Original Article

A Comparison of Pre- and Postbreakfast Tooth Brushing in Caries Prevention through the Estimation of *Streptococcus mutans* Counts: A Prospective Clinical and Microbiological Study

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Abstract

Objectives: The purpose of this study was to compare the efficacy of prebreakfast tooth brushing (PrBTB) and postbreakfast tooth brushing (PoBTB) with or without a prebrushing rinse in caries prevention through the estimation of *Streptococcus mutans* count. **Materials and Methods:** Sixty consenting dental students were divided into three age-matched groups of 20 each and instructed to follow prebreakfast, postbreakfast tooth brushing, and prebreakfast rinsing plus PoBTB using the Bass technique. Plaque samples were collected at the start and at the end of every quarter for a total of 6 quarters (18 months) for the evaluation of *S. mutans* colony-forming unit (CFU) counts. The decayed missing filled surface (DMFS) index of participants was also recorded and compared at the beginning and end of the study period. **Results:** The post-breakfast tooth brushing group with pre-breakfast rinse (RPoBTB) and without pre-breakfast rinse (PoBTB) showed a highly significant reduction in total *S. mutans* CFU counts per ml (38% and 29% respectively) at the end of the study. The changes in DMFS value were not significant and did not show any correlation with the *S. mutans* counts. **Conclusion:** Our study revealed that PoBTB with or without a prebreakfast rinse reduces the total counts of the cariogenic bacteria *S. mutans* more efficiently than PrBTB. Although further proof in the form of clinical trials is essential, this study provides the proof of concept for a minor change in the tooth brushing habit, which can significantly enhance caries prevention.

Keywords: Colony-forming units, dental caries, microbial count, postbreakfast tooth brushing, prebreakfast tooth brushing, rinsing, *Streptococcus mutans*

Introduction

Dental caries is a multifactorial ecological disease in which diet, host, and the microbial flora interact over a period of time in such a way as to initiate demineralization of the tooth enamel with resultant caries formation.[1] Microbial plaque is a critical factor in the multifactorial etiology of caries. Acquired pellicle is an indispensable component of dental plaque formed just before or concomitant with the bacterial colonization that may facilitate the plaque formation.[2] *Streptococcus mutans* (*S. mutans*) is the chief initiator of caries; a major habitant of supragingival plaque. *S. mutans* possess several abilities, such as the ability to adhere to the salivary pellicle on enamel, produce acidic intermediate metabolites, build glycogen reserves, and synthesize

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extracellular polysaccharides, which are essential in dental caries initiation and propagation.[3]

Plaque removal by daily tooth brushing is recognized as an indispensable means of caries prevention.[1-3] The most commonly advised tooth brushing protocol is a twice-daily brushing protocol once at night and once in the morning. Brushing at night is essential because the dinner's leftover food residues would provide the ideal substrate for *S. mutans* to produce demineralizing acids throughout the night.[4] The reduced salivary secretion during the night (0.1 ml/min at rest compared to 1.0 ml/min stimulated secretion)[2] enables both increased volume and strength of the acid overnight by a combination of reduced clearance and buffering, respectively. It is generally agreed that night brushing is the most effective when completed after dinner or just before sleep.

On the other hand, no consensus exists on the most beneficial timing for the morning tooth brushing. Some dentists advise their patients to brush upon waking up, and others recommend brushing after breakfast.[5] Water rinsing cleans the oral cavity by increasing the oral clearance,[6] which can be assumed to help reduce dental caries.

This study was designed to compare pre and postbreakfast tooth brushing (PoBTB) and an additional rinse along with postbrushing on caries prevention through variations in *S. mutans* counts.

Materials and Methods

The study was conducted in 2009-2010. Declaration of Helsinki and Informed Consent was obtained. The study was conducted on 60 dental students. Equal numbers of age-matched male and female dental students were included in the study after obtaining informed consent. Clearance for the study was obtained from the institutional ethical board. Students with ages ranging from 19 to 28 years with a minimum of 20 natural fully erupted permanent teeth were included in the study. Students suffering from acute caries, periodontitis, fluorosis, or any major systemic illnesses and those undergoing orthodontic therapy or long-term antibiotic therapy were excluded from the study. The students were divided randomly into three equal groups of 20 each. The groups were as follows:

1. Prebreakfast tooth brushing group (PrBTB)
2. PoBTB group
3. Prebreakfast rinsing followed by PoBTB group (RPoBTB).

Bass brushing technique was demonstrated to all participants at the beginning of the study. The participants were instructed to brush with the Bass technique using soft toothbrushes and fluoridated toothpaste. All participants were asked to brush at night just before bed, change toothbrushes every 3 months, or when the bristles were damaged, whichever was earlier. Oral prophylaxis was performed on every participant to ensure a uniform oral hygiene status at the start of the study.

Two weeks after oral prophylaxis, the baseline decayed missing filled surface (DMFS) score[7] was calculated, and the plaque samples for the baseline colony-forming unit (CFU) counts of *S. mutans* were collected.

Microbiological sampling and counts

One side mandibular first molar was chosen for every sample collection in each subject. The plaque samples were collected from the middle third of the lingual surface; the sample collection site was chosen because of ease of access with a sterile toothpick. After collection, the toothpick ends with the plaque samples were placed in test tubes containing 2 ml of brain heart infusion broth. The test tubes with the samples were incubated at 37°C for 24 h to propagate bacteria. 100 μL of the diluted sample (1:10 dilution)[8] was transferred to Mitis Salivarius Bacitracin (MSB) Agar media, a selective and differentiating media for *S. mutans* containing Mitis Salivarius Agar base, 20% sucrose, 1% potassium tellurite, and 0.2 units/ml of bacitracin. The MSB agar plates were placed in an anaerobic candle gas jar and then incubated at 37°C for 48 h. The *S. mutans* colonies were identified based on individual colonies being 0.5–1 mm in diameter, usually gray-translucent to white, circular or irregular, sometimes with a rough surface[1] urther confirmation of colonies was done by biochemical tests of positive mannitol fermentation and negative arginase test.[9] Total CFU of *S. mutans* was counted using a colony counter [Figures 1a-c and 2]. The total CFU counts were expressed as CFU per ml.

Statistical analysis

The results were analyzed using one-way analysis of variants for multiple comparisons followed by *post hoc* Tukey's test for group-wise comparison. An intragroup comparison was made by paired *t*-test. Spearman's rank correlation coefficient was done to analyze the correlation between DMFS and CFUs per ml counts of *S. mutans* at baseline and the end of the study.

Results

The present study aimed to compare the effectiveness of PrBTB, PoBTB and RPoBTB in caries prevention by comparison of total *S. mutans* colony-forming unit counts and DMFS values. A total of 60 dental students were divided into three groups, i.e., PrBTB, PoBTB, and RPoBTB, comprising 20 subjects.

Changes in *Streptococcus mutans* colony-forming unit counts per ml at different time intervals observed in three groups

The baseline *S. mutans* CFU counts per ml for RPoBTB, PoBTB, and PrBTB groups ranged from 102.9–107.9 [Table 1 and Figure 3]. The quarterly CFU counts of the PrBTB group showed a seesaw variation through the quarters. The counts showed a gradual increase through the 1st two quarters, a decline through the 3rd quarter, and subsequent rise through the remaining three quarters. Overall, the changes were not significant from that of the baseline till the end of the study. The PoBTB group showed a gradual decrease in the CFU counts per ml till the last quarter, when it increased slightly.

Table 1: Changes in total microbial counts (colony-forming units per ml) at different time intervals in three groups

Groups	Number of subjects	Baseline	3rd month	6th month	9th month	12th month	15th month	18th month
PrBTB	20	107.9±16.0	116.1±14.5	120.7±11.4	96.8±31.4	101.8±29.8	102.1±21.6	100.7±19.3
PoBTB	20	108.4±18.5	97.8±12.8	97.2±13.0	75.2±23.3	74.4±26.3	74.2±26.1	77.0±16.9
RPoBTB	20	102.9±17.0	99.8±15.5	96.1±13.7	72.2±26.0	64.2±23.4	64.1±12.4	63.9±12.6

PrBTB: Prebreakfast tooth brushing group, PoBTB: Postbreakfast tooth brushing group, RPoBTB: Prebreakfast rinsing followed by postbreakfast tooth brushing group

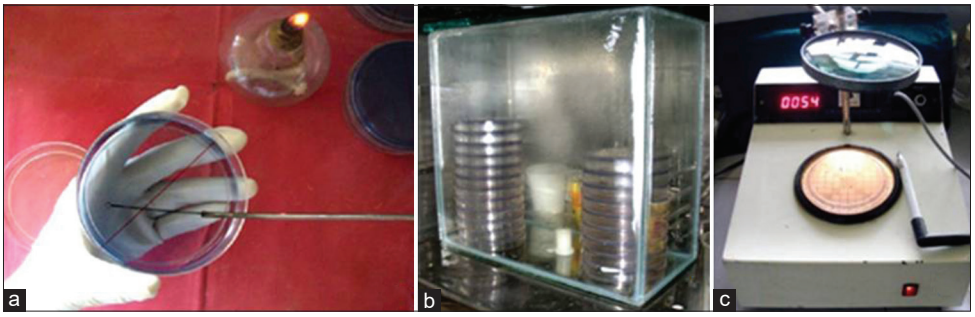

Figure 1: Photograph showing (a) inoculation of plaque samples in Mitis Salivarius Bacitracin agar by Z-streak method, (b) inoculated agar plates in anaerobic candle gas chamber, (c) colony counting machine

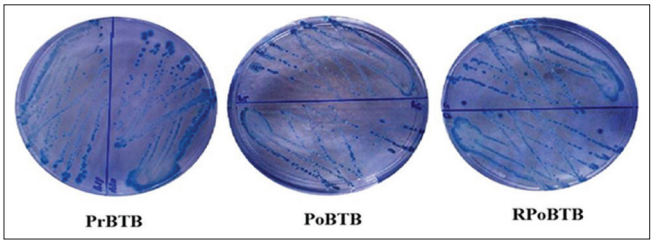

Figure 2: Culture plates showing bacterial colonies in various study groups

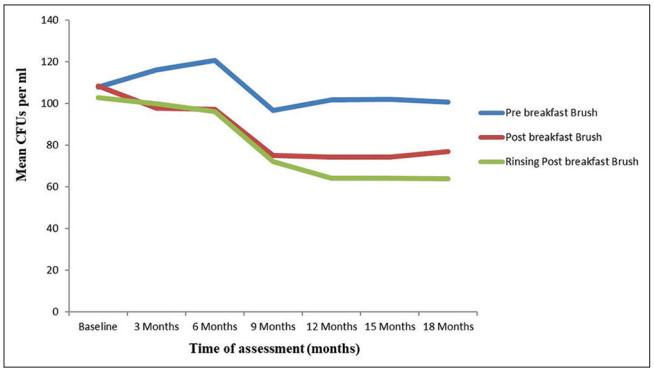

Figure 3: Graph showing microbial counts (colony-forming unit/ml) in various study groups

The decline was more pronounced at the end of the 3rd quarter and 4th quarters.

In terms of intragroup comparison, the PrBTB group showed a statistically insignificant reduction of 7% in the CFU counts per ml at the end of the study. The PoBTB group showed a highly significant ($P < 0.001$) total *S. mutans* CFU count reduction amounting up to 29% by the end of the study. The RPoBTB group also showed a highly significant ($P < 0.001$) total CFUs decrease of 38%, which was the maximum in the study [Table 2 and Figure 4].

Intergroup comparison of colony-forming unit counts per ml

The intergroup comparison was made by *post hoc* Tukey's test. The difference in the baseline CFU counts per ml between all the groups was statistically insignificant [Table 2 and Figure 4]. While all groups showed a decline compared to the baseline, there were highly significant differences in decline levels. The fall in numbers was the least in the PrBTB group at 7%, followed by 29% in PoBTB and 38% in the RPoBTB group [Figure 5]. The counts in the PoBTB group were significantly lower than the PrBTB group. There was no statistically significant difference between CFU counts per ml of the PoBTB and RPoBTB group.

Variation in decayed missing filled surface value at the end of the study as compared to baseline

By the end of the study, there were insignificant changes in the DMFS scores of groups [Table 3 and Figure 6]. One occlusal pit lesion in the PrBTB group, two new buccal pit lesions in the PoBTB group, and one extraction and new occlusal caries in the RPoBTB groups were the cause of the changes [Table 4]. Spearman's rank correlation coefficient, to evaluate any correlation between DMFS and CFUs per ml at the end of the study, gave statistically insignificant values [Table 5].

Discussion

A prospective microbiological study was carried out on 3 equally divided groups, i.e., PrBTB, PoBTB, and RPoBTB groups to determine the effect of time of tooth brushing in the morning on *S. mutans* counts and caries. Plaque samples from all subjects were collected quarterly (3 months) for one and a half years (18 months).

The study demonstrated a statistically significant difference in CFUs between the three groups, with the subjects of RPoBTB

Table 2: Intragroup and intergroup comparison of microbial counts (colony-forming units per ml) in various groups

Groups	Baseline CFUs	18th month CFUs	Difference	Percentage reduction	Significance *t**, *P*
PrBTB	107.9±16.0	100.7±19.3	7.2±27.5	7%	1.18, 0.25 (NS)
PoBTB	108.4±18.5	76.9±16.9	31.4±21.2	29%	6.61, <0.001 (HS)
RPoBTB	102.9±17.0	63.8±12.6	39.0±17.1	38%	10.19, <0.001 (HS)
ANOVA F**	0.63	25.6<0.001 (HS)	11.01<0.001 (HS)	-	-
P	0.54 (NS)				
Difference between groups*** (*P*)					
PrBTB - PoBTB	0.99 (NS)	<0.001 (HS)	<0.05 (S)	-	-
PrBTB - RPoBTB	0.63 (NS)	<0.001 (HS)	<0.001 (HS)	-	-
PoBTB - RPoBTB	0.57 (NS)	<0.05 (S)	0.53 (NS)	-	-

*Intragroup comparison - paired *t*-test, **Intergroup comparison - one-way ANOVA, ****Post-hoc* Tukey's test, *P*≤0.05 statistically significant. PrBTB: Prebreakfast tooth brushing group, PoBTB: Postbreakfast tooth brushing group, RPoBTB: Prebreakfast rinsing followed by postbreakfast tooth brushing group, CFU: Colony-forming unit, S: Significant, NS: Not significant, HS: Highly significant, ANOVA: Analysis of variants

Table 3: Intragroup and intergroup comparison of decayed, missing, filled surfaces on the tooth in various groups

Groups	Baseline DMFS	18th month DMFS	Difference in DMFS	Significance	
				*t***	*P*
PrBTB	1.50±2.06	1.55±2.04	0.05±0.22	1.00	0.33 (NS)
PoBTB	1.50±1.85	1.60±1.85	0.10±0.31	1.45	0.16 (NS)
RPoBTB	3±3.03	3.25±3.20	0.25±1.12	1.00	0.33 (NS)
ANOVA F*	1.07	1.43	0.47		
P	0.35 (NS)	0.25 (NS)	0.63 (NS)		

*Intragroup comparison - paired *t*-test, **Intergroup comparison - one-way ANOVA *P*≤0.05 statistically significant. PrBTB: Prebreakfast tooth brushing group, PoBTB: Postbreakfast tooth brushing group, RPoBTB: Prebreakfast rinsing followed by postbreakfast tooth brushing group, DMFS: Decayed, missing, filled surfaces on the tooth, ANOVA: Analysis of variants, NS: Not significant

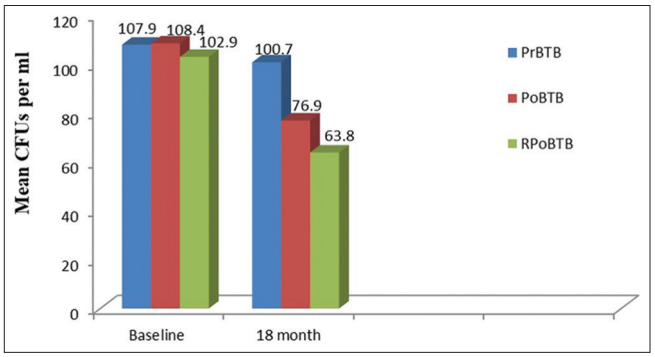

Figure 4: Graph showing microbial counts (colony-forming units per ml) at baseline and at the end of the study in three groups

group showing the most significant reduction. However, the difference in the DMFS value between baseline and at the end of study for the three groups was statistically insignificant. The above results were indicative that RPoBTB in the morning was far superior to PrBTB in terms of reducing the overall *S. mutans* CFU counts over a period of time. PrBTB removes bulk of the overnight accumulated plaque[5,10,11] and *S. mutans*, just prior to the breakfast. Even the most effective brushing, will not be able to remove all the cariogenic bacteria and few cariogenic bacteria will be left on the tooth surface. [5] Breakfast provides sucrose to the remaining S. mutans helping them to both recolonize and start the lactic acid production using their intrinsic enzymatic machinery activity.[3] This recolonization will continue throughout the day till the next scheduled tooth brushing, i.e., at night and growth is supplemented by substrate provided by intervening meals (the lunch and dinner) and snacks. Hence, the time duration for which *S. mutans* is active in acid production is 13–17 h, i.e., time interval between breakfast and night tooth brushing. We believe this to be the explanation for the high *S. mutans* CFU counts per ml in the PrBTB group at the end of the study as compared to other two groups. However, in the PoBTB group, the time duration for *S. mutans* to act upon substrate is decreased to 8–10 h, as PoBTB removes bulk of *S. mutans* along with food remnants left over from breakfast. Hence, effectively the substrate supplies to the left over *S. mutans* is cut off till lunch or till next snack is consumed. As the samples were collected just prior to lunch, the total CFU counts per ml gave a highly significant reduced value compared to baseline value for PoBTB group. In the RPoBTB group, the further reduction can be explained on the basis that in this group, PoBTB was further supplemented by prior water rinsing. Prebreakfast water rinsing in the RPoBTB group facilitated the dilution and removal of some of the buildup bacteria and acids[12,13] accumulated overnight in addition to the previously explained effect of PoBTB.[13,14] This is a very likely explanation of the greater decrease in total CFU counts per ml in this group as compared to the PoBTB group. The difference in the total CFUs per ml between PoBTB and RPoBTB groups was not significant, suggesting that additional rinsing has no significant implication in reducing total CFUs per ml when coupled with PoBTB. Our findings could not be compared with other studies since similar studies on timing of tooth brushing and microbial estimation are not available in existing published literature.

The PrBTB group showed a slight increase in CFU counts per ml between the baseline to 6th month followed by a prominent decline from 6th to 9th month and then a near plateau phase till the end of study period. PoBTB and RPoBTB groups showed

Table 4: Variation in decayed, missing, filled surfaces on the tooth value at 18th month as compared to baseline in various groups

Groups	Total baseline DMFS	18th month DMFS	Variation
PrBTB	30	31	+1 (a buccal pit lesion in 1 subject)
PoBTB	30	32	+2 (occlusal pit lesion each in 2 separate subjects)
RPoBTB	60	65	+5 (an extraction in 1 subject and an occlusal caries in another subject)

PrBTB: Prebreakfast tooth brushing group, PoBTB: Postbreakfast tooth brushing group, RPoBTB: Prebreakfast rinsing followed by postbreakfast tooth brushing group, DMFS: Decayed, missing, filled surfaces on the tooth

Table 5: Correlation between decayed, missing, filled surfaces on the tooth and colony-forming units per ml value at baseline and 18th month

Values	Group	Spearman's rank correlation coefficient	Significance (*P*)
Baseline	PrBTB	0.166	0.484 (NS)
	PoBTB	0.232	0.325 (NS)
	RPoBTB	−0.044	0.853 (NS)
18th month	PrBTB	0.060	0.841 (NS)
	PoBTB	−0.053	0.824 (NS)
	RPoBTB	−0.371	0.107 (NS)

NS: Not significant, $P \leq 0.05$ statistically significant, PrBTB: Prebreakfast tooth brushing group, PoBTB: Postbreakfast tooth brushing group, RPoBTB: Prebreakfast rinsing followed by postbreakfast tooth brushing group

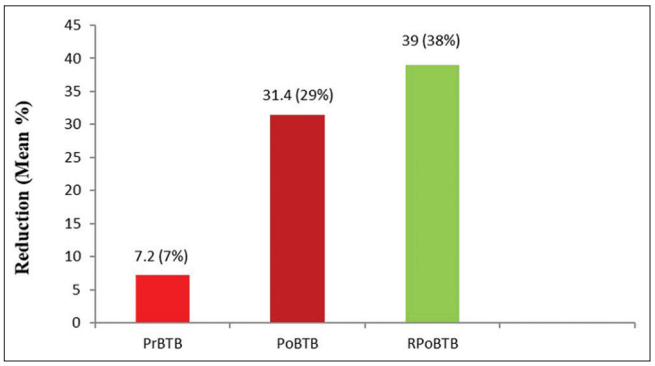

Figure 5: Graph showing mean reduction of microbial counts (colony-forming units per ml) in three groups

a decline in the CFU counts throughout with more prominent decrease between 6th and 9th month. The introduction of Bass technique[13] in all groups could be expected to cause some loss of brushing efficacy in the initial months, demonstrated by the rise in CFU counts per ml in PrBTB group between the baseline to the 6th month. The CFU counts per ml of PoBTB and RPoBTB group, however, showed a slight decline in the same period, which might have been possibly due to the additional effect of the postbreakfast brushing time negating the loss of brushing efficacy. The prominent reduction in all the groups between 6th and 9th month demonstrated increase in efficiency of Bass technique with practice. The near plateau in the counts achieved from 12th month onward may be attributed to the microbial adaptation to the changing oral environment due to the new technique and timing of brushing.

The difference in DMFS value at the baseline and at the end of the study did not give any significant result. This could be explained by the time duration required for caries development.[14] The average time for incipient caries to develop into clinical caries is 18 ± 6 months,[2] which can explain the insignificant difference in DMFS value at the end of the study. Further, it could be attributed to overall improved technique in maintaining the oral hygiene producing an effective inhibitory effect on microbes. The use of fluoridated tooth paste also probably added to not significant DMFS difference. No significant correlation was noted between DMFS and CFUs per ml value in all the three groups. In the PrBTB group, one subject showed a new lesion, in the PoBTB group, two subjects developed new lesions, and in the RPoBTB, one subject underwent extraction for a preexisting deep carious tooth and another one developed an occlusal caries at the end of study period. This could probably be attributed to the multifactorial etiology of caries which were not considered in the study such as dietary details, previous oral hygiene measures, and variation in caries susceptibility of individual subjects. To the best of our knowledge, similar study was not available in existing literature for comparison of findings warranting further research.

Limitation of the study and future scope

PoBTB and RPoBTB groups showed a very marginal rise in CFU counts per ml in the last month, thus pointing to a possible microbial adaptation. This demonstrates a need for a longer duration of the study to develop a clear picture of the *S. mutans* adaptation that might follow in the postbreakfast brushing groups. The participants were not asked about their existing oral hygiene habits, which reflected in the baseline CFU count. Few cases were outlier in result in terms of CFU counts per ml because of which average of one group was higher (in RPoBTB group there was a preexisting deep carious tooth). Probable reason for this microbial count could be attributed to diet or lack of following of instructions given to the study group. Another shortcoming of the study is that at the beginning of the study, treatment should have been given to each participant as existence of caries could act as a source of infection in oral cavity. In addition, setting a maximum DMFS score for the inclusion of subjects in the study will ensure that subjects are equally distributed in the groups and thus preventing any bias being introduced due to variation in caries susceptibility and activity.

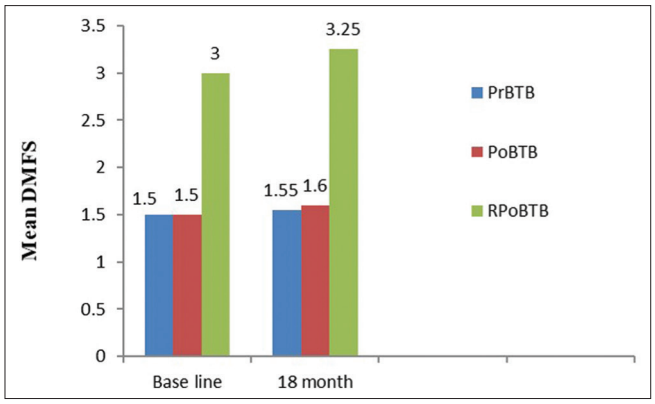

Figure 6: Graph showing Decayed missing filled surface values at baseline and at the end of the study in three groups

Conclusion

Timing of morning brushing has a highly significant effect in the reduction of *S. mutans* counts and PoBTB is the most effective in this regard. Additional water rinsing can reduce the *S. mutans* counts further; however, the reduction is not significantly more than when brushing alone is used. There is a severe scarcity of available literature on the effects of brushing in relation to timing of tooth brushing and caries development. This necessitates further research work in this area of cariology.

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Conflicts of interest

There are no conflicts of interest.

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