

Correlation of salivary levels of *Streptococcus mutans* with the conditions of oral hygiene in schoolchildren between the ages of 6 and 12

*Correlação dos níveis salivares de *Streptococcus mutans* com as condições de higiene bucal em escolares de 6 a 12 anos*

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ABSTRACT

Objective

To evaluate the microbiological salivary levels of *Streptococcus mutans* by correlating them with conditions of oral hygiene by measuring rates of Oral Simplified Hygiene and the plaque index in schoolchildren between 6 and 12 years of age, enrolled in a State school in the city of Campina Grande, Paraíba.

Methods

The sample consisted of 25 students of both genders. An observational study was carried out using comparative, statistical and descriptive procedures, by direct observation through clinical and microbiological analysis. We first measured the simplified oral hygiene index and the plaque index and immediately afterwards the saliva was collected from the schoolchildren in order to perform a count of the colonies of *Streptococcus mutans*.

Results

There was a normal oral hygiene and the mean simplified oral hygiene index equal to 1.7 is in line with the average of 1.6 for the plaque index. A quantitative analysis of the number of colony forming units of *Streptococcus mutans* showed a variation of between 0.1×10^4 CFU/ml and 7.7×10^4 CFU/ml. These data correlated with the index card shows a weak correlation in which only 11.7% of the variability of the plaque index is explained by the amount of *Streptococcus mutans* (CFU/ml $\times 10^4$).

Conclusion

It was shown, therefore, that the Plaque Index present in these schoolchildren, is not related to the amount of salivary microorganisms of the type *Streptococcus mutans* (CFU/ml $\times 10^4$).

Indexing terms: Students. Oral hygiene. *Streptococcus mutans*.

RESUMO

Objetivo

Avaliar os níveis salivares de *Streptococcus mutans* correlacionando-os com as condições de higiene bucal através da mensuração dos Índices de Higiene Oral Simplificado e Índice de Placa Bacteriana em escolares de 6 a 12 anos de idade matriculados em uma escola Estadual do município de Campina Grande, Paraíba.

Métodos

A amostra foi constituída de 25 escolares de ambos os sexos. Realizou-se um estudo observacional com procedimento comparativo, estatístico e descritivo, através da observação direta por meio do exame clínico e análise microbiológica. Primeiramente foi mensurado o Índice de Higiene Oral Simplificado e o Índice de Placa Bacteriana e logo após foi coletada a saliva dos escolares para contagem das colônias de *Streptococcus mutans*.

Resultados

Observou-se uma higiene bucal regular sendo a média de Índice de Higiene Oral Simplificado igual a 1,7 estando de acordo com a média do Índice de Placa Bacteriana que foi de 1,6. A análise quantitativa do número de unidades formadoras de colônia de *Streptococcus mutans* revelou uma variação de $0,1 \times 10^4$ UFC/ml a $7,7 \times 10^4$ UFC/ml. Esses dados correlacionados com o Índice de Placa demonstram uma correlação fraca na qual apenas 11,7% da variabilidade do índice de Placa Bacteriana é explicada pela quantidade de *Streptococcus mutans* (UFC/ml $\times 10^4$).

Conclusão

Com isso, foi demonstrado que o Índice de Placa presente nos escolares não está associado à quantidade de micro-organismos salivares do tipo *Streptococcus mutans* (UFC/ml $\times 10^4$).

Termos de indexação: Estudantes. Higiene bucal. *Streptococcus mutans*.

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INTRODUCTION

The correlation between dental biofilm and tooth decay is not in dispute, bearing in mind that the prior existence of biofilm is of fundamental importance to the start of the carious process¹.

Oral microbiota are formed by a complex ecosystem composed of bacteria, fungi, viruses and protozoans, the *Streptococcus* being the most prevalent microorganism². In the main, these oral bacteria produce glucosyltransferase (GTF), which uses saccharose as a substrate to synthesize extracellular polysaccharides, thereby facilitating adhesion and the accumulation of oral bacteria on the dental³ surfaces.

Amongst the *Streptococcus* species, the mutans group plays an important role in the establishment of dental caries and may cause a broad assault on the deciduous dentition when its establishment occurs prematurely. As well as being directly linked to the development of the carious process, the saliva levels of this group of bacteria are dependent on the number of unerupted² teeth. In corroboration of this statement, the literature evaluates the prevalence of these microorganisms in infants aged between 12 and 31 months and their link to the formation and severity of dental⁴ caries. Amongst the main results encountered, they noted that 80.3% of the children studied presented *S. mutans*, of which 22% exhibited high salivary levels of these bacteria. A number of authors have conducted research of the same level, evaluating the prevalence of children between the age of 8 and 60 months who were carriers of *S. mutans*, in which they observed that 92.5% of these children in the study presented this bacteria in the dental biofilm⁵.

It may be stated, therefore, that saliva functions as a system that is indicative of dental biofilm, since the planktonic microbiota detected comes from the biofilm, and *S. mutans* is found in everybody's non-stimulated saliva⁶. Therefore, according to current studies, the increase in the plaque index is proportional to the number of Colony Forming Units (cfu) of *S. mutans* detected in the saliva and that the saliva flow presents a significant link with the average number of *S. mutans* cfu in the dental biofilm⁷.

The reduction in dental biofilm through good oral hygiene is directly related to a better oral health condition. Motivation in preventive education programs is hugely important in the stimulation of the reduced formation

of dental biofilm and gingival⁸ bleeding, therefore the participation of the professional in enlightening the patient is just as important as restoration treatment.

The aim of this study was to evaluate the microbiological saliva levels of *S. mutans* through a correlation with conditions of oral hygiene in schoolchildren enrolled in a state school in the city of Campina Grande, in the state of Paraíba, Brazil.

METHODS

This study was conducted on a total of 25 children aged between 6 and 12, enrolled in the Aplicação state school located in the city of Campina Grande, Paraíba. The following inclusion criteria were established for the children taking part in the study: they must have 20 dental elements, not carry out mouthwash with oral antiseptics and there must be satisfactory systemic conditions.

The clinical examinations were carried out in the dental consulting rooms of the institution itself, during normal school hours, by just one examiner and complying with infection control criteria. At this point, the measurement was performed of the Simplified Oral Hygiene Index (OHI-S) and the Bacterial Plaque Index, which were recorded on pre-prepared record cards, and then the saliva was collected for a microbiological examination.

The OHI-S proposed by Greene & Vermillion⁸ was used to measure oral hygiene. The recommended dental elements manifested themselves with a solution of 2% basic fuchsin. For the upper elements, the vestibular surfaces were examined of the first permanent molars and the right central incisor, while in the lower elements, the lingual surfaces of the first permanent molars and vestibular surface of the left central incisor were examined. The values of the plaque accumulation found received the following marks: 0 = surface free from plaque; 1 = $\frac{1}{3}$ of the surface covered in plaque; 2 = $\frac{2}{3}$ of the surface covered in plaque; 3 = whole surface covered in plaque, thereby obtaining the average for the elements examined.

In order to quantify the Bacterial Plaque Index, the proposal put forward by Loe was used⁹. According to this author, all teeth may be evaluated or just six representative teeth: 16, 12, 24, 36, 32, 44. The four surfaces of the elements were examined, always in the cervical region, where there is usually more dental biofilm formation. For

the application of this index, the following scoring was observed: 0 = no plaque in the gingival area; 1 = a thin film of plaque adhering to the free gingival margin adjacent to the area of the tooth; 2 = accumulation inside the gingival groove, on the gingival margin and/or the gingival margin adjacent to the surface of the tooth; 3 = abundance of softened material inside the gingival groove and/or at the gingival margin adjacent to the surface of the tooth.

The *S. mutans* count was performed using the technique prescribed by Gold et al¹⁰.

The saliva samples required for determining the viable *Streptococcus* cell count were collected without stimulation and kept in ice until the commencement of the microbiological procedure for counting, with the aim of preventing the multiplication of bacteria, the mesophiles that grow at room temperature. Accordingly, the period between collection and streaking was no more than three hours.

The cultivation was carried out with the aid of a pipette by withdrawing 0.5 ml of previously collected saliva, and was then placed in a test tube containing 4.5 ml of BHI broth (DIFCO), and homogenized in a tube shaker for 30 seconds. Successive decimal solutions were then carried out in a sterilized 0.85% saline solution and 0.1 ml of each dilution was placed on a Petri dish containing Agar mitis salivarius added to 5% saccharose, 0.2 international units of bacitracin and 0.1g of potassium telluride. The dishes were incubated for 72 hours at 37°C in microaerophiles.

The dish reading was carried out through a standard count of viable *Streptococcus* colonies (CFU/ml) under a stereoscopic microscope. The colony count was performed with the aid of a colony counter. The value was then converted based on the amount inoculated 0.1ml, and the dilution factor for counting, presenting the final result in CFU/ml of saliva. The final result in CFU/ml of saliva was given by the conversion of the inoculated quantity (0.1ml) and the dilution factor.

All the children, parents and/or persons responsible first took part in a lecture in which they were informed about the study, its risks and benefits, receiving from the examiner and monitor the free and informed consent document. They were instructed on how to maintain oral hygiene and its importance in controlling tooth decay and periodontal disease, making it possible to enjoy better oral health.

Those responsible for the schoolchildren participating in the study signed the consent form, whose ethical stance complied with the resolution for research using human beings no. 196/96 of the Brazilian National

Health Council. The study followed all the ethical principles contained in the Helsinki Declaration (2000) and was registered with the Committee on ethics and research at the state university of Paraíba, under reference number 0051.0.133.000-05.

RESULTS

The OHI-S values obtained from the schoolchildren, on average, distributed by sex and age range, are shown in Table 1.

Table 2 illustrates the descriptive analysis of the Plaque Indices (PI) and *S. mutans* Count (CFU/ml x 10⁴), and here it is possible to observe the maximum and minimum values, the mean and the standard deviation of these variables. The plaque index shows a mean of 1.6 and standard deviation of 0.23, the low standard deviation value showing little variability in the data observed in minimum and maximum values of 1.1 and 2.1, respectively. The *S. mutans* (CFU/ml x10⁴) count has an average of 1.7 and standard deviation 2.02, unlike the PI, the deviation is greater due to the variation between the minimum and maximum values of 0.1 and 7.7 respectively.

The dispersion diagram illustrated in Figure 1 shows the correlation (intensity of association) between the variables, and from this it can be seen that the correlation between the variables is small (Table 3) which demonstrates the degree of correlation between the Plaque Index (PI) and the *S. mutans* count (CFU/ml x10⁴).

The coefficient of linear regression (*r*) between the two variables is 0.342, which indicates a weak, though positive correlation; the coefficient of determination (*r*²) is 0.117 and it is estimated that just 11.7% of the variability in the Plaque Index (PI) is explained by the amount of *S. mutans* (CFU/ml x10⁴).

Based on these results, it is possible to see that the variables do not have a very strong linear association, i.e. the existing Plaque Index for schoolchildren is not associated with the quantity of salivary microorganisms of the *S. mutans* type (CFU/ml x10⁴).

Table 1. Distribution of schoolchildren according to sex, age and OHI-S levels.

	Age and OHI-S					
	Age 6 - 7		Age 8 - 9		Age 10 - 12	
Sex	n	OHI-S	n	OHI-S	n	OHI-S
Female	4	1.4	2	2	4	1.7
Male	4	1.6	5	1.5	6	1.8

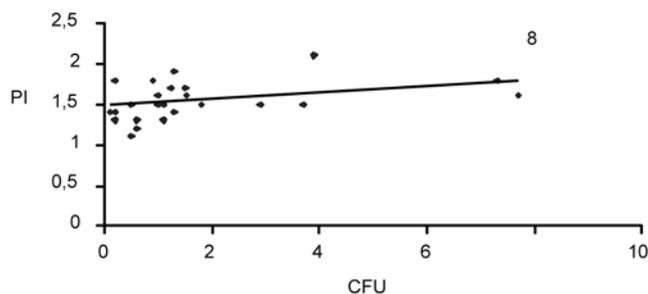
Table 2. Descriptive analysis of the Plaque Indices and the *S. mutans* Count (CFU/ml x10⁴). Campina Grande (PB), 2009.

	n	Minimum	Maximum	Mean	SD±
PI	25	1.1	2.1	1.552	0.23473
CFU	25	0.1	7.7	1.695	2.01505
n valid	25				

Table 3. Model of Linear Regression between the Plaque Index and the *S. mutans* Count (CFU/ml x 10⁴). Campina Grande (PB), 2009.

R	R2	Adjusted R2	Std. Error of estimate
0.342	0.117	0.079	0.225

CFU is independent

**Figure 1.** Relationship between the Plaque Indices of each school child with the number of Colony Forming Units of *S. mutans* (CFU/ml x 10⁴). Campina Grande (PB), 2009.

DISCUSSION

The majority of studies point to the Streptococcus of the mutans group as being the main etiological agent of dental caries and this microorganism can be linked to non-oral infections, principally bacterial endocarditis^{2,7,9}.

In the present study, 25 schoolchildren, aged between 6 and 12, were examined with the aim of measuring the indices of Simplified Oral Hygiene (OHI-S) and Bacterial Plaque (PI) and correlating them with the evaluation of the microbiological salivary levels of *S. mutans* (CFU/ml). As far as gender is concerned, 15 (60%) of the schoolchildren were male and 10 (40%) female.

The overall OHI-S mean was 1.7. The majority of the schoolchildren examined (60%) presented OHI-S scores between 0 and 1.9 (< 2); the next largest group presented scores of between 2 and 3. By correlating the OHI-S with sex, it was found that a larger number of schoolchildren of the female sex presented OHI-S of between 2 and 3 (≥ 2), the mean being 0.68, and a higher level of OHI-S was found in the 8 to 9 age range. As far as the male sex is concerned, the majority presented OHI-S between 0 and 1.9 (mean of 1.72), and schoolchildren in the 10 to 12 age range presented a higher level of OHI-S. These results were high in comparison with another study in which those schoolchildren examined in the 10 to 12 age range presented OHI-S ≥ 2 in 50% of the sample¹¹.

The quantitative analysis of the number of Colony Forming Units of *S. mutans* in the schoolchildren studied showed a variation of 0.1 x 10⁴ CFU/ml to 7.7 x 10⁴ CFU/ml, and these data, correlated with the PI index, show a weak correlation in which just 11.7 % of the Plaque Index (PI) variability can be explained by the quantity of *S. mutans* (CFU/ml x10⁴). Accordingly, it was demonstrated that the Plaque Index present in the schoolchildren is not linked to the quantity of salivary microorganisms of the type *S. mutans* (CFU/ml x10⁴). These results contradict the study by Almeida et al.¹¹ who verified, through a colorimetric microbiological analysis of 21 schoolchildren, the lack of a relationship between caries activity and levels of *S. mutans*, but which diverges from another two studies found in the literature which show a strong association in the results¹².

This weak correlation may be explained by the use of fluoridated dentifrices around three times a day, thereby promoting an adaptation of the oral environment, with the control of bacterial flora. According to Rodrigues & Guedes-Pinto¹², the correct hygiene to be practiced after meals reduces the buildup of bacterial plaque, and consequently prevents the cariogenic action by the plaque.

CONCLUSION

Given the results presented above, we may conclude that the schoolchildren evaluated presented deficient oral hygiene conditions, where the majority (50%) of those in the 10 to 12 age range presented OHI-S ≥ 2. The oral hygiene of the schoolchildren in the study is normal, presenting a moderate Bacterial Plaque index, which demonstrates the need for greater care with oral hygiene, by means of educational preventive programs.

The study found no relevant link between the numbers of *S. mutans* (CFU/ml x10⁴) and the OHI-S and PI indices in the schoolchildren, which may be explained by the reduced sample numbers, by the absence of any association of these numbers with the daily diet of the study participants or by the difference in the products used (fluoridated or not fluoridated) in the daily oral hygiene routine.

Collaborators

JV PEREIRA e DQC GOMES were responsible for guidance and the wording of the article. PKO SILVA was responsible for statistical analysis and wording of the article. RS OLIVEIRA, GTG FLORINDO e RO COSTA were responsible for the bibliographic survey, data collection and wording of the article.

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