Caries risk in children: determined by levels of mutans streptococci and Lactobaccilus

Luis Alejandro Aguilera Galaviz* / Gloria Premoli** Anajulia Gonzalez*** / Rafael Aguilar Rodriguez****

Lactobacullus sp. and S. mutans are microorganisms with cariogenic capacity, however, their presence do not determine the presence of dental caries. We evaluated the relationship between the presence of Lactobacillus sp. and S. mutans and dental caries in a schoolchildren population. The relation PI-DMFT have a value of significance p = 0.001489. In dental caries risk evaluation, the S. mutans and Lactobacillus sp. detection in saliva is a good predictor and contributing to the caries development.
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INTRODUCTION

The factors related to the development of dental caries are of great relevance. The microorganisms with cariogenic capacity do not determine the presence of dental caries. It is necessary to have suitable substrates and the physiological conditions in the host to allow implantation and survival of the microorganisms, and finally, the development of caries. The activity of caries is associated with the presence of Lactobacillus sp. and Streptococcus mutans in dental plaque and saliva. This kind of flora produces low pH in the plaque and subsequent demineralization of the tooth.[1] Other studies in children with 6 to 36 months age, showed the predominant species in the teeth with caries are S. mutans and A. Israel and secondly S. sobrinus and A. actinomycetemcomitans.[2]

* Luis Alejandro Aguilera Galaviz, Instituto de Investigaciones Odontológicas, Unidad Académica de Odontología. Universidad Autónoma de Zacatecas, México.
[2] Centro de Investigaciones Odontológicas, Facultad de Odontología. Universidad de los Andes Venezuela.
** Gloria Premoli, Centro de Investigaciones Odontológicas, Facultad de Odontología. Universidad de los Andes Venezuela.
***Anajulia Gonzalez, Centro de Investigaciones Odontológicas, Facultad de Odontología. Universidad de los Andes Venezuela.
**** Rafael Aguilar Rodriguez, Instituto de Investigaciones Odontológicas, Unidad Académica de Odontología. Universidad Autónoma de Zacatecas, México.

Send all correspondence to Dr. Luis Alejandro Aguilera Galaviz, Paseo a la Feria Edificio Primavera, Torre A Apt. A 41, Merida, Venezuela, CP. 5101.

Voice: 58 274 8084215
E-mail: aguileragalaviz@hotmail.com

Lactobacullus sp. and *S. mutans* are not competing in the ecosystem. They have a complex relationship. Data generated from mixed cultures showed the coexistence and establishment of synergic activities between these two species.[3] Studies with populations of children under dental control have been associated the presence of these microorganisms and dental caries, demonstrating that the applications of fluoride and the hygiene considerably diminish the number of mfc of both microorganisms.[4]

On the other hand, the prevalence of dental caries in populations on the basis of genetic background, do not explain the susceptibility or resistance to implantation of potentially cariogenic bacteria. The evidence suggest that dental caries is a multifactorial disease, where there is relationship between genes of the immune system (HLA haplotypes), the enamel alterations, diet, hygiene, caries experience, even within people with the same genotype.[5]

Finally comparing populations with caries and caries-free, it is observed that the patients with better hygiene habits present low concentration of *Lactobacillus sp.*, and the patients with active caries present bacteria with greater capacity of adhesion to hydrophobic substances, greater agglutination in the presence of salts and less production of inhibiting substances for other species.[6] It is suggested in dental caries risk evaluation to determine the qualitative characteristic of dental plaque flora and their components. Also, the adhesion of *S. mutans* by means of antigen Ag I/II represent pathogenic mechanisms, and increases the risk to decay.[7] The aim of this research is to establish the relationship between the presence of *Lactobacillus sp.*, *S. mutans* and the presence of dental caries in a schoolchildren population of Zacatecas, Mexico

The study had 150 schoolchildren of both sexes, low

middle class status (data provided from socioeconomical evaluation by the school administration), from the elementary school General Enrique Estrada, with a total population of 547 students. It is located in the urban zone of the city of Zacatecas, Mexico, and was randomly selected. The ages of the children ranged between 10 to 13 years. Children were eliminated from the study if they used antibiotic therapy or syrups or suspensions drugs administration with high carbohydrate content during, and a month before the beginning of the study.

Determination of Decaying Missing Filled Teeth (DMFT) and Plaque Index (PI) was done using the standard diagnostic criteria between the dentists, who participated in this study. They checked ten children three times and the results were analyzed calculate to the coefficient of variation between the group and establish an agreement value of 85-95%. Once standardized, the criteria for DMFT and the registry were in agreement with the criteria established by the World Health Organization.[8] The dental plaque index used the criteria established by Green and Vermillion.[9]

Streptococcus mutans levels in saliva

S. mutans levels in saliva was done using Dentocult SM (Orion, Diagnostica, Finland), following the instructions of the manufacturer. A disc impregnated with bacitracin, within the tube that contain selective culture media for *S. mutans*. To sample saliva the strip was rotate 10 times on the surface of the tongue and put it into the tube with media, and incubated at 37°C during 48 hours. The results of the strip were compared with the chart of the manufacturer. The data were codified as follows: code 0 and 1 is $<10^5$ ufc/ml, code 2 is $>10^5 < 10^6$ ufc/ml , code 3 is $>10^6$ mfc/ml *S. mutans* in saliva.

Lactobacillus sp. detection

The presence of *Lactobacilos sp.* in saliva was done with Dentocult LB (Orion, Diagnostica Finland) in agreement with the instructions of the manufacturer. The salivary glands were stimulated by chewing parafilm and the saliva was collected in a conic tube (Corning 15ml). The saliva sample was cultured at 37°C for four days and the readings were done comparing the culture against the chart in the kit. The samples were coded as follows: code 0 is 10^3 mfc/ml, code 1 is 10^4 mfc/ml, code 2 is 10^5 mfc/ml, code 3 is 10^6 mfc/ml *Lactobacilus* in saliva.

Saliva buffer capacity

To determine the flow rate and buffer capacity of saliva, the patient was told not eat food one hour before the test. The stimulation of the salivary glands was done by chewing parafilm for 5 minutes. Then, the saliva was collected in a tube to take the reading and reported in ml/min. After that buffer capacity was determined using Dentobuff Strip (Orion Diagnostica, Finland). The results of the strip were codified according to the following scheme: blue is code 0 with High buffer capacity (pH ≥ 6.0), Green is code 1 with adequate buffer capacity and Yellow is code 2 with low buffer capacity (pH < 4).

Statistical methods

The collected data from the DMFT, PI, microbiology test, buffer capacity and flow rate was codified as described before and registered. The analysis was made using the ANOVA test in EPINFO 6.0. The variables were expressed in terms of the mean.

RESULTS

One hundred and fifty children examined of both sexes between 10 to 13 years old with a mean of 11.26 years old and standard deviation of 0.77, from the total were 45.4 % females and 54.6% males. Caries prevalence was recorded according with "WHO oral health surveys," the DMFT by sex was calculated and the relation between DMFT and sex determine was as follows 128 for males and 188 for females. Considering the relation male/females the DMFT was greater for females than males. The percentage distribution according with the DMFT was 44.66 % presented zero, 14% equal to 1, 12% a DMFT of 2, 11.33% a DMFT of 3, 10.66 % of 4 and the 7.32 % with a value bigger or equal to 5. (Figure 1)

In our population study 73.3% have a Plaque Index of 2, 18.66% of 1 and 7.33 equal to 3. The relation between PI-DMFT have a value of significance p = 0,001489, a= 0.05 (Table 1)

Table 1. Relation between DMFT and Plaque Index

Plaque Index	*N*	*%*	*DMFT Mean*	*SD*	
0	*0*	–	–	–	*ANOVA*
1	28	19	1.00	1.51	p = 0.001489
2	111	74	1.58	1.79	*a* 0.05
3	11	7	2.73	2.05	
Total	150	100			

We analyzed the buffer capacity in order to know if it represented a key factor. Children with adequate buffer capacity (code 1) presented a DMFT of 1.8 compared to those in which the buffer capacity was high (code 0), nevertheless, there is no significant statistical association for this group (Table 2).

Table 2. Buffer capacity according to DMFT.

Buffer Capacity	*N*	*$*	*DMFT Average*	*Standar Deviation*	*ANOVA*
0	142	95	1.54	1.82	p = 0.4893
1	8	5	1.88	1.88	*a* 0.05
Total	150	100	1.55	1.8	

Figure 1

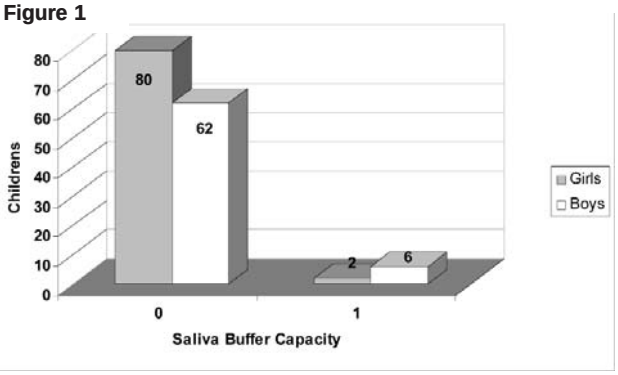

Figure 1. Distribution according with buffer capacity and gender.

Figure 2

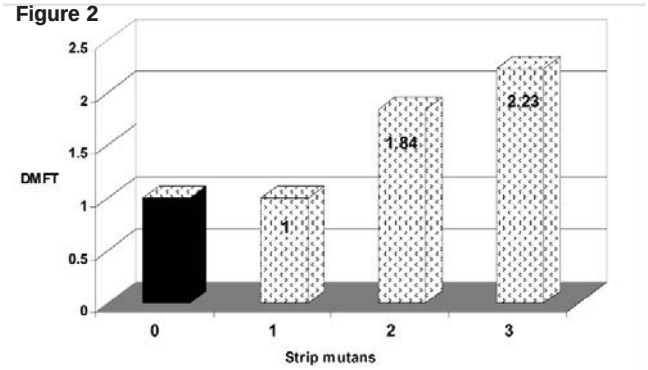

Figure 2. Mean DMFT distribution in relation with *S. mutans* concentration in ufc/ml. ANOVA p = 0.00134; *a* 0.05

The relationship between the DMFT and *S. mutans* concentration in saliva, found that 41.33 % has $>10^5 < 10^6$ mfc/ml *S. mutans* in saliva 2 (see Table 3).

Table 3. *S. mutans* distribution according to number of patient examined.

S.mutans code	*N*	*%*
0	28	18.66
1	35	23.33
2	62	41.33
3	25	16.66
Total	150	99.98

Those with 10^6 mfc/ml of *S. mutans* had the DMFT bigger than those with less than 10^3 mfc/ml of *S. mutans*. (Figure 2).

The relationship between the presence of *Lactobacillus sp*. and dental caries was expressed as the mean of DMFT in relation to mfc/ml of *Lactobacillus sp.* in saliva. It was found that those with more than 10^6 mfc/ml present a DMFT of 2, and those with 10^3 mfc/ml present a DMFT of 1.33. The association between the *Lactobacillus sp.* concentration and DMFT have a low statistical significance p=0.037606; a0.05 (Figure3).

Finally, we found *Lactobacillus sp.* in 64% of the population of study the most of them were code 2 and only 36% do not have any growth or less than 10^3 mfc. (Table 4)

Table 4. *Lactobacillus sp* distribution according to number of patient examined.

Lactobacillus code	*N*	*%*
0	54	36
1	33	22
2	38	25.33
3	25	16.66
Total	150	99.99

Figure 3

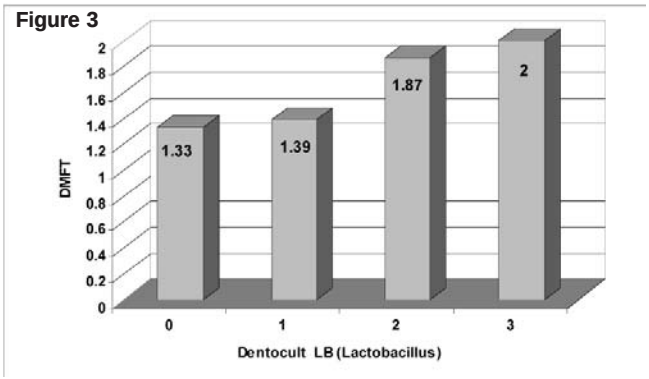

Figure 3. Relation between Lactobacillus and the mean of DMFT, ANOVA p = 0.037606; *a* 0.05.

DISCUSSION

Oral disease is a major public health problem due to the high prevalence and incidence in all regions of the world and the greatest impact on the socially marginalized populations.[10] The evaluation of caries risk is important in children. It represented an opportunity to improve hygiene, diet, and the implementation of prevention programs with flour applications, sealants and periodic examination and lifestyles changes in a exposed population. We evaluated 150 schoolchildren ages between 10 to 13 years old for caries experience in relation with of *S. mutans* and *Lactobacillus sp.* In this study, it was found that the whole group has a mean of DMFT of 1.58 and the caries free population is 44.66%. These findings represent a low caries experience compared with others studies done in Mexico City, which have reported a variation between 2.5-5.1.[11,12] The World Health Organization reported that in Mexico City for 12-year-old children a DMFT of 2.7 to 4.4, which was higher than our findings.[10]

The caries risk evaluation must take several factors into consideration. One of them is the presence of *S. mutans* considered the main etiological agent in dental caries. In the present study, it was found that most of the population is positive and only 18.66% of the populations have less than 10^5 mfc/ml of *S. mutans*. Considering the prevalence of these microorganisms, it was expected to have more caries. Our data are in agreement with those founding by Ramos-Gomez *et al*.[13] in which children with early childhood caries have a high levels of *S. mutans* in saliva compared with dental healthy population.

Lactobacillus sp. is considered a factor in determining the predisposition to develop cavities as a first step in colonization, interact with other microorganisms in the adherence to oral tissues. In our findings 36% of people have less than 10^3 mfc/ml of *Lactobacullus sp.* and only the 16.66 % have more than 10^6 mfc/ml. The number of lactobacilli was lower in saliva of subjects with good oral health, and with statistical significant between *Lactobacillus sp.* and DMFT. These findings are in agreement with Branbilla *et al.*[14] They found that 21% of the people from 9 to 13 year old presented *Lactobacillus sp.* in saliva, which is similar in our study, and also in terms of the correlation between DMFT and *Lactobacillus sp.* in saliva.

When we evaluated the buffer capacity, we did not find significant relevance as a protector factor, in our group all of them have high or adequate buffer capacity, and in relation to DMFT no statistical significant was found (p= 0.4893).

Accordingly with our study, the most significant parameters evaluated are the SIP, *S. mutans* and *Lactobacillus sp.* in relation to DMFT. In this context, the evaluation of this microorganism in saliva, represent a tool for the evaluation of caries risk. As described by previous studies, the combination of various tests is essential to obtain a final diagnosis, considering the multifactorial etiology of dental caries.[15]

In the present study, it is important to notice the source of the population. In a previous study done by Irigoyen *et al.*[10] they were trying to find a relation between the social status and the caries experience. They found a DMFT of 2.5 to 5.1 in a nine to twelve year old population, which is similar with our data for the same population and range of age and social status.

Lactobacillus sp. and *S. mutans* play a role in the formation of biofilms and *S. mutans* promote the growth of this specie.[16] Because of the acidogenic and acidophilic properties of those species, and the maintenance of pH balance in dental plaque to avoid the demineralization of teeth, it is important to evaluate the presence of both species. It is controversial whether to evaluate the presence of these microorganisms in plaque or saliva. However, it was demonstrated that plaque samples collected from four proximal surfaces at two interdental spaces in the primary molars, were significantly better than detecting *S. mutans* and *Lactobacillus sp.* in saliva.[17]

However, we demonstrated that in the case of dental caries risk evaluation with children at the age of our population of study, it is better to use a less time consuming strategy, with an accurate result. Testing *S. mutans* and *Lactobacillus sp.* in saliva is easy, fast, and it is an important predictor to dental caries risk.[18] Our results show a significant correlation among DMFT and *S. mutans* in saliva (p= 0.00134) also in the case of *Lactobacillus sp.* the significantly is high (p= 0.03760). For caries risk evaluation, saliva sampling and the evaluation of several parameters related with dental caries included the clinical judgment are powerful tools for caries risk evaluation and activity determination, in addition to the evaluation of diet, saliva flow, buffer capacity. Also, Koga *et al.*[19] propose that the immune response plays an important role in a clinical study about dental caries, in which high levels of IgG antibodies in serum against *S. mutans* and *Lactobacillus sp.* have a correlation with low dental caries.

CONCLUSION

For dental caries risk evaluation, *S. mutans* and *Lactobacillus sp.* detection in saliva is a good predictor contributing to the caries development.

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