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# Oral colonization by *Streptococcus mutans* and its association with the severity of periodontal disease in adults

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## ABSTRACT

**Background:** *Streptococcus mutans* (*S. mutans*) is associated with the onset of caries. Since root exposure in patients affected by periodontitis leads to higher caries rates, progressively more severe forms of periodontal disease might associate with elevated counts of *S. mutans*. **Aim:** To determine whether increasingly destructive forms of periodontal disease are associated with higher counts of *S. mutans* in untreated patients. **Methods:** 206 subjects aged 20-75 were classified into three groups according to the severity of periodontal disease: 1) gingivitis, 2) chronic slight periodontitis and 3) chronic moderate or chronic severe periodontitis. *S. mutans* counts (cfu/mL) were obtained by direct counting on selective agar plates from saliva samples. A cumulative proportional logistic regression model was adjusted for *S. mutans* counts. **Results:** The model failed to show differences by gender, but periodontal diagnosis had a significant effect on *S. mutans* counts depending on age. While in the group with moderate and severe periodontitis the probability of having high counts of *S. mutans* significantly increased with age, the probability remained unchanged in individuals with chronic slight periodontitis or gingivitis. **Conclusion:** High *S. mutans* levels appear directly co-associated with increased severity of periodontal disease at older ages in untreated patients. **Rev. Clin. Periodoncia Implantol. Rehabil. Oral Vol. 4(1); 9-12, 2011.**

**Key words:** Periodontitis, periodontal disease severity, *Mutans Streptococci*, *Streptococcus mutans*, caries, root caries.

## INTRODUCTION

Oral biofilms usually comprise beneficial microorganisms for the host as they exclude colonization by pathogenic species<sup>(1,2)</sup>. Under certain circumstances, however, oral environmental changes may modify the composition and metabolic activities of the bacterial consortium leading to predominance of pathogens<sup>(1)</sup>. High counts of *Mutans streptococci* (MS), *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus*, have been associated with higher prevalence of coronal caries in temporary and permanent dentitions<sup>(3-7)</sup> and also root caries<sup>(8-11)</sup>. In spite of the widely acknowledged association between increased levels of MS and higher caries experience, other studies have argued against this association<sup>(12,13)</sup>. Colonization and pathogenicity of these facultative Gram(+) cocci derive from their capacity to synthesize extracellular polysaccharides<sup>(14)</sup>. Indeed, adult subjects colonized by biofilm-forming *S. mutans* strains have higher caries experience than individuals without these types of strains<sup>(12)</sup>.

It remains unclear whether *S. mutans* increases colonization during periodontitis. Studies have shown, on the one hand, that gingival inflammation does not associate with higher levels of *S. mutans*<sup>(15)</sup> and higher recoveries of the microorganism have been found in treated periodontal patients during follow-up<sup>(5,16)</sup>. Likewise, periodontally treated subjects show more *S. mutans* than untreated controls<sup>(11)</sup>. Recent studies, however, report that untreated patients with periodontitis have high recovery rates of *S. mutans* from saliva, tongue dorsum, buccal mucosa and supra and subgingival plaque<sup>(17)</sup>. Moreover, studies assessing the biofilm composition of root caries have reported more elevated counts of *S. mutans* when compared with sound root surfaces<sup>(10)</sup>. Given the high prevalence of root caries in periodontally treated patients with root exposure<sup>(18)</sup>, it has been hypothesized that high levels of MS may act as a predictor for the onset of carious lesions in this type of patients<sup>(11)</sup>. Indeed, more root caries have been described during the follow-up of periodontally treated patients with elevated counts of *S. mutans* than those with lower number of the microorganism<sup>(19)</sup>.

The aforementioned evidence allows speculating that untreated periodontal patients with more severe forms of periodontitis, who in many cases show more root exposures, might have more cariogenic flora

than those subjects with milder periodontitis or gingivitis. An association between *S. mutans* colonization and the severity of periodontal disease has not been reported. The aim of this cross sectional study was, therefore, to compare salivary *S. mutans* counts in untreated patients with gingivitis and different degrees of severity of periodontal disease.

## MATERIALS AND METHODS

**Subjects.** 206 patients (53 males and 153 females) between 20 and 75 years-old were randomly selected to participate in the study. Patients included had to refrain from eating, rinsing or smoking two hours before the exam. Likewise, participants could not be under antibiotics therapy or oral antimicrobials at the moment of the exam. Sample size represented all the adult patients under treatment at the time of the study who met the eligibility criteria for the study protocol. Patients were chosen among those requesting dental treatment at the Dental Clinics of the University of Talca, Talca, Chile. Participants signed an informed consent form to enroll the study, which was approved by the ethics committee of the University of Talca. A comprehensive dental examination was carried out by dental students in all the patients, including radiographs. To be eligible for the study, participants must be older than 20 years old, not have been treated for periodontitis, have at least 25% of their teeth in the mouth, have a gingival index (Löe and Silness) at least of 1 and hygiene index (Silness and Löe) lower than 50%. Two previously calibrated and experienced periodontists (Dr. S. Matus and Dr. M. Nally) examined the patients and verified the accuracy of the student's first diagnosis. Periodontal diagnosis criteria were based on the classification of the American Academy of Periodontology<sup>(20)</sup>. Individuals were classified as having gingivitis or periodontitis. The latter was subsequently divided into two categories according to the severity of the disease. Diagnostic categories were defined based on the level of attachment loss, as follows:

- 1) 0 mm: gingivitis associated with dental plaque only (gingivitis) (n=92),
- 2) 1-2 mm: generalized chronic slight periodontitis (CSP) (n=56) and
- 3) Generalized chronic moderate (3-4 mm) or severe (5 or more mm) periodontitis (CMSP) (n=58).

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S. mutans Culture and Counts

To obtain the number of colonies of *S. mutans*, each patient chewed a piece of paraffin wax for 2 min and expectorated the saliva into sterile glass tubes. Immediately after samples were collected, saliva was homogenized for 30 seconds, diluted 1:1000 (v/v) in sterile distilled water and an aliquot of 50 µL sowed on selective Trypticase–yeast–cysteine–sucrose–bacitracin agar plates (TYCSB) (Merck, Darmstadt, Germany) modified by addition of 20% sucrose (w/v) (Merck, Darmstadt, Germany) for *S. mutans*, as described<sup>(13)</sup>. Plates were incubated at 37°C for 48 h in anaerobic jars (Gen Box Anaer; bioMérieux, Marcy-l’Etoile, France), followed by phenotypical colony identification, considering as *S. mutans* only those adherent, irregular and sugar grain-like colonies, as described elsewhere<sup>(21)</sup>. Colonies growing on the plates were counted adjusting for the dilutions to obtain the final number of *S. mutans* expressed as cfu/mL. Counts of *S. mutans* were grouped into three categories: <1 x 10<sup>5</sup>; 1 x 10<sup>5</sup> to 1 x 10<sup>6</sup> and >1 x 10<sup>6</sup> cfu/mL.

Statistical Analysis

Using two cut-off points, Cochran-Armitage test for trend was used to determine whether the proportion of patients with >1 x 10<sup>5</sup> cfu/mL or >1 x 10<sup>6</sup> cfu/mL followed a linear trend with increasing severity of periodontal disease. Moreover, a proportional cumulative risk logistic regression model was adjusted to evaluate the effect of gender, age and periodontal diagnosis on the number of *S. mutans*, divided into three counting categories: <1 x 10<sup>5</sup>; 1 x 10<sup>5</sup> to 1 x 10<sup>6</sup> and >1 x 10<sup>6</sup> cfu/mL, with a significance level of 5%.

RESULTS

The study group of patients consisted of 74.3% of females and 25.7% males. 44.7% of the participants were diagnosed with gingivitis, 27.2% with CSP and 28.2% were classified as having CMSP (Table 1). Median age of the participants was 34.5 years-old. Only the CSP group showed differences of age between men and women (p=0.008) (Table 1).

Table 1. Patient distribution by age, gender and periodontal condition.

Periodontal Diagnosis	Age		p value	Total Median (IQR) n - %
	Male	Female		
	Median (IQR) n - %	Median (IQR) n - %		
Gingivitis	22.5 (5.5)	25.0 (12.0)	0.084	24 (10.5)
	24 - 45.3	68 - 44.4		92 - 44.7
CSP*	33.0 (12.5)	39.0 (7.0)	0.008	38 (9.8)
	13 - 24.5	43 - 28.1		56 - 27.2
CMSP**	43.5 (12.0)	41.5 (17.3)	0.626	42.0 (16.3)
	16 - 30.2	42 - 27.5		58 - 28.2
Total	30.0 (18.5)	36.0 (17.0)	0.065	34.5 (19.0)
	53 - 100.0	153 - 100.0		206 - 100

IQR: Interquartile Range; \*CSP: Chronic Slight Periodontitis; \*\*CMSP: Chronic Moderate and Severe Periodontitis. # Mann-Whitney U test.

Cochrane-Armitage test showed that the proportion of patients with high *S. mutans* counts (>1 x 10<sup>6</sup> cfu/mL) progressively and linearly increases from gingivitis to the two degrees of severity of periodontal disease (p=0.0123) (Figure 1, solid line). When the cut-off point used was set to a level >1 x 10<sup>5</sup> cfu/mL, however, the linear trend was not observed (p = 0.8066) (Figure 1, dotted line).

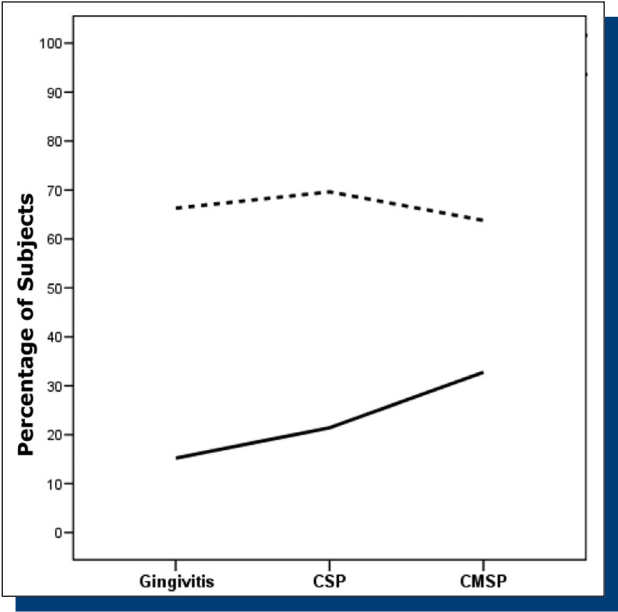


Figure 1. Cochrane-Armitage test results for patients with *S. mutans* counts >1x 10<sup>6</sup> cfu/mL (entire line) and >1x 10<sup>5</sup> cfu/mL (dotted line) by periodontal condition.

\*CSP: Chronic slight periodontitis.

\*\*CMSP: Chronic moderate and severe.

The adjusted cumulative proportional risk model for the three categories of *S. mutans* count failed to show differences by gender. By age, the model showed significant differences (p=0.0052) on the microbial counts, depending on the periodontal diagnosis, nonetheless. Hence, the probability of having higher counts of *S. mutans* at older ages was significantly higher for CMSP patients when compared with CSP and gingivitis (p=0.0134 and p=0.0022, respectively). While probability of high counts of the cariogenic species tends to increase with age in patients with CMSP (Figure 2 Panel C), the likelihood of high counts is unaffected in subjects with gingivitis and CSP (Figure 2 Panel A and B). On the other hand, probability of low counts of *S. mutans* (<1 x 10<sup>5</sup>) decreases with age in CMSP (Figure 2 Panel C), but increases with age in patients with gingivitis and CSP (Figure 2 Panel A and B), without differences between the two latter diagnoses (p=0.8087).

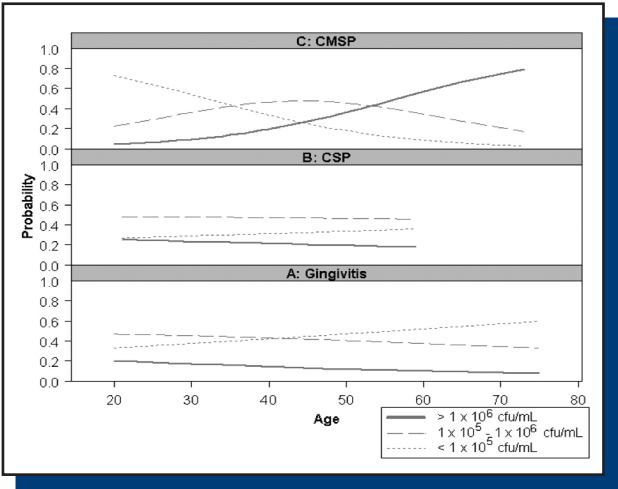


Figure 2. Adjusted cumulative proportional risk model for the probability of high, moderate and low count of *S. mutans* by age in gingivitis (Panel A), CSP (Panel B) and CMSP (Panel C).

\*CSP: Chronic slight periodontitis.

\*\*CMSP: Chronic moderate and severe.

## DISCUSSION

Pathogenicity of the biofilms relies, in part, on the environmental factors to which they are exposed<sup>(1)</sup>. Periodontal diseases provide substrates for bacteria that differ from those colonizing under healthy conditions<sup>(22)</sup>. In the subgingival biofilm of less destructive forms of periodontal disease, Gram positive species, including *Streptococcus* and *Actinomyces*, are expected to be isolated<sup>(23)</sup>. Conversely, more destructive periodontitis produces periodontal pockets, which favor the establishment of anaerobic flora<sup>(24)</sup>. Based on the aforementioned issues, it is reasonable to hypothesize that more severe forms of periodontal disease create different ecological niches for the proliferation of *S. mutans*. In this study, it was found that patients with gingivitis or milder forms of periodontal disease are more likely to have low counts of *S. mutans* than subjects with more severe forms of periodontitis, who showed significantly higher counts of the cariogenic species, in both cases, in an age-dependent manner (Figure 2).

It has been reported that microbial flora changes after periodontal therapy, from one more predominantly periodontopathogenic to one more cariogenic<sup>(11,25)</sup>. Despite the changes in proportion of *S. mutans* before and after the therapy, the bacterium persists within the biofilm of the treated patients<sup>(16)</sup>. Microbiological changes due to periodontal treatment may affect the incidence of caries, particularly root caries. Periodontally treated patients show higher rates of root caries, even over 80%, which is higher in subjects with high counts of *S. mutans*<sup>(19)</sup>. *S. mutans* has been isolated more frequently from root caries than from non-carious root tissues and it is believed to participate in the etiology of the lesions<sup>(8-10,26)</sup>. Whether there is an association between progression of periodontal disease and a higher proportion of *S. mutans* has not been reported. Importantly, our study was performed from saliva rather than from root surfaces samples. *S. mutans* in saliva characterizes the overall colonization by the microorganism, but it may misrepresent the presence of the bacterium on the root surfaces or in the carious lesions. Further studies must consider this issue in their experimental design.

A higher presence of *S. mutans* in untreated patients with chronic moderate or severe periodontitis, as we show here, may derive from a lower oxygen tension within the periodontal pockets<sup>(27)</sup>, which favors growth of microaerophilic species, such as *S. mutans*. On the other hand, oral biofilm comprises hundreds of species coexisting in the same ecosystem<sup>(28-30)</sup>. Predominance of one species over the other

may be the result of interspecies competition<sup>(31)</sup>. When one species predominates, it may exclude others and create new conditions for the remaining microorganisms. Thus, in the oral biofilm multiple species can co-aggregate to colonize the tooth surface<sup>(32)</sup>, or provide nutrients to other bacteria by metabolization of substrates<sup>(33)</sup>. After cleaning the teeth, *Streptococci* compete for colonizing saliva-coated enamel or dentin<sup>(34)</sup>, in some cases mediated by the expression of adhesion molecules<sup>(30)</sup> or antibacterial molecules<sup>(2,12)</sup>. Periodontitis-associated microorganisms can coexist with *S. mutans* and survive acidic conditions constrained by saccharolytic flora by interspecies interactions. For example, by fermenting glutamic and aspartic acids, *F. nucleatum* y *P. intermedia* produce ammonium, which increases pH protecting periodontopathogens such as *P. gingivalis*<sup>(35)</sup>. It is important to state, though, that several microniches may exist in the mouth, each having different composition of species within the consortium of the oral biofilm. Lower oxygen tension in deeper periodontal pockets might exclude *S. mutans* in some of the microniches, which can explain why the differences observed are not too large when the different severities of the disease were compared. However, higher overall colonization by *S. mutans* associated with more severe periodontitis in untreated patients, measured in whole saliva, may be the result of several ecological changes in the oral environment of this type of patients. Hence, the mechanism by which *S. mutans* levels increase with severity of periodontitis, requires further investigation.

Findings of the present investigation are in line with a reported higher risk for root caries in periodontitis patients<sup>(36)</sup>. More severe forms of periodontitis at older ages might represent an increased risk for root caries, suggesting a need for more effective caries control measurements in people affected by periodontal disease.

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