

THE BUFFER CAPACITY OF NIGHT SALIVA SECRETION ON STREPTOCOCCUS MUTANS DYNAMICS IN YOUNG CHILDREN: A COMPREHENSIVE STUDY

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ABSTRACT

Introduction Saliva is a clear, watery fluid that is produced by the salivary glands in the mouth. It plays a crucial role in maintaining oral health and facilitating the process of digestion. The bacterial load in nighttime saliva can be influenced by factors such as oral hygiene practices, diet, and overall oral health. **Aim of the study** The aim of the study is to uncover the potential connection between the presence of Streptococcus mutans bacteria and the buffering capacity of saliva, and how this connection impacts the risk of dental caries in children with ages under 5 years old. **Material and methods** Three groups of young patients with ages between 3 and 5 years old were formed during the regular dental check-up, in the Pedodontics Clinic of the Faculty of Dental Medicine, UMFST "G.E. Palade". From each of the we have collected the saliva early in the morning, without the children having their teeth brushed using special sterile plastic cups. We have tested the buffer effect of the saliva using specific test and for the determination of bacterial saliva parameters we used Karies Screen Test. **Results** Group A – 43 (40.18%), included children who presented at least 3 dental caries, group B – 37 (34.57%) of children had 1 or 2 carries, and the control group of children – 27 (25.23%), with intact dentition without carious processes. 39 (36.44%) children had high density of Streptococcus Mutans colonies > 10 units CFU/ml saliva form group A, 35 (32.71%) children presented low to medium density < 10 units CFU/ml saliva from group B and for the group control all 27 (25.23%) children presented very low density < 5 units CFU/ml saliva. The hights buffer capacity scores were obtained for the group control. **Conclusions** Children with higher buffer capacity in their saliva tend to exhibit better oral health, including a lower prevalence of dental caries. The buffering capacity of saliva plays a protective role by neutralizing acids produced by oral bacteria, such as Streptococcus mutans, thus contributing to reduced tooth decay.

Key words: *buffer capacity, saliva secretion, Streptococcus Mutans, caries lesions*

INTRODUCTION

Saliva is a clear, watery fluid that is produced by the salivary glands in the mouth. It plays a crucial role in maintaining oral health and facilitating the process of digestion. Saliva is composed of various substances, including water, electrolytes, enzymes, proteins, and other compounds. The primary function of saliva is to keep the mouth moist, which aids in speaking, chewing, and swallowing. It lubricates the oral tissues, making it easier to move food around in the mouth [1]. Saliva contains

enzymes, such as amylase, which begin the process of breaking down carbohydrates in the mouth. Amylase starts the digestion of starches into simpler sugars, preparing them for further digestion in the stomach and intestines [2].

It has a buffering capacity that helps maintain the pH balance in the mouth. It neutralizes acids produced by bacteria and foods, which can help prevent tooth enamel erosion and dental decay [3,4]. It also contains minerals like calcium and phosphate, which can help remineralize and

strengthen tooth enamel that might have been demineralized by acids. Saliva contains antimicrobial proteins and antibodies that help control the growth of harmful bacteria in the mouth [5]. These components contribute to oral hygiene and protection against infections and also growth factors and enzymes that assist in the initial stages of wound healing within the mouth.

Salivary composition can vary based on multiple factors such as age, hydration, diet, and individual health. Insufficient saliva production, known as xerostomia or dry mouth, can lead to discomfort, difficulty swallowing, and an increased risk of dental problems [6,7].

Conversely, excessive saliva production can also occur due to various reasons, and it might cause drooling or difficulty in speaking and swallowing [8].

Thus, mainly for young children under 5 years old saliva is a critical bodily fluid that contributes to various aspects of oral and overall health, including digestion, oral hygiene, and the maintenance of a healthy oral environment. Saliva secretion during the night in children under 5 years old exhibits several distinct physical properties, which can differ from daytime saliva secretion [9]. Nighttime saliva secretion in young children is often thicker and more viscous compared to daytime saliva. This can be attributed to reduced swallowing frequency during sleep, allowing saliva to pool in the oral cavity and become more concentrated [10]. The flow rate of saliva during the night is generally lower than during the day due to reduced oral activity and swallowing while asleep. This reduced flow rate can contribute to the pooling of saliva in the mouth. Saliva plays a vital role in maintaining oral pH balance. During the night, the pH of saliva can decrease slightly due to reduced salivary flow and buffering capacity, potentially creating an environment that is conducive to

the growth of oral bacteria [11]. Nighttime saliva secretion might have a slightly reduced buffering capacity due to reduced flow and overall activity, potentially contributing to increased vulnerability to acid-related oral issues.

The bacterial load in nighttime saliva can be influenced by factors such as oral hygiene practices, diet, and overall oral health [12]. Children with a higher bacterial load of potentially harmful bacteria like *Streptococcus mutans* might have an increased risk of tooth decay if proper oral care is not maintained [13].

It's important to remember that the physical properties of nighttime saliva secretion can vary widely among individuals and are influenced by various factors. Maintaining good oral hygiene practices, including brushing before bedtime and promoting proper hydration, can help support oral health in young children. Regular dental check-ups are also crucial for monitoring and addressing any oral health concerns [14].

The study aims to uncover the potential connection between the presence of *Streptococcus mutans* bacteria and the buffering capacity of saliva, and how this connection impacts the risk of dental caries in children with ages under 5 years old [15]. The ultimate goal would be to improve our understanding of the underlying mechanisms that influence oral health in order to develop more effective preventive and treatment strategies.

MATERIAL AND METHODS

The clinical trial began on January 5, 2023 and was carried out for a period of 6 months, a period used to form the three groups (batches) of children: the group of target children and the control group (control) after, receiving the Decision of the Scientific Research Ethics Commission

no.2425/2023. These groups of young patients were formed during the regular dental check-up, together with their parents, in the Pedodontics Clinic of the Faculty of Dental Medicine, UMFST "G.E. Palade".

To start the study, we obtained the written approval of the parents, after informing them and presenting the working protocol, the hypothesis and the purpose of the research. The clinical examination and data recording was performed by specialized dentists present in the clinic together with Dr. Stoica Oana, Pedodontics specialist.

We consulted a number of 129 children, both boys and girls, who had to fit the following inclusion criteria: ages between 3 and 5 years,

the presence of temporary teeth in the full formula (maximum deviation of 2 teeth), a cooperative behavior, the signed consent of at least one parent and the possibility of returning to the consultation and saliva collection. Collecting saliva from children, especially young ones, was a bit challenging due to their age and cooperation level.

The saliva was collected early in the morning, without the children having their teeth brushed using special sterile plastic cups following the next steps:

1. Chewing a paraffin capsule for 3 minutes,
2. Swallow saliva in the first minute,
3. Collect the saliva for 5 minutes,
4. 1ml of saliva was put in the plastic bottle with the solution and shake,
5. After 5 minutes we determined the pH value by immersing the measuring strip and comparing it to the recommended standard coloured strip (Fig.1).

RESULTS AND DISCUSSIONS

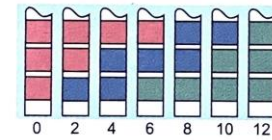
Results

From the total of 129 clinically assessed

Conversion table
Test pad colour at 2 minutes

Green	4 points
Green / Blue	3 points*
Blue	2 points
Red / Blue	1 point *
Red	0 points

Examples



*Where a colour combination provides an unclear result, use intermediate scores.

Interpreting the result. Combined total

Combined total	Buffering ability of Saliva
0-5	Very low 
6-9	Low 
10-12	Normal / High 

Figure 1. Standard coloured strip showing the buffer capacity of saliva.

The determination of bacterial saliva parameters with Karies Screen Test using the producers recommended following steps:

1. Remove the double dip and pull off the foil
2. Wet the side with agar completely with saliva
3. Place NaHCO_3 on the bottom of the tube
4. Two days of incubation time at 37°C
5. Compare with evaluation chart (Fig.2).



Figure 2. Standard low and high density of Streptococcus Mutans colonies.

children both, boys and girls, only 107 met the criteria and were included in our study and were divided into three groups as

follows: group A – 43 (40.18%), included children who presented at least 3 dental caries on 3 different teeth, group B – 37 (34.57%) of children who sporadically had 1 or 2 caries on different teeth, and the control group of children – 27 (25.23%), with intact dentition without carious processes.

For each child included in all three groups, the collected saliva early in the morning was tested for the buffering capacity, following the steps presented previously, recommended by the manufacturer and the results are included in the table 1 and the buffer capacity tests/strips are presented in figure 3.

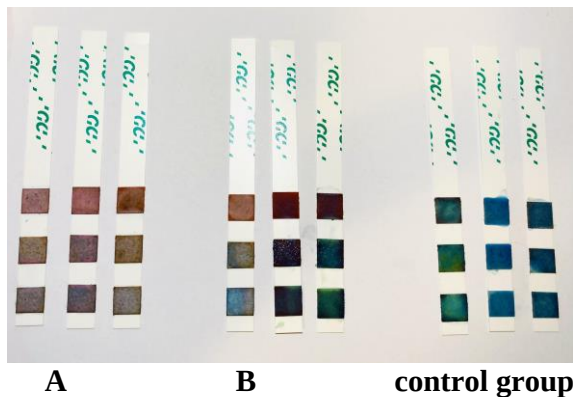


Figure 3. Saliva buffer tests results for all three groups A, B and control group.

For the evaluation of the density for *Streptococcus Mutans* colonies, after two days of incubation time at 37° C, we compared all 107 Karies Screen Test with evaluation chart given by the producer. For the three groups of children included in the study we obtained the following results for group A: 39 (36.44%) children had high density of *Streptococcus Mutans* colonies > 10⁵ units CFU/ml saliva (Fig.4) and 4 (3.73%) children had low to medium density < 10⁵ units CFU/ml saliva. For group B: 35 (32.71%) children presented low to medium density < 10⁵ units CFU/ml saliva (Fig.5) and 2 (1.86) presented very low density < 5⁵ units CFU/ml saliva and for the group control all 27 (25.23%) children presented very low density < 5⁵ units CFU/ml saliva.



Figure 4. High density of *Streptococcus mutans* colonies > 10⁵ units CFU/ml saliva.

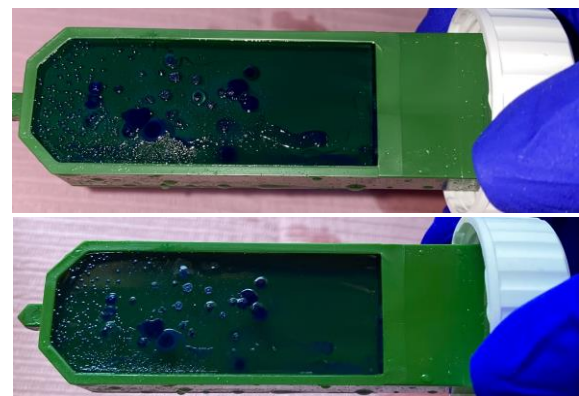


Figure 5. Low density of *Streptococcus mutans* < 10⁵ units CFU/ml saliva.

Discussions

The results of the *Streptococcus Mutans* colony density evaluation after two days of incubation time at 37°C have provided valuable insights into the prevalence of these colonies in different groups of children.

The discussion can focus on the potential impact of saliva's buffering effect on the observed results [16].

Saliva plays a crucial role in maintaining oral health by providing various protective functions, including buffering capacity. Salivary buffering capacity refers to the saliva's ability to resist changes in pH levels in response to the introduction of acids or

bases. This buffering capacity helps to maintain the oral environment within a relatively stable pH range, which is essential for preventing dental caries (cavities) and promoting overall oral health [17].

In your study, the three groups of children (Group A, Group B, and the control group) showed distinct variations in *Streptococcus Mutans* colony density. The density of these colonies is a significant indicator of potential oral health issues, as *Streptococcus Mutans* is a primary contributor to dental caries formation.

Group A had a relatively high percentage of children (36.44%) with high *Streptococcus Mutans* colony density (> 10 units CFU/ml saliva), while Group B had a substantial portion (32.71%) of children with low to medium density (< 10 units CFU/ml saliva). The control group showed a lower overall density, with 25.23% of children having very low density (< 5 units CFU/ml saliva).

The buffering capacity of saliva is relevant to these results because it affects the oral environment's pH [18].

Saliva's ability to buffer against acidic conditions helps in preventing the demineralization of tooth enamel, which is a key step in the formation of dental caries. When the buffering capacity of saliva is compromised, the oral environment becomes more acidic, creating conditions conducive to the growth of acid-producing bacteria like *Streptococcus Mutans*. This can lead to higher colony densities and an increased risk of dental caries [19].

The results you've obtained might indicate

a potential correlation between the buffering capacity of saliva and the observed *Streptococcus Mutans* colony densities. Children in Group A with high colony densities might have reduced salivary buffering capacity, making their oral environment more susceptible to the growth of cariogenic bacteria.

On the other hand, the control group's higher percentage of children with very low colony densities could suggest a healthier oral environment, possibly due to a more effective buffering capacity of saliva [21].

It's important to note that while the buffering capacity of saliva is a contributing factor, other factors such as oral hygiene practices, dietary habits, genetics, and microbial interactions also play significant roles in the development of dental caries. Therefore, a comprehensive understanding of all these factors is necessary to draw more conclusive insights [22].

The observed variations in *Streptococcus Mutans* colony densities among different groups of children may be linked to the buffering capacity of saliva, which influences the oral environment's pH stability. Further research and analysis are needed to fully elucidate the relationship between salivary buffering capacity and the prevalence of *Streptococcus Mutans* colonies, taking into consideration other contributing factors as well.

Combined total	Group A	Group B	Control group
0 - 5 points	1 point – 9 children (8.41%) 2 points – 8 children (7.47%) 4 points – 10 children (9.34%) 5 points – 9 children	-	-

	(8.41%)		
6 - 9 points	6 points – 7 children (6.54%)	8 points – 7 children (6.54%) 9 points – 9 children (8.41%)	-
10 - 12 points	-	10 points – 11 children (10.28%) 11 points – 10 children (9.34%)	10 points – 8 children (7.47%) 11 points – 13 children (12.14%) 12 points – 6 children (5.60%)

Table 1. Results for the buffering capacity of the saliva for all three study groups.**CONCLUSIONS**

1. Children with higher buffer capacity in their saliva tend to exhibit better oral health, including a lower prevalence of dental caries. The buffering capacity of saliva might play a protective role by neutralizing acids produced by oral bacteria, such as *Streptococcus mutans*, thus contributing to reduced tooth decay.

2. Children with lower buffer capacity could show a higher prevalence of *Streptococcus mutans* colonization, which is a known risk factor for dental caries the buffering capacity of saliva has a significant impact on the oral environment's acidity.

3. Higher buffer capacity might lead to better acid-neutralizing ability, helping to

maintain a balanced pH and preventing enamel demineralization.

4. Children with healthier oral hygiene habits and diets rich in minerals might exhibit higher buffer capacity. Educating parents and caregivers about the importance of maintaining good oral hygiene practices and providing children with tooth-friendly diets might be recommended to enhance buffer capacity and reduce dental caries risk.

5. The findings might be relevant for dental practitioners, helping them assess the oral health risk of young children. Children with lower buffer capacity and higher *Streptococcus mutans* colonization could be identified as high-risk individuals who require more tailored preventive measures.

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