

Dental Caries and Level of Cariogenic Bacteria in Preschool Children and their Mothers, Residents in Lima-Peru

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Abstract

Dental caries is the result of bacterial metabolic activity in dental biofilm with the bacterium *Streptococcus mutans*, identified as the primary etiological factor for dental caries and *Lactobacillus* plays an important role in the progression of the disease. The purpose of the study was to evaluate the level of cariogenic bacteria, *S. mutans* and *Lactobacillus* in 30 children and their respective mothers. Children affected with dental caries had high levels of *Streptococcus mutans* in relation to children free of dental caries (60% and 20% respectively), however, the difference was not significant for *Lactobacillus*. Both children with dental caries and without dental caries had mothers with high levels of *Streptococcus mutans*.

Keywords: *Streptococcus mutans*; *Lactobacillus*; Dental caries; Saliva; Cariogenic bacterium; Transmissible

Introduction

The human fetus inside the uterus is in a sterile condition, but as soon as it passes through the birth canal, it acquires microorganisms from the vagina and fecal microorganisms [1]. The colonization of the sterile oral cavity begins from the moment of birth. After a few hours, the sterile cavity is colonized by facultative and aerobic bacteria, although in low numbers. On the second day, anaerobic bacteria can also be detected in the infant's edentulous mouth. The number of oral bacteria gradually increases as a result of exposure to external environmental microbial sources [1]. *Streptococcus mitis* have been identified as the first dominants to colonize the oral cavity of newborns. *Actinomyces* spp. and *Staphylococcus* spp. they are also initial occupants of the oral cavity. After dental eruption, a more complex oral microbiota is established. Species that settle in and attack deciduous teeth after eruption include *Streptococcus*

sanguinis, *Lactobacillus* spp. and *Streptococcus oralis*. In the first year of life, *S. mutans* and *Streptococcus sobrinus* [1]. Dental caries is the result of bacterial metabolic activity in the dental biofilm. The latest national study of oral health in Peru, carried out by the Ministry of Health between 2012 and 2014, shows that the presence of dental caries in children with deciduous dentition is 59.1% [2]. In this way, dental caries behaves as one of the most prevalent infectious diseases in humans and, at the same time, it is one of the main public health problems worldwide.

The development of dental caries requires as a prerequisite the existence of microbial communities on the surface of the tooth. However, its mere presence in the teeth is not always enough to show visible signs of dental caries. Other factors are quite involved in the progression of this disease [3]. Indicators of risk,

associated with early childhood dental caries, include colonization with *S. mutans*, poor tooth brushing and frequent consumption of sugar. Maternal psychosocial and behavioral factors are known to influence a child's risk for many conditions; however these factors have not been sufficiently addressed in the etiology of early childhood dental caries [4]. Berkowitz and Jordan, in 1975, suggested for the first time the possibility of transmission of microorganisms from the mother's saliva to the child. To do this, they used the mutacin typing method and demonstrated that the microorganisms in the samples taken from the children's mouths were similar to those found in the mothers' mouths [5]. In multiple investigations, an identical pattern of chromosomal DNA has been recorded in the bacteria of children and their mothers, such as in the study carried out by Berkowitz [6], in which he compared the production of bacteriocin by *S. mutans*, isolated from the mouth of 20 pairs of mothers and children and concluded that the correspondence of microorganisms was statistically remarkable. Likewise, in molecular typing experiences it has been verified that the acquisition of oral *Streptococcus* and Gram-negative species in a child's biofilm comes predominantly from his mother (vertical transmission). Children's *S. mutans* genotypes were found to be identical to their mothers in 71% of 34 infant-mother pairs, while no evidence of father-child transmission was detected [3]. In addition, research shows a high correlation between colonization by *S. mutans*, and the number of teeth present in the child's oral cavity, since this placement depends on the appearance of retentive areas. Thus, the greater the number of teeth and, consequently, the greater the age of the children, the greater the probability that colonization by *S. mutans* will increase successfully [7].

Also, other microorganisms participate in the process. Within these, *Lactobacillus* play a leading role in the progression of dental caries. It is the case that the absence of retentive sites in the teeth is not an obstacle for them to lodge in any sporadic interstice of the oral cavity. *Lactobacillus* are capable of producing large amounts of acids at a low pH, forming a highly acid dental plaque that favors dental demineralization, due to the presence of sucrose and cariogenic carbohydrates, as well as due to the porosity of the plaque matrix. dental plaque possesses [8]. However, these bacteria have shown minimal contagiousness between individuals and there is little correlation between their presence in the saliva of the mother/child pair. Therefore, it is generally assumed that the increase in the frequency of *Lactobacillus* is associated with the constant ingestion of carbohydrates [9]. The *S. mutans* count is used as a diagnostic aid to select patient groups at risk for dental caries. Counts higher than 100,000 CFU / ml of *Streptococcus*, in saliva, are considered indicators of dental caries risk, lower counts agree with a minimal tendency to contract the disease [10,11]. Although it is recognized that dental caries has a multifactorial origin, where sociodemographic, psychosocial and behavioral factors may participate [12], the microbial factor, associated with aciduric and acidogenic bacteria, would be increasing the risk of caries in children and adolescents, being the species of *S. mutans*, *Streptococcus sobrinus*, *Lactobacillus* spp and some species of

Candida, isolated in the saliva of these patients, which would have the greatest influence [12-14]. Thus, it has also been possible to determine that in the oral cavity of a baby, *S. mutans* increases its presence from 30% at 3 months of birth to 80% at 24 months in children with primary dentition, with transmissibility being decisive in this case. by the mother. The risk of dental caries in children is greater when a mother has a high level of *S. mutans* in her saliva, as well as if she is accustomed, due to ignorance, to frequent inoculation [15]. Therefore, the developed study aims to determine the relationship between dental caries and the level of cariogenic bacteria in preschool children and their mothers.

Materials and Methods

An analytical, cross-sectional and comparative case-control study was carried out, which included 30 children from 3 to 5 years of age and their respective mothers. Oral examinations were performed on all of them and unstimulated saliva samples were taken. The children were preschoolers from the Tupac Amaru Educational Center in the district of La Victoria in Lima-Peru, who met the following inclusion criteria: not presenting systemic disease two months prior to the study and having the informed consent of the parents for the participation of their children. Children receiving or having received antibiotic therapy were not admitted, in the form of syrup with a high sugar content and/or iron supplements in the last 6 months, including drugs that interfere with salivary secretion, as well as those who had undergone dental prophylaxis within the last month from the date of completion of the procedure inclusion test. Clinical dental check-ups were performed by a single previously trained investigator. Artificial light, flat dental mirrors No. 5, explorer, cotton forceps were used. The presence or absence of dental caries in the children (color changes and cavitated lesions in the teeth) and the index of teeth with dental caries, missing and filled (DMFT) of their respective mothers were recorded.

For the determination of *S. mutans* levels, 3 ml of unstimulated saliva was obtained from each child and each mother. The samples were deposited in sterilized polypropylene millimeter test tubes and were taken to the laboratory at 4 °C. They were processed within two hours of collection. The sowings were carried out by placing, using 0.1 ml micropipettes, 2 ml of mitis salivarius broth with bacitracin in test tubes, which were then incubated on an inclined plane at 37°C. After 24 hours, the readings taking into account the number of adherent colonies. For the determination of *Lactobacillus* levels, we used the Rogosa SL11 agar system, supplemented with 1.32 mL of glacial acetic acid. The bivariate analysis of the results was carried out using the Person's Chi square test, based on the contingency tables prepared. The research work complied with the protocols and permits of the Directorate of the Graduate School of the Federico Villareal National University and was carried out in 2019.

Results

Most children with dental caries had high levels of *S. mutans*, while most children without dental caries had low levels of

these microorganisms. It was noted that there was a statistically significant relationship ($p < 0.05$) between the level of *S. mutans* and the presence of dental caries in children (Table 1). The majority of children with dental caries showed low levels of *Lactobacillus*, observing that all children without dental caries also showed low levels of this microorganism. It was verified that there was no statistically significant relationship ($p > 0.05$) between the level of *Lactobacillus* and the presence of dental caries in children (Table 2). Most children with and without dental caries had mothers with high levels of *S. mutans*. It was found that there was no statistically significant relationship ($p > 0.05$) between the existence of dental caries in children and the levels of *S. mutans* in their mothers (Table

3). Most children with and without dental caries had mothers with low *Lactobacillus* levels. It was found that there was no statistically significant relationship ($p > 0.05$) between the manifestation of dental caries in children and the levels of *Lactobacillus* in their mothers (Table 4). The majority of children with and without dental caries had mothers with high levels of *S. mutans* and the majority of children with and without dental caries had mothers with low levels of *Lactobacillus* (Table 5). The average DMFT of mothers whose children had dental caries was noted to be 9.5 ± 5.2 , while the DMFT of mothers without dental caries was 3.5 ± 2.2 . There was a statistically significant difference ($p < 0.05$) in the DMFT of both groups of mothers (Table 6).

Table 1: Relationship between the level of *S. mutans* and the presence of dental caries in children.

S. mutans levels	Presence of dental caries	
	Yes	No
Low	6 (40%)	12 (80%)
High	9 (60%)	3 (20%)
Total	15(100%)	15 (100%)

Pearson chi square = 5

p-value = 0.03

Table 2: Relationship between the level of *Lactobacillus* and the presence of dental caries in children.

Lactobacillus levels	Presence of dental caries	
	Yes	No
Low	12 (80%)	15 (100%)
High	3 (20%)	0 (0%)
Total	15(100%)	15 (100%)

Pearson chi square = 3.33

p-value = 0.07

Table 3: Relationship between the presence of dental caries in children and the levels of *S. mutans* in their mothers.

S. mutans levels of mothers	Presence of dental caries in children	
	Yes	No
Low	4 (26.7%)	5 (33.3%)
High	11 (73.3%)	10 (66.7%)
Total	15 (100%)	15 (100%)

Pearson chi square = 0.16

p-value = 0.69

Table 4: Relationship between the presence of dental caries in children and levels of *Lactobacillus* in their mothers.

Mothers' Lactobacillus levels	Presence of dental caries in children	
	Yes	No
Low	10 (66.7%)	14 (93.3%)
High	5 (33.3%)	1 (6.7%)
Total	15 (100%)	15 (100%)

Pearson chi square = 3.33

p-value = 0.07

Table 5: Relationship between the level of *S. mutans* and *Lactobacillus* from mothers according to the presence of dental caries in children.

Presence of dental caries in children	S levels . mothers mutans		Mothers' Lactobacillus levels	
	Low	High	Low	High
Yes	4 (26.7%)	11 (73.3%)	10 (66.7%)	5 (33.3%)
No	5 (33.3%)	10 (66.7%)	14 (93.3%)	1 (6.7%)

Table 6: Relationship between the presence of dental caries in children and the DMFT index in their mothers.

Presence of dental caries in children	DMFT of mothers
Yes	9.5 ± 5.2
No	3.5 ± 2.2

Kolgomorov-Smirnov test: $p > 0.05$

Shapiro-Wilk test: $p > 0.05$

Levene's test: $p > 0.05$

Student's t-test = 3.8

p-value = 0.001

Discussion

The main microorganism responsible for dental caries at the beginning is *S. mutans*, but in the progression of the disease it is *Lactobacillus* that predominates. Studies indicate that the early colonization of the child's mouth by *S. mutans* is through adult saliva. These bacteria can be quantified in saliva, so their counts have been used in numerous studies to relate the prevalence and incidence of dental caries. In the present research work, the relationship between the presence of dental caries and the level of cariogenic bacteria in preschool children and their respective mothers, residing in Lima-Peru, was evaluated. High degrees of infection by *S. mutans* ($>10^6$ CFU/ $>10^5$ ml/saliva) mean a high risk of dental caries and transmission of the microorganism [16]. Regarding the relationship between the level of *S. mutans* and the presence of dental caries in preschool children, we found that the group of children with dental caries had higher levels of *S. mutans* compared to the group of children without dental caries. (60% and 20% respectively). These results agree with Al Shukairy et al. who found that children with dental caries had higher levels of *S. mutans* compared to the group of children without dental caries (80% and 16.7% respectively), observing quite similar results [17]. Ge et al. also concluded that the severity of dental caries in children was related to the levels of *S. mutans* [18]. Gispert et al., in a longitudinal study, found that children with a pronounced level of *S. mutans* infection had a higher incidence of dental caries compared to the other control group, corroborating the correspondence between the level of infection and the activity of dental caries. Therefore, this conclusion is of considerable value in the prognosis of dental caries risk [19]. Regarding the relationship between the level of *Lactobacillus* and the presence of dental caries in preschool children, we found that the group of children with dental caries had higher levels of *S. mutans* compared to the group of children

without dental caries, but these differences were not statistically significant (20% and 0% respectively). Al Shukairy et al., found that children with dental caries had higher levels of *S. mutans* compared to the group of children without dental caries (60% and 13.3% respectively), these differences were statistically significant [17].

Another objective was to evaluate the relationship between the presence of dental caries in children and the levels of *S. mutans* and *Lactobacillus* in their mothers. We noted that the majority of children with and without dental caries had mothers with high levels of *S. mutans*, we did not see significant differences, agreeing with Al Shukairy et al. Similarly, there were no significant differences between the presence of dental caries in children and the levels of *Lactobacillus* in their mothers, since most children with and without dental caries had mothers with low levels of *Lactobacillus*. Regarding the presence of dental caries in children and the DMFT index in their mothers, we found that the DMFT of mothers whose children had dental caries was higher than that of mothers of children without dental caries (DMFT 9.5 and 3.5 respectively), with statistically significant differences. We agree with Ersin et al., who observed that the DMFT index of mothers was a factor for the development of dental caries in children. They also mention a great relationship between maternal education and the colonization of *S. mutans* [20]. Various investigators have evaluated other cariogenic bacteria such as *Streptococcus sobrinus*, *Streptococcus sanguinis*. So Ge et al. conclude that the interaction of *Streptococcus sobrinus* with *S. mutans* is a significant factor associated with the state of dental caries in children, suggesting that the levels of these two microorganisms play an important role in the development of dental caries [18]. It is valid to mention that other researchers have also tried to relate the transmission from the mother to her children. We will cite a few.

In a study with the participation of 16 mother-child pairs to analyze the colonization profile of *S. mutans* and *Streptococcus sobrinus*, in the oral cavity of children, over a period of 20 months, they concluded that the genotypes of *S. mutans* and *S. sobrinus* acquired from maternal or alternative sources may show effective persistence in the oral cavity and/or transient detection in the mouth of children [21]. In order to find out more evidence of the transmission of *S. mutans* from caries-free mother to child, Ravikumar D. et al. developed a study to identify the genetic characteristics of *S. mutans* strains isolated from mothers free of dental caries and children with active caries and without active caries from 3 to 6 years of age. We found that the *S. mutans* were closely related and could have been transmitted vertically from their mothers [22]. In a well-known work on the salivary microbiome, it was concluded that in the first two years of life it is dynamic in nature since it is influenced by different factors such as the mother's state of health, the type of feeding of the child (breastfed or no), including environmental factors. All these situations can intervene in the assembly of the salivary microbiome in childhood [23]. Damle SG. et al. in an investigation on the transmission of *S. mutans* in mother-child pairs, they pointed out as a conclusion and suggestion the existence of a vertical transmission from mother to children. They determined that feeding habits, gum cleanliness, and the number of erupted teeth in children had a significant effect on *S. mutans* colonization [24].

Conclusions

- a. There is a relationship between the level of *S. mutans* and dental caries in children. (Most children with dental caries: high level of *S. mutans*. Most children without dental caries: low level of *S. mutans*.)
- b. There is no relationship between the level of *Lactobacillus* and dental caries in children. (Most children with dental caries: low *Lactobacillus* level. Children without dental caries: low *Lactobacillus* level.)
- c. There is no relationship between levels of *S. mutans* in mothers and their children with dental caries. (Most children with or without dental caries had mothers with high levels of *S. mutans*.)
- d. There is no relationship between the level of *Lactobacillus* in mothers and their children with dental caries. (Most children with or without dental caries had mothers with low *Lactobacillus* levels)
- e. The DMFT Index of mothers of children with dental caries (9.5 ± 5.2) is higher than the Index of mothers of children without dental caries (3.5 ± 2.2). The difference is statistically significant ($p < 0.05$).

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Authorship Contributions

LMC was responsible for the conception and design of the study. All coauthors participated in the analysis of the information, writing, critical review and final approval of the manuscript.

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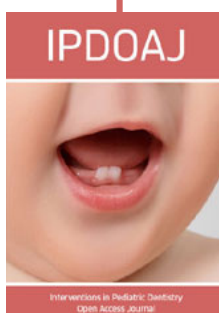
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