

## The Relationship between the Salivary pH, Flow Rate, and the Number of Oral *Streptococci* in Elementary School Age Children

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### Abstract

Salivary pH, flow rate, and the number of oral Streptococci play a role in the occurrence of diseases in the children's oral cavity. Monitoring of salivary pH, flow rate, and the number of oral Streptococci is essential in preventing caries in children and will be able to provide appropriate educational efforts towards children and their parents. The study was aimed to analyze the relationship between salivary pH, flow rate, and the number of oral Streptococci in elementary school age children.

A descriptive survey. Research population was elementary school students aged 9 – 10-years-old. The sampling method was total sampling. The research sample was as much as 30 people. Saliva was collected for 10 minutes. Measurement of the pH value was performed using a pH meter. The salivary flow rate was counted by the saliva volume/collection time formula. Salivary volume was collected by a salivary tube with a volume assessment indicator issued by the Gc Buffer™ strip. Oral Streptococci was counted by calculating the cultured colonies on blood agar plates using a bacteria colony counter. Data analysis was performed using statistical software and Spearman correlation statistical tests to see the relationships between variables.

Average salivary pH was 7.1, the salivary flow rate was 0.17, and the number of oral Streptococci was 108 — the relationship tests between all three variables were found to be higher than 0.05 (p-value > 0.05).

No relationship was found between the salivary pH, flow rate, and the number of oral Streptococci in elementary school age children.

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### Introduction

*Streptococcus* is a Gram-positive bacteria genus, with a spherical shape and forming a chain-like formation in the microscopic image, which belongs to the phylum *Firmicutes*. *Streptococcus* is classified as alpha-hemolytic, beta-hemolytic or gamma-hemolytic, according to their appearance on blood agar. Mechanism of alpha-hemolysis involves the occurrence of heme iron reaction by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)

towards the *Streptococcus*, resulting in a greenish tinge on the blood agar.<sup>1</sup>

Alpha-hemolytic *Streptococcus* used to be known as the 'Viridans group' due to the greenish color produced by the partial hemolysis process. However, the alpha-hemolytic reaction is not entirely occurred amongst different strains of individual *Streptococcus* species. Therefore, the term 'Viridans' is no longer used. These organisms are now more commonly known as oral *Streptococcus*. Overall, *Streptococcus* are divided into six groups, namely the *Mitis*, *Anginosus*, *Salivarius*, *Mutants*, *Bovis*, and *Pyogenic*.<sup>1</sup>

The human oral *Streptococcus* is commensal bacteria which often inhabit the gastrointestinal and genitourinary tracts, as well as the oral mucosa and tooth surfaces. In healthy individuals, *Streptococcus* are able to form more

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than 50% of the oral microbiota and generally possess low pathogenic potential. However, one group of oral *Streptococcus* can invade the bloodstream, and have the potential of causing infective endocarditis (IE). Other oral *Streptococcus*-associated conditions including odontofacial infections, brain abscesses, and abdominal infections.<sup>1</sup>

Dental caries is one of the most common chronic diseases among children. The occurrence of dental caries is known to be associated with the presence of polymicrobial colonization of both acidogenic and aciduric bacteria on the teeth surfaces, especially the *Streptococcus mutans* group, which is part of oral *Streptococcus* and *Lactobacillus acidophilus*, known as the primary etiological agent of dental caries.<sup>2</sup> Caries risk factors include the presence of a high number of cariogenic bacteria, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, inappropriate methods of baby feeding, and poverty.<sup>2,3</sup> Reducing these microorganisms will be able to cause a significant decrease in dental caries.<sup>2</sup>

Research on the direct effects of adventure honey proves that honey can prevent dental caries in the future.<sup>4</sup>

*Streptococcus mutans* is the main cariogenic microorganism present in the oral cavity; these pathogens are able to damage the tooth surface and produce acids faster than the acid neutralization capacity of the biofilm in an oral environment below the critical pH value (less than 5.5) which destroys the tooth enamel.<sup>2</sup>

Saliva plays an important role in oral health such as maintains the integrity of the oral hard and soft tissues and also protects the oral tissue against bacterial, fungal and viral infections. Saliva plays a critical role in the prevention of dental caries as the balance controller between demineralization and remineralization in a cariogenic environment.<sup>2</sup>

Saliva has been reported as the physiological fluid which plays a role in making a diagnosis. The collection is simple, does not require special skills and injures the tissue, and has the same content as blood plasma. These conditions make saliva as an ideal alternative sample that can be taken into account. Diagnosis can be made from saliva samples can be determining the agent (cause) of local and systemic infections, hormone levels, and medications.<sup>5</sup>

Analysis of saliva can help monitors disease and provides many advantages in detecting protection of oral cavity disease and systemic diseases earlier, to accelerates the diagnosis process and reduces the health service costs.<sup>5</sup>

Descriptions above encourage us to research saliva and the influence of oral *Streptococcus*. This study was aimed to analyze the relationship between salivary pH, flow rate, and the number of oral *Streptococci* in elementary school age children.

### Materials and methods

The research type was descriptive with survey techniques. The study population was elementary school children aged 9 – 10-years-old. The inclusion criteria were children aged 9 – 10-years-old at the time of the research with caries and getting permission from their parents. The exclusion criteria were children with medication which caused a disturbance in salivation and children who did not want to follow the research procedure. The sample was taken using the total sampling technique. The research samples were as much as 34 children who fulfilled the inclusion criteria. The study was conducted at their school in accordance with the research ethical principles required by the research ethics committee of the Faculty of Medicine Universitas Padjadjaran, Indonesia.

Unstimulated saliva was collected using the spitting technique. Unstimulated whole saliva technique is a salivary collection technique that does not use any organ stimulation.<sup>6</sup> The spitting technique was performed by allowing the saliva to accumulated at the floor of the mouth then spit into the salivary tube. Salivary was collected for 10 minutes.<sup>5</sup> The salivary pH value was measured using the pH meter.<sup>6</sup> The salivary flow rate was measured using the volume/collection time formula. The salivary volume was collected with a salivary tube with a volume assessment indicator issued by Gc Buffer™ strip.<sup>6</sup> The oral *Streptococci* was calculated by counting the cultured colonies on the blood agar plate using the bacteria colony counter.<sup>7</sup>

All data were analyzed using statistical software by calculating the mean, maximum value, minimum value, and standard deviation, also by the Spearman analytic test. The correlation was observed after obtaining a

significant correlation. The correlation results (r) were interpreted according to the level of relationship which was strong (= 0.7-1), moderate (= 0.5-0.7), or low (= -0.5), after counting a significant correlation (or) significance value for consideration.

## Results

The salivary pH and flow rate and the number of oral *Streptococci* in elementary school-aged children are presented in the table below:

Variable	Mean	Minimum	Maximum	Std. Deviation
Salivary pH	7.4	6.6	8.2	.42
Salivary flow rate (ml/min)	0.56	1.0	9.5	2.53
Oral <i>Streptococci</i> (CFU)	108	20	308	73.65

**Table 1.** Minimum, Maximum, Mean and Standard Deviation Value of Salivary pH and Flow Rate, and The Number of Oral *Streptococci* in Elementary School-Aged Children with Dental Caries (N=34).

Table 1 shows that the average salivary pH value was 7.4, the average salivary flow rate was 0.56 ml/minute, and the number of oral *Streptococci* was 108 colonies. The table above also shows that the average salivary pH value was slightly alkaline, the average salivary flow rate was quite high, and the number of oral *Streptococci* was also quite high.

Variable	Correlation Coefficient	Sig. (2-tailed)	N
Salivary pH & Salivary flow rate (ml/min)	-0.192	0.27	34
Salivary pH & Oral <i>Streptococci</i> (CFU)	-.266	0.128	34
Salivary flow rate (ml/Minute) & Oral <i>Streptococci</i> (CFU)	-.138	0.438	34

**Table 2.** Relationship Test between Salivary pH and Flow Rate and The Number of Oral *Streptococci* in Elementary School-Aged Children.

Table 2 shows no significant relationship between salivary pH and flow rate with salivary pH; salivary pH with oral *Streptococci*; and salivary flow rates (ml/min) with oral *Streptococci*.

## Discussion

Table 1 shows the average pH value was 7.4. This value indicated that the average pH value was in the high-level group, in accordance

with previous research stated that pH value was categorized as the low-level group in  $\leq 5.3$ , the medium-level group in  $>5.3-6.3$ , and the high-level group in  $>6.3$ .<sup>8</sup> The average salivary pH value of non-stimulated saliva ranges from 6.10-6.47.<sup>9</sup> The pH value of 7.4 was categorized as the high-level group. A high-level pH value shows the alkaline pH. Studies had reported the importance of alkaline pH for deposition of calcium phosphate, thereby promoting plaque mineralization which stimulated the calculus formation.<sup>10</sup>

All mineral phase of calculus is more soluble at a lower level pH value than a higher level. When the pH value is above the critical level, it will be saturated thus increases the tendency for calculus to deposit and the remineralization of white-spot enamel lesions. Therefore, calculus formation is most likely to occur when the pH value level remains well above the critical level for long periods.<sup>11</sup> The measurement of plaque and salivary pH may be a better alternative in the diagnosis of the caries risk<sup>7</sup> and calculus formation which causes gum and periodontal disease<sup>8</sup> in children.<sup>7,8</sup>

In patients with heart disease using warfarin treatment. Salivary albumin and C-reactive protein levels increase which causes the periodontal condition to worsen so instructions for oral hygiene are needed.<sup>12</sup>

Table 1 shows that the average salivary flow rate was 5.5 ml/minute. This result was consistent by the range of normal salivary flow rates. The normal flow rate for unstimulated saliva is above 0.1 ml/min, but there is also another study which mentions 0.26 ml/min or between 0.12-0.16 ml/min. We take the range from 0.1 to 0.26 ml/min as the normal salivary flow rate.<sup>9,13</sup> The average salivary flow rate was above 0.26 ml/min, which showed that all respondents had quite high salivary flow rate. An adequate salivary flow rate is critical to the preservation and maintenance of oral tissue.<sup>13</sup> The measurement of salivary flow rate is very individual and ideally recorded after the age of 15-years-old. The report stated that there must be monitoring in children before the age of 15-years-old because the unstable salivary condition thus needed to be rechecked after the age of 15-years-old.<sup>14</sup>

Table 1 shows that the number of oral *Streptococci* was 108 colonies. This result was consistent with the study of Ling and Nasidze

stated that dental caries was characterized by detectable changes in the bacterial profile of saliva mostly in young populations. The rapid development of advanced molecular methods has provided valuable insights into the oral microbiome and complex biofilm compositions.<sup>7</sup>

In research on the biofilm *Streptococcus mutans* is proven by reducing the value of surface roughness after polishing metal base, acrylic resin, and valplast will be followed by a decrease in levels of CFU *S. mutans*.<sup>15</sup>

The number of colonies in Table 1 was counted from saliva on the blood agar plate by a traditional identification method. This method was consistent with the method used in Belstrom's study, stated that more than 700 oral bacterial species had been identified, of which only 50% is cultivable with traditional agar-based methods. Detection of the caries-related bacterial profiles have been investigated with various molecular techniques, and most studies analyzed samples collected directly from the caries lesions. However, being easy and inexpensive to obtain, saliva is an almost ideal biological secretion for oral microbial studies in health and disease.<sup>7</sup>

The maximum value of oral *Streptococci* detected was as much as 108 colonies. This result indicated that there was found a high amount of oral *Streptococci* in unstimulated saliva of elementary school-aged children. This result was consistent with the research conducted by Soro et al. stated that unstimulated saliva showing larger oral bacterial detection than the stimulated saliva.<sup>16</sup>

Table 2 shows that there is no significant relationship between salivary pH and salivary flow rate ( $R=-0.192$  ( $p<0.005$ )). This result, however, was different from Trima's study stated that the relationship of the salivary flow rate and salivary pH was quite significant ( $R=-0.221$  ( $p>0.01$ )).<sup>9</sup> This result may occur because the salivary flow rate of and pH were differed individually, and influenced by many things, such as the masticatory muscle movements in the oral cavity and chemical stimulation, the sampling temperature, and the sampling season.<sup>9,5</sup>

Table 2 also shows that there is no significant relationship between salivary pH and the number of oral *Streptococci* (CFU), as well as the relationship between salivary flow rate (ml/min) and the number of oral *Streptococci* (CFU). These unrelated results did not mean that the caries problem in children has been

thoroughly researched. Identifying caries-high-risk children is essential for the planning of oral health care and preventing future diseases. The results in Table 1 shows that there is still caries risk in elementary school-aged children, namely children with low salivary flow rate. This result will also help tertiary institutions when planning outreach activities to focus on communities that are most vulnerable to dental caries, one of the pillars for the success of a community service program.<sup>13,17</sup>

The maximum number of oral *Streptococci* counted was up to 308 colonies (Table 1). The result required a follow-up checking of dominant bacteria species in the respondents. This step is critical because oral bacteria play an essential role in body homeostasis and the bacterial genus *Streptococcus* is the dominant microflora commonly found in the oral bacterial community. Their ability to establish biofilms in the oral cavity by outcompeting other bacteria and producing bacteriocin.<sup>18</sup>

## Conclusions

No relationship was found between the salivary pH, flow rate, and the number of oral *Streptococci* in elementary school age children.

## Declaration of Interest

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