ORIGINAL RESEARCH

Identification and Correlation of *Streptococcus mutans* and *Streptococcus sanguinis* in Caries-active and Caries-free Children: A PCR Study

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Abstract

Aim and objectives: Dental caries is currently considered an ecological imbalance within the oral biofilm leading to the dissolution of the tooth's hard tissues. It has been traditionally thought that two species belonging to the *Streptococci* group, *Streptococcus mutans* (SM) and *Streptococcus sanguinis* (SS), are the etiologically responsible for the onset of dental decay.

Materials and methods: The present *in vivo* study was conducted on 40 children with caries-active (CA) and caries-free (CF). They were allocated into two groups, group I (CA) = 20 and group II (CF) = 20. The whole saliva was collected into the vials with buffer solution and was stored in cold storage. Polymerase chain reaction (PCR) was done to identify and correlate SM and SS in CA and CF children.

Results: Comparison of mean SM level between CA and CF groups showed a statistically significant result at $p = 0.001$. Spearman's correlation between caries score and SM showed a strong correlation of 0.77 between caries score and SM, which was statistically significant at $p = 0.001$. Similarly, SS and caries scores showed a weak correlation of 0.22. Simple linear regression analysis to SM and caries score showed a significant increase of 4.74 units for 1 score increase in caries score, which is statistically significant.

Conclusion: The presence of SM levels in children with caries is significant, whereas, in CF children, SS levels are present in increased levels. A strong correlation was seen between caries scores and SM. The simple linear regression analysis predicts a statistically significant increase by 4.74 units per increase of 1 score of caries at $p < 0.001$. As caries increase, SM count increases, but SS count decreases; as SS count increases, there is a reduction in SM counts.

Keywords: Dental caries, Linear regression equation, Oral biofilm, Polymerase chain reaction, *Streptococcus mutans*, *Streptococcus sanguinis*.

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Introduction

Dental caries is a very common, complicated, multifaceted illness that is impacted by socioeconomic, environmental, genetic, and microbial aspects of the host. When the homeostasis in the interface is disturbed by a high-sugar diet and the presence of bacteria that produce acid in the dental biofilm, the teeth become demineralized. A solid/liquid interface is where the dental biofilm is made up of structured bacterial communities. Although people of all ages can develop caries, it has been shown that early colonization with the cariogenic SM in newborns and young children may enhance the severity of the lesions and predispose them to future caries. Additionally, even though some nations are seeing a decline in caries prevalence, the disease affects 60–90% of kids worldwide, with low-income nations bearing the brunt of the condition.[1]

According to the present theory, the ecological shift in the microbial composition towards cariogenic species caused by sucrose plays a significant part in the development of dental caries. Certain conditional infections thrive in the cariogenic environment, where they more effectively compete with commensal microorganisms for the tooth surface, ultimately causing disease. Oral *Streptococci* make up 80% of the initial colonizers during early biofilm development but only makeup 20% of the supragingival bacteria in the oral biofilm.

The commensal microorganisms in the oral biofilm are advantageous to the host because they prevent the colonization of harmful germs that could cause disease.[2] Certain conditional infections thrive in the cariogenic environment, where they more effectively compete with commensal microorganisms for the tooth surface, ultimately causing disease.[3] A few *Streptococci* are linked to the development and spread of carious lesions. Although SM plays a crucial part in the development of caries, there is debate concerning the relationship between the rise in SM and the prevalence of caries.[4]

With the advent of the tooth, SS is found on the enamel as a typical commensal.[5] Conversely, as the amount of SM rises, SS slows down its colonization, which results in a decrease and delayed colonization of SS.[6] In addition, it is thought that the formation of hydrogen peroxide (H_2O_2) in SS over SM *in vitro* acts as an inhibitory mechanism.[7]

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The ratio between the SM and SS can be used to determine the caries risk. A high number of SS has been connected with children who do not have cavities, and the smaller the ratio, the lower the risk.[8]

A precise and sensitive method for the detection and quantification of distinct species and bacterial populations can be achieved using quantitative real-time PCR (qRT-PCR) using species-specific primers.[9]

There are very few studies supporting the difference in the ratio between SM and SS. Hence the aim of the study is to identify, evaluate and correlate the amount of SM and SS present in the CF and CA children using PCR.

Materials and Methods

A total of 40 children between the ages of 3–6 who visited the Department of Pediatrics and Preventive Dentistry participated in the current *in vivo* investigation. The patients were chosen by the random sample method. The clinical procedures, hazards involved, and answers to all of the patients' inquiries were briefly explained to 40 patients with CA and CF. The parents or guardians of the children taking part in this study gave their informed consent.

Inclusion Criteria

Children with four or more than four cavitated restorable lesions and children with CF.

Exclusion Criteria

Children who were on antibiotics within the past 3 months, children who received fluoride topical application during the last 48 hours, children who require special health care needs, children with existing restorations on any surface of the tooth, pulpal involved teeth, and children undergoing any kind of interceptive orthodontic treatment. Clinical examination of the children was performed using a plain dental mirror and explorer on the dental chair with optimal light. The presence of decayed, missing or filled teeth was scored according to World Health Organization criteria. Children were randomly allocated to one of the groups using the flip coin method into an experimental group (CA) I—*n* = 20 children and a control group (CF) II—*n* = 20 children. The unstimulated whole saliva was taken from the patient's mouth and was collected into the vials with buffer solution, and the samples were stored in cold storage. All the samples were subjected to PCR analysis and assessed for SM and SS.

Polymerase Chain Reaction (PCR) Procedure

The RajaRajeswari Dental College and Hospital, Bengaluru, Karnataka, India, in Bangalore's lab performed the PCR procedure for molecular biology. RT-PCR analysis was performed on "SM and SS."

Deoxyribonucleic Acid (DNA) Extraction

A purified Invitrogen DNA isolation kit (PurelinkTM DNA extraction kit, Applied BioSystems, India) was used to extract DNA from the saliva samples in a highly effective manner. DNA was isolated using the traditional "proteinase K" technique. It was 55°C in the water bath. Prior to being centrifuged for 2 minutes at 10000 RPM, the samples were vortexed for 5 seconds. Then a sterile microcentrifuge tube received 20 µL of proteinase K. 200 µL of saliva was placed in a sterile micro centrifuged tube.

The tubes were held in a water bath at 55°C for 2 hours, occasionally vortexed, until the process was finished. The lysate was mixed thoroughly with 20 µL of ribonuclease-A using a brief vortex before being incubated for 2 minutes at room temperature. To create a homogeneous solution, 200 µL of Purelink™ genomic lysis/binding buffer was added and well mixed by vortexing. Then, 200 µL of 96–100% ethanol was added and thoroughly mixed by vortexing for 5 seconds to create a homogeneous solution, and the purification technique was used right away.

Deoxyribonucleic Acid (DNA) Purification

The centrifugation technique is based on a spin column and takes between 10 and 15 minutes to complete. It is intended to remove genomic DNA. The Purelink™ spin-column was taken out of its packaging and put in a pristine collection tube. To the spin-column was loaded the whole lysate created using the Purelink™ genomic lysis/binding buffer and ethanol. The column was centrifuged at room temperature for 1 minute at 10000 RPM. After that, the spin column was put into a pristine Purelink™ collecting tube that was included with the package. The column received 500 µL of "wash buffer-1" made with ethanol.

At room temperature, the column was centrifuged for 3 minutes at its top speed. The collecting tube was thrown away. A sterile 1.5 mL microcentrifuge tube was used to contain the spin column. The column received 40 µL of the Purelink™ genomic elution buffer, which was then incubated at room temperature for 1 minute. To extract purified genomic DNA in the tube, the column was then centrifuged at maximum speed for 1 minute at room temperature. Until it was used, purified DNA was kept at –20°C.

Ploymerase Chain Reaction (PCR) Analysis

In this investigation, custom SYBR Green test reagents from Applied Biosystems in India were employed. The following primer sequence for SM was chosen for the study:

Streptococcus mutans (SM) forward—5′-GCCTACAG CTCAGAGATGCTATTCT-3′, SM reverse— 5′-GCCATAC ACCACTCATGAATTGA-3′, SS forward—5′-TGC TAT CTT TCC CTA GCA TG-3′, SS reverse—5′-GGT ATT CGG TTT GAC TGC-3′, 16S ribonucleic acid (two sets) forward primer—3′-TCCTACGGGAGGCAGCAGT-5′, reverse primer—5′-GGACTACCAGGGTATCTAATCCTGTT-3′.

In a nutshell, a reaction volume of 20 µL was created by mixing SYBR Green Universal PCR Master Mix (10 µL, one), forward and reverse primers (1 µL, one each) for the appropriate organism, extracted DNA from an unidentified sample (3 µL, one), and nucleus-free water. The RT-PCR conditions were as follows—stage held at 95°C for 10 seconds, then 40 cycles of shuttle heating, each lasting 15 seconds at 95°C and 1 minute at 60°C. The melt curve step lasted 15 seconds at 95°C, 1 minute at 60°C, and 15 seconds at 95°C. The endogenous control was 16S RNA. (Applied Biosystems, India, SYBR Green assay reagents).

Statistical Analysis

The statistical analyses will be carried out using the Statistical Package for the Social Sciences for Windows, version 22.0, released in 2013 by Armonk, New York, United States of America, IBM Corp.

Statistically Descriptive

While the frequency distribution for continuous data will be expressed as frequency, mean, and standard deviation (SD), that for categorical data will be expressed as number and percentage (SD).

Statistical Inference

Whitney–Mann *U* test was used to compare the average SM and SS levels between the two study groups. The level of significance will be set at $p < 0.05$. And any further pertinent tests will be handled in accordance with what is appropriate if they are discovered during data processing.

Results

The present *in vivo* study was done to identify and correlate SM and SS in group I (CA) and group II (CF), and quantification was done using the PCR method. Samples were collected, and the mean count was obtained using PCR. The results were tabulated and statistically analyzed.

Age-wise distribution among the two groups shows the mean and SD of age ranging from 3–6 years in group I is 4.53 and 1.15, respectively and in group II, the mean and SD of age ranges from 4.95 and 0.84, respectively. Gender-wise distribution in group I had males ($n = 10$) and females ($n = 10$) 50% each, and in group II had males ($n = 11$) and females ($n = 9$). No significant difference was seen between in gender distribution (Table 1).

The comparison of mean SM and SS levels between the two groups was made using the Mann–Whitney *U* test. In the SM group between caries and CF children, statistically significant results were seen at a $p = 0.01$. Here SM count was significantly higher in caries children than in CF children. In the SS group between caries and CF children also, statistically significant results were seen at a $p = 0.01$. Here SS count was higher in CF children than in caries children (Table 2).

In our study, Spearman's correlation test was done to assess the relationship between caries scores and SM and SS in the caries group. In our study, we found a strong correlation between SM and caries score of 0.77, which was statistically significant with a *p*-value of <0.001. Between SS and caries, the score showed a weak correlation which was not statistically significant (Table 3).

Simple linear regression analysis was done to predict the SM by caries scores which showed a statistically significant increase by 4.74 units per increase of 1 score of caries at $p < 0.001$. Variability in SM levels will be able to explain the caries score by 34%, where 34% is regression mode as R^2 (prediction level) or how closer we are to the prediction levels. SM levels don't increase just because caries scores have increased. SM can increase by so many other factors; one such contributory factor would be the caries score. If there is an increase in SM levels, how much is the contribution of caries score? SS contributing by this analysis, we know that 34% of your variability in SM levels is determined by caries score. Another 66% of SM increase can be of various other reasons. But in 3–6 years old children, when we determined this particular study, we would be able to narrow down that about 4.64 unit of SM will be increased because of 1 score increase in caries score.

Table 1: Age and gender distribution among two groups

Age and gender distribution among the two groups						
		Caries (group I)		*Non-caries (group II)*		
Variable	*Category*	*Mean*	*SD*	*Mean*	*SD*	*p*-value
Age	Mean and SD	4.53	1.15	4.95	0.84	0.19[a]
	Range	3–6		4–6		
		n	%	*n*	%	
Sex	Males	10	50%	11	55%	0.75[b]
	Females	10	50%	9	45%	

a, independent student *t*-test; b, Chi-squared test

Table 2: Shows comparison of mean SM and SS levels between 2 groups using the Mann–Whitney *U* test

Comparison of mean SM and SS level between 2 groups using Mann–Whitney U test						
Microbe	*Group*	*N*	*Mean*	*SD*	*Mean difference*	*p-value*
SM	Caries	16	21.212	32.412	20.000	0.01*
	Caries-free	17	1.212	1.073		
SS	Caries	16	1.533	2.211	−3.104	0.01*
	Caries-free	17	4.636	7.119		

*Statistically significant

Table 3: Spearman's correlation test to assess the relationship between caries scores and SM and SS in the caries group

Spearman correlation test to assess the relationship between caries scores and SM and SS in the caries group				
Group	*Variable*	*Values*	*SM*	*SS*
Caries	Caries Score	ρ	0.77	0.22
		p-value	<0.001*	0.41
		N	16	16

*Statistically significant

Interpretation

For every 1 score increase in caries score, the SM level will significantly increase by 4.74 units in the caries group (<0.0001). The variability in SM levels will be able to explain by caries scores of 34%.

Prediction Equation

SM = Caries × 4.74 – 2.01 (Table 4).

The graphical representation shows the mean SM levels between the two groups. The lowest count of SM with a mean score of 1.212 was seen in the CF group, followed by the highest mean score of 21.212 seen in the CA group (Fig. 1).

The graphical representation shows the mean SS level between the two groups, with the lowest mean value of 1.533 in the caries group and the highest mean score of 4.636 in the CF group (Fig. 2).

Scatterplot depicting the relationship between caries scores and SM level. As the SM levels increase, caries scores also increase (Fig. 3).

Scatterplot depicting the relationship between caries scores and SS level. As the caries score increases, SS levels decrease (Fig. 4).

Discussion

Early childhood caries (ECC), commonly referred to as nursing caries, baby bottle tooth decay, or rampant caries is a severe public health issue affecting children. It is very common, particularly among disadvantaged communities in emerging nations. The multifaceted etiology of ECC led to the adoption of the term. In an effort to focus on the many factors (such as socioeconomic, behavioral, and psychosocial) that contribute to caries at such young ages, the term "ECC" was first used in a 1994 workshop sponsored by the Centers for Disease Control and Prevention. In a child who is 72 months of age or under, ECC is described as "the presence of one or more decaying (non-cavitated or cavitated lesions), missing teeth (due to caries), or filled tooth surfaces in any primary tooth. Any trace of smooth-surface caries in children under the age of three indicates severe-ECC.[10] As a result, the study's objective was to use PCR to detect and correlate SM and SS in the saliva of children with CA and CF.

Streptococcus mutans (SM) is a mutans *Streptococci*-related gram-positive facultative anaerobic bacteria. The main causative agents are SM, which are also the most common isolates from the human oral cavity. Understanding how SM metabolizes sucrose is crucial to understanding the disease process because SM has been identified as the primary cause of tooth caries. Many of the enzymes in SM use sucrose as a substrate. SS belongs to the *viridans*

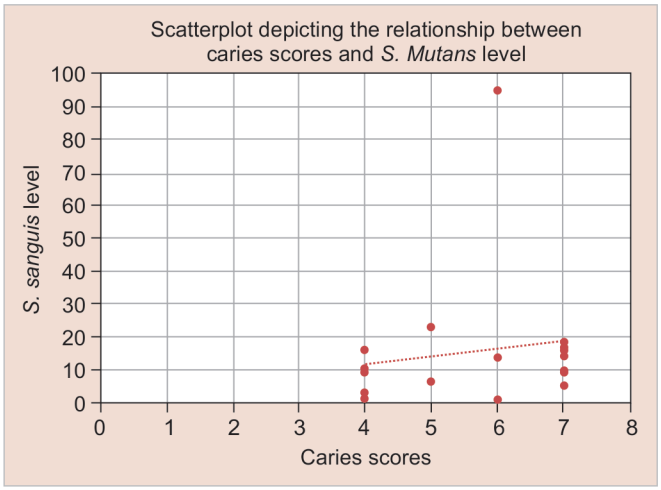

Fig. 2: Shows mean SS level between two groups

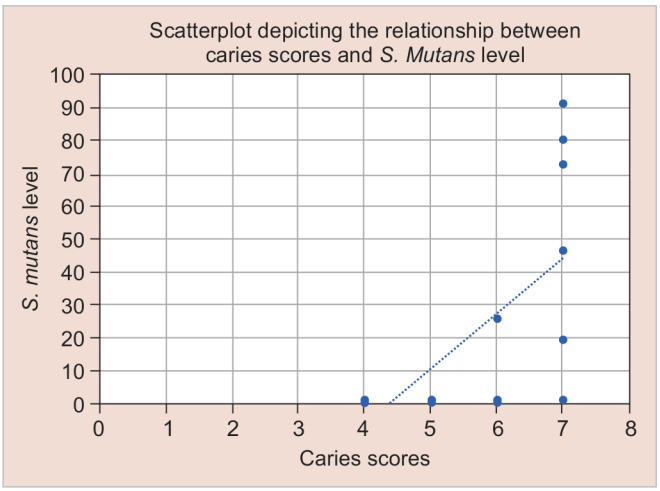

Fig. 1: Shows the mean SM levels between two groups

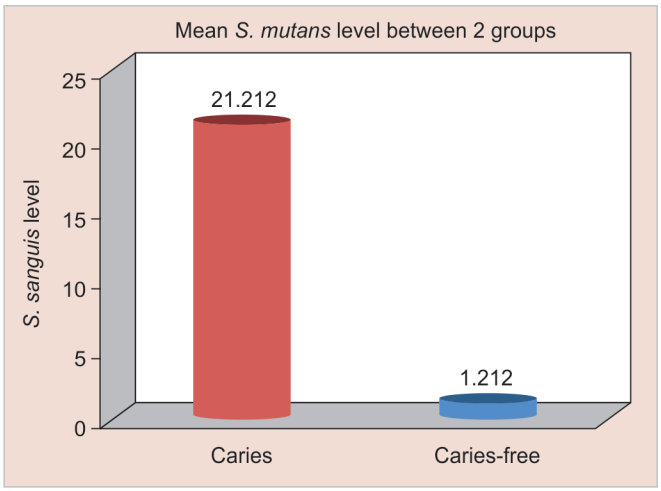

Fig. 3: Shows scatterplot depicting the relationship between caries scores and SM level

Table 4: Simple linear regression analysis to predict the SM by caries scores

Simple linear regression analysis to predict the SM by caries scores						
Group	Individual variable	*β*	*Standard error*	*t*	*p-value*	R^2
Caries	Constant	–2.01	4.79	–0.419	0.68	0.34
	Caries score	4.74	1.19	3.970	0.001*	

*Statistically significant

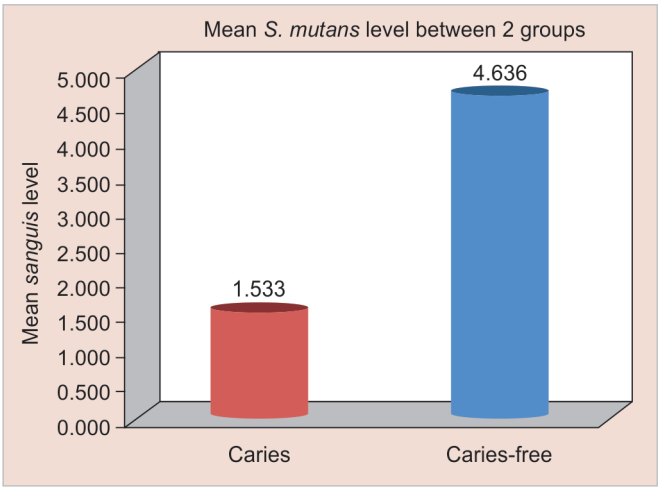

Fig. 4: Shows scatterplot depicting the relationship between caries scores and SS level

Streptococcus group of bacteria and is a gram-positive facultative anaerobic coccus species. SS is a common component of saliva and dental plaque in a healthy human mouth, where it alters the environment to make it less friendly for other *Streptococcus* strains that cause cavities, including SM. In order to prevent the growth of dental caries, SS produces H_2O_2 as an antibacterial agent to thwart competitive niche-vulnerable species like SM during the first biofilm formation phase. Expression and synthesis of oxygen-dependent pyruvate oxidase in SS.

The oral biofilm is made up of commensal microorganisms that are helpful to the host rather than pathogenic germs that cause disease.[2] Some *Streptococci* are responsible for the beginning and development of carious lesions. The fundamental role that SM play in the pathogenesis of caries is debatable, as is the relationship between the rise in SM and the prevalence of caries.[10]

With the emergence of the tooth, SS is present as a typical commensal microorganism on the enamel.[5] Early SS colonization causes SM colonization to slow down and decline, but as SM populations rise, SS colonization rates decline.[6] H_2O_2 generated by SS is thought to act as an inhibitory mechanism against SM *in vitro*.[7] By measuring the ratio between the SM and SS, the risk of caries can be evaluated. The risk of caries is inversely correlated with the ratio of SM to SS. Children without caries have been found to have higher numbers of SS.[6]

Research on caries is particularly interesting in the antagonistic action of SS against SM, and both *in vitro* and clinical investigations have looked into this topic in great detail.[1] Further research into how competition might shape the community's ultimate makeup and its overall impact on the host is warranted, given the putatively advantageous role of SS in the dental biofilm. Our study demonstrates that in the oral biofilm of CF participants, SS is statistically superior to SM. Numerous studies have been done on this subject in adults, but there is not enough clinical proof of this antagonistic interaction between the two bacterial species in children.[11] Therefore, we made the decision to test the idea that in children with different caries histories, SS and SM levels would be inversely correlated.

Several types of samples were used to investigate the relationship between SM and human dental decay, including (1) paraffin-stimulated saliva samples, (2) pooled occlusal and approximal plaque, (3) plaque that had been scraped out of individual occlusal fissures, (4) gingival crevicular fluid, and (5) blood samples. Saliva serves as a representative of the mouth's worldwide colonization and enables a broad inference about the colonization of the species.[1] Therefore, in our study, we used unstimulated whole saliva samples to identify and correlate the amount of SM and SS in both groups, which was in agreement with our study and studies using unstimulated and stimulated saliva samples concluded that total antioxidant capacity was higher in unstimulated saliva therefore un-stimulated saliva is more accurate in quantifying the cariogenic microorganisms.[12,13] Children with ECC had higher amounts of SM in their dental plaque, according to a study that used qRT-PCR on samples of dental plaque from those children.[14]

There are several benefits to using PCR, or polymerase chain reaction. It provides results quickly and is quite easy to use and comprehend. The process can create millions to billions of clones of a certain product for sequencing, cloning, and analysis. It is highly sensitive. The benefits of qRT-PCR are similar to those of PCR, with the addition of the ability to quantify the produced result. As a result, it can be used to examine changes in gene expression levels in tumours, microbes, or other disease states. It is also a popular technique for quickly making millions to billions of copies of a particular DNA sample, which enables researchers to take a very small amount of DNA and amplify it to a quantity that is big enough to study in depth. It is essential to many genetic tests, such as the analysis of old DNA samples and the detection of infectious pathogens. In a series of temperature-changing cycles, copies of very small amounts of DNA sequences are exponentially amplified by PCR. Today, PCR is a widely used and frequently essential method in medical laboratory research for a wide range of purposes, including biological research and criminal forensics.[15] So, in our investigation, PCR was utilized to precisely identify the microorganisms in the saliva.

In this study, we used entire saliva samples that had not been stimulated in order to determine and correlate the levels of SM and SS in both groups using PCR. To maximize the representation[16] of the species in mature biofilm saliva, we urged children in our study to forgo brushing and to eat their meals at least 18 and 12 hours before sample collection. Whole, unstimulated saliva was then collected. The objective of the current *in vivo* investigation, which involved 40 children aged 3-6, was to test the theory that SS and SM levels are inversely correlated in children with different caries histories.

Despite the study's limitations, the findings seem to confirm that SS and SM in saliva have an antagonistic relationship. According to the findings of our study, children with a long history of caries, as shown by multiple carious lesions, have more *Streptococci* in their saliva than children without such a history. SM was also substantially more prevalent in saliva from CA groups compared to CF groups.[17] In this study, we discovered significant levels of SM in children with CA and substantially lower levels in children with CF, supporting this connection. In this investigation, we discovered that children without a history of caries had greater levels of SS when compared to people with multiple carious lesions, which is consistent with earlier studies described in children.[18] A possible explanation for why SM was more prevalent in CA saliva in our study is that it has the ability to outcompete commensal species by creating bacteriocins, also known as mutacins. Mutacins I and IV, which inhibit SS, are produced by SM under specific environmental conditions. These competitive mechanisms that are mediated by the environment have a significant impact on the makeup of the oral biofilm and may help to explain the prevalence of cariogenic species that has been seen in clinical trials.

When SS was compared to people with multiple carious lesions, we discovered that children without a history of caries had greater levels of SS. Production of H_2O_2 in SS is controlled by external factors in order to maintain a healthy biofilm consistent with its phenotypic traits. Even though *Streptococci* produce H_2O_2 often, SS is resistant to its own H_2O_2 and inhibits the growth of cariogenic SM. These complex traits of SS may be crucial in maintaining the right balance.

Since there may be other factors influencing the dynamics of the oral film, it is challenging to determine that the predominance of SS *in vivo*, as in our work, is simply caused by higher H_2O_2 generation. Nevertheless, this may be the likely mechanism at play. It's interesting to note that SS inhibits multiple SM genes associated with virulence in addition to its inhibitory effect on SM. H_2O_2 may therefore play a role in both direct killing and controlling the expression of SM virulence genes. In our investigation, the Mann–Whitney *U* test was used to compare the mean SM and SS levels between the two groups. A statistically significant outcome was seen in the SM group comparing children with CA and CF with a *p*-value of 0.01. A statistically significant outcome was also observed in the SS group between children with CA and CF, with a *p*-value of 0.01. In this investigation, we discovered that the SM levels in the CA group were higher than those in the CF group, which was consistent with earlier studies.[6] These findings were consistent with earlier research in preschoolers, where it was discovered that SM in the CA group was much higher than in the CF group.[19,20] Studies revealed high levels of SM in some CF youngsters, which is in contrast to the aforementioned findings.[8,21]

To determine the link between caries scores and SM and SS in the caries group, Spearman's correlation was performed. In our research, we discovered a significant link between the SM and caries score of 0.77, with a statistical significance level of 0.001. The link between the caries score and the SS was 0.22. Similar to other earlier studies[17], these findings showed that SM counts are more prevalent in children with caries, whereas SS counts are more prevalent in the CF group.[1,22] A risk factor for severe early childhood caries with significant caries activity was the presence of SM.[23]

In our investigation, the SM by caries scores were predicted using straightforward linear regression analysis. The outcome of caries was substantially correlated with the interaction between SS and SM, according to the findings of the linear regression analysis. The results not only confirm that the presence of SM may not be the only marker for an increased risk of developing caries, but they also imply that the interaction between SM and SS may be a significant factor in how often children develop caries. We calculated the constant and the standard error for our investigation. SM will increase by 4.74 units for every increase of 1 score in the oral cavity.

The SM levels considerably increase in the CA group by 4.74 units, which is statistically significant with a *p*-value of 0.001. The variation in SM levels will be able to account for 34% of the variation in caries scores. Prediction For SM, the equation "SM = Caries × 4.74 − 2.01" was developed. Similar to our work, where elevated levels of SM were found in the CA group[7,24] by linear regression analysis.[25,26]

Dots are used in a scatter plot (also known as a scatter chart or scatter graph) to show the values of two different numerical variables. Each dot's location on the horizontal and vertical axes represents a data point's values. To view relationships between variables, utilize scatter plots. In our study, a scatterplot illustrating the correlation between caries scores and SM level was created, demonstrating that when SM levels rise, caries scores rise along with them. The scatterplot showing the link between the caries score and the SS level reveals that as the caries score rises, the SS level falls. The use of SS counts as a biomarker in place of SM counts as a caries risk factor represents a novel strategy, yet it is possible that the number of colonies in saliva or in the biofilm is insufficient to distinguish risk.

In order to assess risk, it is important to consider the proportion of both SM and SS in a single individual. In our study, we evaluated this proportion and discovered that as SM counts significantly decreased, SS counts significantly increased, and vice versa; as SS counts significantly increased, SM counts significantly decreased. Additionally, these findings imply a connection between SS's capacity to create H_2O_2 and the absence of caries. These results help us comprehend the intricate dynamics of dental biofilm in both health and illness. Additional research on the SS characteristics may reveal some cutting-edge anti-caries tactics.

The results of our investigation strongly imply that SM may be more prevalent than SS in the saliva of children who have had caries. However, in the saliva of people who have never had caries, SS seems to outweigh SM. In addition, our work hypothesizes the process by which the two main indigenous microorganisms compete with one another to cause disease or maintain dental health, as well as the relative amounts of SS and SM in predicting caries formation in children using a linear regression equation.

Conclusion

The following inferences can be taken from the current *in vivo* study, which was carried out to detect and correlate SM and SS in CA and CF children: The very straightforward PCR technique is a highly sensitive and precise tool for identification. As a result, it was utilized to analyze, identify, and quantify microorganisms in children with CA and CF. Children with caries had substantial levels of SM, whereas children without caries had increased levels of SS. There is a significant link between caries scores and SM. According to the results of the simple linear regression analysis, the SM and Caries scores increased statistically significantly by 4.74 units at *p* 0.001 with a 34% variance in SM levels, with 34% serving as the regression mode. The scatterplot also demonstrates that whereas SS declines as caries grows in SS, SM increases as caries increase in SM.

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