COMPARISON OF *STREPTOCOCCUS MUTANS* AND *STREPTOCOCCUS SOBRINUS* LEVELS AND DENTAL HEALTH BEFORE AND FOR 1 YEAR AFTER COMPREHENSIVE DENTAL TREATMENT UNDER GENERAL ANESTHESIA AMONG CHILDREN WITH SEVERE DENTAL CARIES

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Abstract. Dental caries is a major health problem among young children and can be challenging to treat. In this study we aimed to compare *Streptococcus mutans* and *Streptococcus sobrinus* levels and dental health before and for 1 year after comprehensive dental treatment under general anesthesia among children with severe dental caries in order to determine the immediate and long-term benefits of this procedure. Study subjects were recruited from the Pediatric Dental Clinic, Faculty of Dentistry, Mahidol University, Bangkok, Thailand. Inclusion criteria for study subjects were: being Thai, aged 1-5 years and having severe early childhood caries (S-ECC) requiring comprehensive dental treatment under general anesthesia. At the time of the dental treatment and at 1 week, 3 months, 6 months and 1 year following treatment, each study subject was examined and the number of caries and levels of *S. mutans* and *S. sobrinus* DNA using quantitative real-time PCR with fluorescent dye (SYBR green) were determined and recorded. A total of 27 subjects were included in the study, 82% male. The mean (±standard deviation) age of subjects was 3 (±1) years. The mean *S. mutans* levels pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: 10,451, 706, 1,152, 10,515 and 1,623 colony forming units (CFU), respectively. The only time the *S. mutans* level was significantly lower than pre-treatment, was at 1-week post-treatment ($p<0.014$). The mean *S. sobrinus* levels pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: 24, 0, 0, 0 and 0 CFU, respectively. The only time the *S. sobrinus* level was significantly lower than pre-treatment was at 3 months post-treatment ($p<0.0001$). Forty-eight percent of subjects developed new dental caries 3-12 months post-treatment. Among those with new caries, 31% had both *S. mutans* and *S. sobrinus*, 54% had *S. mutans*, 8%

had *S. sobrinus* and 8% had no bacteria detected. In summary, there was a temporary significant decrease in pathogenic bacteria post-treatment but by 6 months the pathogenic bacterial levels were no longer significantly different from pre-treatment levels and nearly half of subjects developed new caries during the one-year post-treatment period. Our results show dental treatment under general anesthesia is not beneficial for long term control of dental caries or control of pathogenic bacterial levels.

Keywords: severe early childhood caries, *Streptococcus mutans*, *Streptococcus sobrinus*, dental treatment, general anesthesia, real-time PCR

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INTRODUCTION

Severe early childhood caries (S-ECC) is a major public health problem word-wide. In the USA and Japan, the prevalences of caries during 2012 and 2013 among children aged 2-5 years were 22% and 17%, respectively (Berry *et al*, 2017). In Thailand, the prevalences of caries in 2017 among children aged 3 and 5 years were 53% and 76%, respectively (Mitrakul *et al*, 2020).

Dental plaque is an adherent aggregation of microorganisms and presents as a biofilm on tooth surfaces. It is a major cause of dental caries (Berkowitz, 2003). The most common bacteria species found in biofilm are mutans streptococci (MS), including *Streptococcus mutans* and *Streptococcus sobrinus* (Berkowitz, 2003). *S. mutans* has been reported to be associated with caries and caries risk (Berkowitz, 2003; Kanasi *et al*, 2010) but may also occur in those without caries. *S. sobrinus* is uncommonly isolated among those without caries (Choi *et al*, 2009; Hirose *et al*,1993; Ahmady *et al*, 1993). *S. sobrinus* is found more commonly in caries on the smooth surfaces of the teeth and *S. mutans* is found more commonly in caries on the occlusal or chewing surfaces of the teeth (Hirose *et al*,1993; Ahmady *et al*, 1993). Children who have both *S. mutans* and *S. sobrinus* have a greater incidence of caries than those who have only *S. mutans* (Okada *et al*, 2002; Hirose *et al*, 1993; Hata *et al*, 2006).

In several studies, *S. mutans* and *S. sobrinus* levels has been reported to be positively associated with the severity of dental caries (Tankkunnasombut

et al, 2009; Loyola-Rodriguez *et al*, 2008). One study among Thai children reported the prevalence of caries among subjects with both *S. mutans* and *S. sobrinus* was the same as the prevalence of caries among subjects with only *S. sobrinus* (Mitrakul *et al*, 2011). However, other studies have reported finding a significant association between having caries and having both *S. mutans* and *S. sobrinus* (van Houte, 1994; Loyola-Rodriguez *et al*, 2008).

Several previous studies have reported a one-time elimination of carious lesions by a comprehensive dental treatment under general anesthesia results in a significant reduction in salivary MS (Chase *et al*, 2004; Morinushi *et al*, 2004; Tanner *et al*, 2011; Twetman *et al*, 1999) but the relapse rate is high and the occurrence rapid (Almeida *et al*, 2000; Chase *et al*, 2004; Foster *et al*, 2006). Some studies have reported this decrease in MS level can last for 6 months after this kind of treatment (Chase *et al*, 2004; Twetman *et al*, 1999; Palmer *et al*, 2012; Vishwanathan *et al*, 2020). Previous studies have reported patients treated this way still have a high incidence of new caries lesions (Klinke *et al*, 2014; Chase *et al*, 2004; Foster *et al*, 2006; Amin *et al*, 2010; Berkowitz *et al*,1997; Eidelman *et al*, 2000; Almeida *et al*, 2000; Sheehy *et al*,1994). However, there are few studies of the long-term effects of comprehensive dental treatment under general anesthesia on levels of MS, especially among Thai children.

In this study we aimed to compare *S. mutans* and *S. sobrinus* levels and dental health before and for 1 year after comprehensive dental treatment under general anesthesia among children with severe dental caries in order to determine the immediate and long-term benefits of this procedure. We hypothesize *S. mutans* and *S. sobrinus* levels post-treatment will be significantly lower than pre-treatment.

MATERIALS AND METHODS

Subject selection

We recruited subjects for this study from the Pediatric Dental Clinic, Faculty of Dentistry, Mahidol University, Bangkok, Thailand. The study was conducted during 1 October 2019-1 October 2020. The minimum number of subjects determined necessary for this study was 47 subjects based on previously described methods (Klinke *et al*, 2014) using the following equation:

$$n = \left[\frac{z_{1-\frac{\alpha}{2}}\sqrt{p_{01}+p_{10}} + z_{1-\beta}\sqrt{p_{01}+p_{10}-(p_{01}-p_{10})^2}}{\Delta} \right]^2$$

where $Z_{1-\alpha/2}$ is the value for standard normal distribution; p_{01} is the estimated prevalence of caries pretreatment obtained from a previous study (Klinke *et al*, 2014); p_{10} is the prevalence of caries posttreatment obtained from the above study; α is the level of statistical significance; β is the probability of making a Type II error; Δ is p_{01}-p_{10}, giving:

$$n = \frac{\left[0.975-\frac{0.05}{2}\sqrt{0.7+0.3}+0.8-0.2\sqrt{0.7+0.3-(0.7-0.3)^2}\right]^2}{(0.7\text{-}0.3)} = 47$$

Inclusion criteria for study subjects were: being Thai, aged 1-5 years and having severe early childhood caries (S-ECC) and having comprehensive dental treatment under general anesthesia.

The diagnosis of S-ECC was based on the American Academy of Pediatric Dentistry (AAPD) criteria, which defines S-ECC as: 1 being aged <3 years with any smooth-surface of the tooth white lesions but no caries; 2 being aged 3 years and having caries in 1 or more teeth, missing a tooth due to caries, having a filled caries lesion on a smooth surface in a primary maxillary anterior tooth or having a decayed, missing or filled teeth involving ≥4 surfaces; 3 being aged 4 years and having 1 or more teeth with a caries, having a missing tooth due to caries, having a filled caries lesion on a smooth surface in a primary maxillary anterior tooth or a decayed, missing, or filled teeth involving ≥5 surfaces; 4 being aged 5 years and having caries in 1 or more teeth, missing a tooth due to caries, having a filled caries lesion on a smooth surface in a primary maxillary anterior tooth or decayed, missing, or filled teeth involving ≥6 surfaces (Anonymous, 2016).

All study subjects were physically healthy other than for tooth problems.

Treatment and follow-up visits

Prior to treatment, a baseline oral examination was performed. At that appointment, subject parents were given hands-on instructions for tooth brushing and flossing using a dental model and were given diet counseling.

The dental treatment was performed under general anesthesia in one session and included extractions, restorations with proper restorative materials, pulp treatment and full mouth prophylaxis. Each subject had 4 follow-up visits after treatment: at 1 week and 3, 6 and 12 months (Fig 1). Dental radiographs were taken at the 6-month and 1-year follow-up visits.

The questionnaire

The parents of each study subject were asked to complete a questionnaire

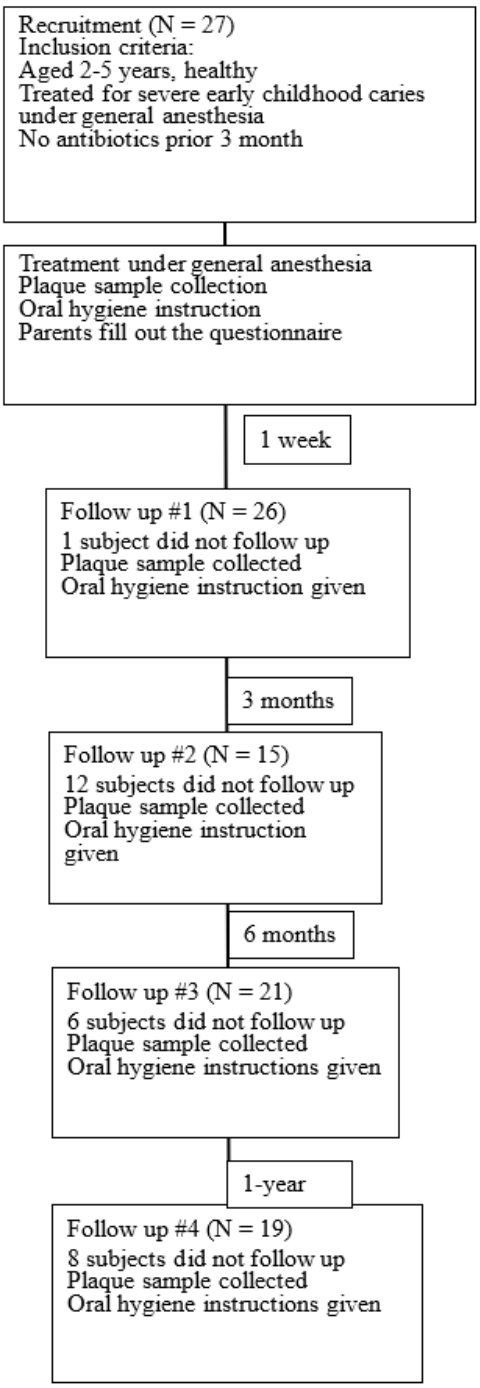

Fig 1 - Flowchart of this study

via a face-to-face interview. All questions had close-ended answers. The questionnaires asked the parental age, career, education level, monthly income and type of dental payment coverage.

Clinical examination, plaque index and modified gingiva index

One examiner in a Master's degree program in pediatric dentistry performed the clinical examinations following World Health Organization's criteria (Ismail *et al*, 2007). The examiner determined and recorded the number of decayed, missing and filled teeth (DMFT) (Ismail *et al*, 2007). The examiner determined and recorded the visible plaque index using the Debris Index simplified from the Oral Hygiene Index described previously (Greene and Vermillion, 1964). The six index teeth surfaces used for the study were the buccal side of the upper right primary molar, the front aspect of the right upper anterior incisor, the buccal side of the left upper primary molar, the lingual side of the left lower primary molar, the front aspect of the left lower anterior incisor and the lingual side of the right lower primary molar. Each tooth surface examined was given a score of from 0 to 3 (Fig 2). The total plaque index for an individual was determined by adding the scores for each of the studied teeth surfaces and dividing that number by the number of teeth examined. A plaque index of 0-0.6 was considered good,

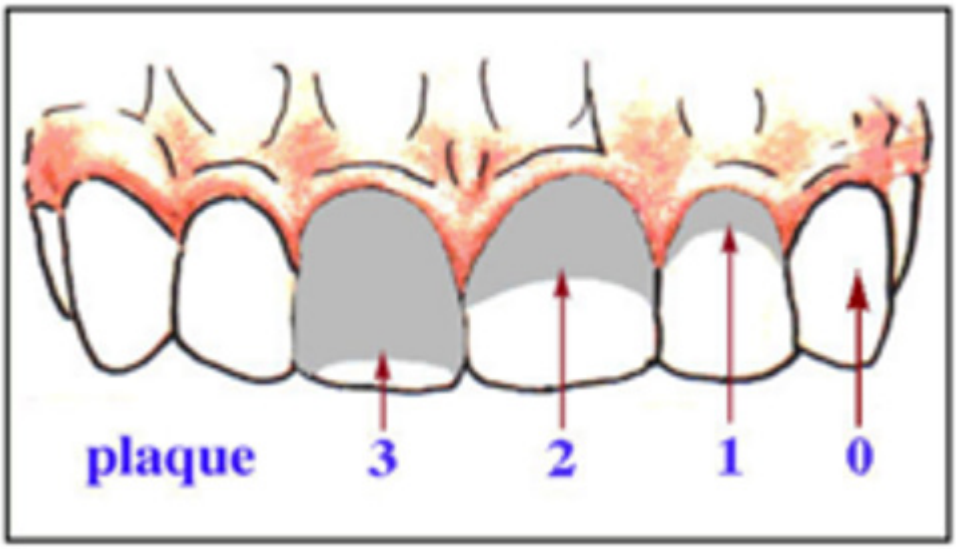

Fig 2 - Criteria for plaque index score

Each area of each tooth was assigned a score from 0 to 3 following Greene and Vermillion, 1964.

0: no debris or stains present; 1: soft debris covering not more than one-third the tooth surface or the presence of extrinsic stains without other debris, regardless of the surface area covered; 2: soft debris covering more than one third but less than two-thirds the exposed tooth surface; 3: soft debris covering more than two-thirds the exposed tooth surface

0.7-1.8 was considered fair and 1.9-3.0 was considered poor.

Plaque sample collection

Each subject was instructed to brush their teeth under parental supervision at 8 PM the night before plaque collection. The subject was then instructed not to eat or drink anything prior to the plaque sample collection. The plaque was collected using a sterile toothpick and the sample was placed in 1 ml Tris-EDTA buffer. All samples were immediately transported on ice to the Oral Biology Laboratory and stored at -20°C until DNA extraction.

DNA extraction

The bacterial DNA of each collected plaque sample was extracted using enzymatic lysis with a commercial kit (Flavogen, Pingtung, Taiwan) as described previously (Mitrakul *et al*, 2016). The extracted DNA concentration and purity were determined using a spectrophotometer at 260/280 nm (Nanodrop 2000C® Thermo Scientific, Wilmington, DE).

Culture condition and standard strains

S. mutans (ATCC 25175) and *S. sobrinus* (ATCC 6715) strains were used as control stains for comparison with PCR products. They were cultured on brain heart infusion agar and in broth. The DNA was extracted from samples cultured overnight. A ten-fold serial dilution, starting from 10^8 to 10^2 CFU/ml, was performed to determine the concentration of the cultured bacteria.

Quantitative real-time PCR

We conducted real-time polymerase chain reaction (RT-PCR) testing to determine the presence of specific bacteria using specific primers (Table 1). The reaction mixture (total volume of 20 µl) contained water (2-9.1 µl), 2X KAPA SYBR® FAST qPCR Master Mix (10µl) (Bio-Rad Laboratories, Hercules, CA), 10 µM forward and reverse primer (0.4 µl) and bacterial DNA (0.1-7.2 µl). The mixture was cycled 40 times using a thermocycler (C1000™ Thermal cycler (Bio-Rad Laboratories, Hercules, CA) and CFX 96 Real-time System (Bio-Rad Laboratories, Hercules, CA). Each cycle consisted of enzyme activation at 95°C for 3 minutes, denaturing at 95°C for 3 seconds, annealing for 20 seconds and extension for 30 seconds.

Table 1

Primers used for the polymerase chain reaction in this study

Primers		Nucleotide sequence 5′ to 3′	Expected amplicon (base pairs)	Annealing Temperature (°C)	Reference
Universal BAC16S	F	5′-TGG AGC ATG TGG TTT AAT TCG A-3′	160	52	Sinsimer *et al*, 2005
	R	5′-TGC GGG ACT TAA CCC AAC A-3′			
Streptococcus sobrinus	F	5′-CGC ACT TGC TCC AGT GTT ACT AA-3′	546	53	Sato *et al*, 2003
	R	5′-GCC TTT AAC TTC AGA CTT AC-3′			
Streptococcus mutans	F	5-AGC CAT GCG CAA TCA ACA GGT T-3′	415	53	Yano *et al*, 2002
	R	5-CGC AAC GCG AAC ATC TTG ATC AG -3′			

F: Forward primer; R: Reverse primer

Melting curves were generated from 60°C to 95°C and read every 0.5°C for 5 seconds (Mitrakul *et al*, 2016).

Agarose gel electrophoresis

The amplified PCR product were then placed on 1.5-2% agarose gel and stained with ethidium bromide and digitally photographed (Molecular Imager ®Gel docTM Systems, Bio-Rad Laboratories, Hercules, CA) (Mitrakul *et al*, 2016).

Statistical analysis

Levels of total bacteria, *S. mutans* and *S. sobrinus*, ratios of *S. mutans*/total bacteria and *S. sobrinus*/total bacteria were compared between pre-treatment and 1-week post-treatment. For data with a non-normal distribution, the Wilcoxon signed-rank test was used to determine significant differences. The paired t-test was used to compare the pre-treatment and 1-week post-treatment plaque indices to determine significant differences. The generalized estimating equation (GEE) was used to determine significant differences in the same factor for total bacteria, *S. mutans* and *S. sobrinus* levels, the percentages of *S. mutans*/total bacteria and the percentages of *S. sobrinus*/total bacteria at the five time-points (pre-treatment, 1 week, 3 months, 6 months and 12 months post-treatment). The GEE was used to determine associations between the plaque index and the bacteria levels and between the DMFT scores and the bacterial levels. The Fisher's exact test was used to assess factors associated with the occurrence of a new cavity at 1 year. Statistical analyses were performed using SAS Studio, version 9.2 (SAS Institute, Cary, NC). A p-value <0.05 was considered statistically significant.

Ethical approval

This study protocol was approved by the Ethics Institutional Review Board, Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University (COA. No. 2019/073.2410).

RESULTS

General information

A total of 27 subjects were included in the study, 82% male. We did not reach the minimum calculated number of study subjects due to the outbreak of coronavirus disease-2019 (COVID-19) requiring the closure of the dental clinic for part of the study. The mean (±standard deviation (SD) age of study subjects was 3 (±1) (range: 2-5) years. The mean (±SD) age of subject mothers was 34 (±5) (range: 25-46) years. Seventy-four percent of subject mothers had a Bachelor's degree. Forty-eight percent of study subjects had a family income >THB50,000 per month. Seventy percent of subject mothers worked outside the home and 30% were housewives (Table 2)

Twenty-six out of 27 subjects (96%) followed up at 1 week, 15 (56%) followed up at 3 months, 21 (78%)

Table 2

Selected subject variables (N=27)

Variables	*n* (%)
Maternal education level	
High school/diploma	3 (11)
Bachelor's degree	20 (74)
Master's degree	4 (15)
Maternal occupation	
Government employee	7 (26)
Private company employee	8 (30)
Merchant	3 (11)
Housewife	8 (30)
Other employee	1 (4)
Monthly family income in Thai Baht	
<30,000	10 (37)
30,000-50,000	4 (15)
>50,000	13 (48)
Dental financial coverage	
Universal Coverage	1 (4)
Civil Servant Medical Benefit Scheme	8 (30)
Self-pay	18 (67)

followed up at 6 months and 19 (70%) followed up at one year.

Total bacterial, *S. mutans* and *S. sobrinus* levels at each visit

The mean *S. mutans* levels pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: 10,451, 706, 1,152, 10,515 and 1,623 colony forming units (CFUs), respectively (Table 3). The mean *S. mutans* level 1 week post-treatment was significantly lower ($p<0.014$) than pre-treatment but none of the others were significantly different from the pre-treatment level (Table 4). The mean *S. sobrinus* levels pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment

Table 3

Oral bacterial levels among study subjects during study period

Bacterial level	Pre-treatment (n = 27)	1 week post-treatment (n = 25)	3 months post-treatment (n = 15)	6 months post-treatment (n = 21)	1 year post-treatment (n = 19)
Total bacteria (CFUs)					
Median	1.37x106	1.38x106	1.52x106	2.26x106	1.83x106
Minimum	9.87x104	2.63x104	3.31x104	6.87x105	4.65x105
Maximum	5.56x106	7.06x106	3.64x106	3.39x106	6.74x106
Streptococcus mutans (CFUs)					
Median	10,451	706	1,152	10,515	1,623
Minimum	0	0	0	0	0
Maximum	402,422	849,643	226,264	285,566	420,053
Streptococcus sobrinus (CFUs)					
Median	24	0	0	0	0
Minimum	0	0	0	0	0
Maximum	96,134	20,121	11,435	54,274	92,417

CFUs: colony forming units

were: 24, 0, 0, 0 and 0 CFUs, respectively (Table 3). The mean *S. sobrinus* level at 3 months post-treatment was significantly lower (p<0.0001) than pre-treatment but none of the others were significantly different from the pre-treatment level (Table 5). The mean total bacteria levels pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: $1.37x10^6$, $1.38x10^6$, $1.52x10^6$, $2.26x10^6$ and $1.83x10^6$ CFUs, respectively (Table 3). The mean total bacteria level 6 months post-treatment was significantly higher (p=0.0487) than pre-treatment but none of the others were significantly different from the pre-treatment level (Table 5).

Plaque index and DMFT scores

The mean (±standard deviation) plaque index scores pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: 2 (±1) (poor), 1 (±1) (fair), 1 (±0.0) (fair), 1 (±1) (fair) and 1.0 (±0.0) (fair), respectively. The post-treatment plaque index levels were significantly lower than the pre-treatment levels at 1 week (p<0.0001), 3 months (p<0.0001), 6 months (p<0.0001) and 1 year (p<0.0001) (Tables 6 and 8).

The mean DMFT (±standard deviation) scores pre-treatment, 1 week, 3 months, 6 months and 1 year post-treatment were: 14 (±4), 14(±4), 14 (±3), 14 (±3) and 14 (±4), respectively (Table 7). The post-treatment DMFT scores at 1 week, 3 months and 6 months were not significantly different from the

Table 4
Mean study subject bacterial levels pre-treatment and 1 week post-treatment

Time	Bacteria levels				
	Total bacteria (Log CFU)	*Streptococcus mutans* (Log CFU)	*Streptococcus sobrinus* (Log CFU)	*Streptococcus mutans*/total bacteria ratio	*Streptococcus sobrinus*/total bacteria ratio
Pre-treatment (n = 27)	6.14	4.02	1.39	0.92	0.003
1-week post-treatment (n = 25)	6.14	2.85	0	0.11	0
p-values	0.178	0.014	0.216	0.05	0.588

CFU: colony-forming unit

Table 5

Comparison of change in bacterial levels among study subjects after treatment

Dependent variable	Regression coefficient and *p*-value				
	Pre-treatment	1 week post-treatment	3 months post-treatment	6 months post-treatment	1 year post-treatment
Total bacteria	6.0834 (p<0.0001)	-0.0663 (p=0.5946)	-0.1114 (p=0.5186)	0.1977 (p=0.0487)*	0.1202 (p=0.2422)
Streptococcus. mutans	10.7707 (p 0.0001)	0.0830 (p=0.9321)	-0.4311 (p=0.4907)	-0.0759 (p=0.8142)	-0.2326 (p=0.4248)
Streptococcus sobrinus	9.0387 (p<0.0001)	-2.0280 (p=0.0867)	-1.4531 (p<0.0001)	-1.0970 (p=0.0527)	-0.4076 (p=0.3044)
% *S. mutans*/total bacteria	0.8916 (p=0.0139)	0.1827 (p=0.8282)	0.0992 (p=0.8929)	0.0913 (p=0.8417)	-0.0233 (p=0.9593)

Note: p-values were calculated using the Generalized Estimating Equation. * indicates significantly difference.

pre-treatment score. The mean DMFT score at 1 year post-treatment was significantly higher (p=0.001) than the 6 month post-treatment score (Table 8).

We found a significant positive association between the plaque index and bacterial level (p=0.0027). We also found a significant positive association (p<0.0001) between the plaque index and the *S. sobrinus*/total bacteria ratio (Table 9). We found no significant association between the DMFT score and the *S. mutans*, *S. sobrinus* and total bacteria levels.

Forty-eight percent of subjects developed new dental caries during the 3-12-month period post-treatment. Of those with new caries, 31% had both *S. mutans* and *S. sobrinus*, 54% had *S. mutans*, 8% had *S. sobrinus* and 8% had no bacteria detected.

DISCUSSION

In our study we found a significant reduction in *S. mutans* and *S. sobrinus* levels at 1 week and 3 months post-treatment, respectively. However, by 6 months post-treatment the levels of these pathogenic bacterial had

Table 6

Mean plaque index and oral hygiene levels among study subjects over time

Examination type	Examination time				
	Pre-treatment	1 week post-treatment	3 months post-treatment	6 months post-treatment	1 year post-treatment
	(*n* = 27)	(*n* = 25)	(*n* = 15)	(*n* = 21)	(*n* = 19)
Mean (± SD) plaque index	2 (±1)	1 (±1)		1 (±1)	1 (±1)
Oral hygiene level	Poor	Fair	Fair	Fair	Fair

SD: standard deviation

Rating of oral hygiene level from plaque index: 0-0.6 = good, 0.7-1.8 = fair, 1.9-3.0 = poor

Table 7

DMFT scores among study subjects over time

DMFT score	Pre-treatment	1 week post-treatment	3 months post-treatment	6 months post-treatment	1 year post-treatment
	(*n* = 27)	(*n* = 25)	(*n* = 15)	(*n* = 21)	(*n* = 19)
Mean ± SD	14±4	14±4	14±3	14±3	14±4
(Min, Max)	(6, 20)	(6, 20)	(10, 20)	(8, 20)	(8, 20)
Median	14	14	14	15	15

DMFT: decayed, missing and filled teeth; Max: maximum; Min: minimum

Table 8

Comparison of plaque index and DMFT scores over time

Dependent variable	Linear regression coefficients and p-values				
	Pre-treatment	1 week post-treatment	3 months post-treatment	6 months post-treatment	1 year post-treatment
Plaque Index	1.8850 (p<0.0001)	-1.1125 (p<0.0001)	-0.9425 (p<0.0001)	-0.8887 (p<0.0001)	-0.8650 (p<0.0001)
DMFT scores	14.000 (p<0.0001)	-0.0169 (p=0.3345)	-0.0120 (p=0.8712)	0.3031 (p=0.0663)	0.9924 (p=0.0010)

DMFT: decayed, missing and filled teeth

Table 9

Assessment of the association between plaque index and bacterial levels and pathogenic to total bacteria ratios

Dependent variable	Independent variable	b^0	b^1	p-value
Plaque Index	lg10(total bacteria)	-1.2344	0.3872	0.0027
	Streptococcus mutans/ total bacteria (%)	1.1142	0.0070	0.2799
	Streptococcus sobrinus/ total bacteria (%)	1.1089	0.0973	<0.0001

Independent variable: levels of total bacteria, percentage of *Streptococcus mutans*/total bacteria and percentage of *S. sobrinus*/ total bacteria

Dependent variable: plaque index

b^0: Beta zero (intercept) or a value of plaque index when independent variable =0

b^1: Beta one (regression coefficient or slope) or the change in plaque index when the independent variable changes one unit

returned to pre-treatment levels. Our findings are similar to those of a previous study (Litsas, 2010).

In our study the mean *S. sobrinus* level was not significantly lower than the pre-treatment level at 1 week post-treatment but it was significantly lower 3 months post-treatment. However, by 6 months post-treatment the mean level was not significantly different from the mean pre-treatment level. The reason for this apparent delay in reduction in the mean *S. sobrinus* level is unclear but a similar finding was reported in a previous study but the significant reduction was seen 6 months post-treatment (Vishwanathan *et al*, 2020).

In our study, nearly half the subjects developed new caries during the one-year period post-treatment, similar to the findings of a previous study (Vinckier *et al*, 2001). We found no significant differences in the mean *S. mutans* and *S. sobrinus* levels between subjects with and without new caries. A reason for this finding could be that many factors are involved in caries development and these other factors had a greater influence on caries development than the levels of studied bacteria. In our study the proportions of subjects with both studied pathogens were relatively low, similar to the results of a previous study (Almeida *et al*, 2000). Other microorganisms, such as lactobacilli, *Actinomyces* spp, or *Candida* spp, may also have been involved in new caries development (Meriç *et al*, 2020).

In our study, the mean plaque index reduced significantly post-treatment and remained significantly lower than pre-treatment levels for a year. A previous study also reported a drop in plaque index with treatment but found the plaque index returned to pre-treatment levels within 3 months (Klinke *et al*, 2014) but our plaque index measurement method differed from that study so these results may not be comparable.

In our study, we found a significant positive association between plaque index and the *S. sobrinus*/total bacteria ratio but not between the plaque index and the *S. mutans*/total bacteria ratio. A possible explanation for this could be the part of the tooth from which plaque was collected. In this study, plaque was collected from all tooth surfaces of infected and non-infected teeth, but mostly from smooth surface areas. *S. sobrinus* mainly colonizes smooth tooth surfaces and causes smooth surface caries but *S. mutans* colonizes the chewing surfaces and caries found in those areas (Choi *et al*, 2009; Mosci *et al*, 1990).

In our study we found no significant association between the mean DMFT score and the bacterial levels throughout the study, similar to the findings of a previous study (Vishwanathan *et al*, 2020).

A major limitation of our study was not having the minimum number of subjects we calculated to be needed for the study due to COVID-19 closures. Our small study sample may have missed significant findings if the number of subjects were larger. Further studies with more subjects and for longer follow up periods are needed to better assess if there is a benefit not significantly evident in the current study. Another limitation was that we evaluated only two species of bacteria which did not reflect the complexity of oral microbiota.

In summary, the mean *S. mutans* level and *S. mutans*/total bacteria percentage decreased significantly 1 week post-treatment but the levels returned to pre-treatment levels thereafter. The mean *S. sobrinus* level decreased significantly at 3 months post-treatment but also returned to the pre-treatment level thereafter. The mean plaque index decreased significantly post-treatment and continued to be significantly lower than the pre-treatment level throughout the study period but this did not result in improved oral pathology. The mean DMFT score was not significantly lower post-treatment. The mean plaque index was significantly positively associated with the total bacteria level and the *S. sobrinus*/total bacteria ratio. In conclusion, our results show dental treatment under general anesthesia is not beneficial for long term control of dental caries or control of pathogenic bacterial levels.

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CONFLICT OF INTEREST DISCLOSURE

All authors declare no conflict of interest.

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