individuals, indicating that they help in maintaining a micro-ecological balance in the oral cavity.[17]

However, studies by Cortelli *et al.* found results that were in accordance to this study. The variation in the results could be attributed to difference in the sampling technique. According to Cortelli *et al.*, unstimulated saliva is a representative of pooled subgingival area in advanced periodontitis patients. Most of the contradictory studies collected stimulated saliva.[18,19]

In this study, unstimulated saliva samples were collected. Furthermore, Preza *et al.* concluded from their study that *S. mutans* count appears directly co-associated with increased severity of periodontitis at older ages and in untreated patients. All patients in the periodontitis group fell in the older age group, untreated, and had severe attachment loss (≥5 mm).[5]

As with the saliva samples, subgingival plaque also showed more colonies of *S. mutans* in periodontitis group when compared to healthy and gingivitis groups. These results are in agreement with studies by Van der Reijden *et al.*[6]

It has been suggested that severe chronic periodontitis show low oxygen tension in deep pockets that favors the growth of microaerophilic species such as *S. mutans*. Reduced oxygen tension can also exclude them from some of the micro niches.[20]

Studies by Rickard *et al.* suggested that planktonic *S. mutans* demonstrate different gene expression than those present in the biofilm. The quorum sensing system of *S. mutans* in the biofilm may enable them to survive with obligatory anaerobes that are present in deeper periodontal pockets.[21]

Thus, in the oral biofilm, multiple species can co-aggregate to colonize the tooth surface or provide nutrients to other bacteria by metabolization of substrates. After cleaning the teeth, *Streptococci mutans* compete for colonizing saliva-coated enamel or dentin mediated by the expression of adhesion molecules or antibacterial molecules. Periodontitis-associated microorganisms can coexist with *S. mutans* and survive acidic conditions constrained by interspecies interactions. Thus, individuality of the host microflora could be responsible for disparity among studies pertaining to *S. mutans* colonization.[6]

When saliva pH was compared between all the three study groups, no statistical significance was seen. However, on comparing the subgingival plaque pH, there was a significant difference between the three groups. Studies by Cortelli *et al.* and Sah *et al.* found that the plaque pH was raised in periodontitis.[22]

In this study also, similar findings were observed. A possible explanation for this could be increased urea concentration in saliva which readily diffuses from saliva to subgingival environment, increased production of ammonia by obligatory anaerobes that are seen in abundance in periodontitis patients, and raised salivary calcium levels that have an affinity to be taken up by the biofilm. All these mechanisms are essential for the survival of obligatory anaerobes such as *Porphyromonas gingivalis*, which is considered as the key periodontal pathogen.[13]

When saliva pH and *S. mutans* count in saliva were correlated, it was found that only the healthy group showed a strong negative correlation. The other two groups showed weak or negligible correlation. Thus, as the saliva pH raises, acidogenic bacteria such as *S. mutans* fail to thrive.[23]

An interesting finding found in this study was that when subgingival plaque pH and *S. mutans* colonies in the plaque were correlated, both healthy and gingivitis groups showed a strong positive correlation. However, periodontitis patients demonstrated negligible correlation between the two.

Despite these correlations, none of them were statistically significant. Thus, based on these findings, it can be suggested that salivary pH may play a role in *S. mutans* colonization within the oral cavity. However, factors other than pH may be responsible for their survival subgingivally.[14,24]

On correlating the counts with periodontal parameters, both healthy and gingivitis groups showed either a negative or no correlation between *S. mutans* in saliva and plaque with GI and PI.

An important observation made in this study was that there was a strong positive correlation between *S. mutans* in saliva in periodontitis group and clinical attachment level. In most of the studies, the *S. mutans* count have positively correlated with clinical attachment level following periodontal therapy, suggesting their possible role in the development of root caries in periodontal maintenance patients.[6]

However, in this study, the count did correlate positively with clinical attachment level, but in untreated patients. The possible explanation for this could be that as the depth of the periodontal pocket increases, there is larger surface area for bacterial colonization. Thus, with increasing inflammation, there is ample amount of nutrients provided for further bacterial multiplication. Hence, an altered host response in periodontitis patients could play a role in bacterial colonization subgingivally.[25]

Conclusion

In this study, a higher colonization of *S. mutans* was seen in saliva and subgingival plaque samples of periodontitis patients. However, subgingival plaque pH did not play much role in their colonization. In addition, a positive correlation was seen between periodontal parameters and *S. mutans* count in chronic periodontitis. However, comparison of all results with other studies was not possible, as disparity between results exists.

Thus, it can be hypothesized from this study that more severe forms of periodontal disease may create different ecological niches for the proliferation of *S. mutans.* The mechanism by which their level increases with the severity of periodontitis needs to be determined. In addition, whether there is any association between periodontal disease progression and a higher proportion of *S. mutans* subgingivally needs further evaluation.

Hence, further studies are needed to elucidate whether they act synergistically with other putative periodontal pathogens or just merely present in the subgingival biofilm ecosystem.

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Conflicts of interest
There are no conflicts of interest.

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