International Journal of Dental Hygiene

Official Journal of the International Federation of Dental Hygienists

ORIGINAL ARTICLE

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The comparative evaluation of the effects of tongue cleaning on salivary levels of mutans streptococci in children

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Dates:
Accepted 25 June 2011

To cite this article:
Int J Dent Hygiene **10**, 2012; 107–112
DOI: 10.1111/j.1601-5037.2011.00522.x
Rupesh S, Winnier JJ, Nayak UA, Rao AP, Reddy V, Peter J. The comparative evaluation of the effects of tongue cleaning on salivary levels of mutans streptococci in children.

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Abstract: *Objectives:* The study compared and evaluated the effects of tongue scraping and tongue brushing on salivary mutans streptococci levels in children. *Methods:* The investigation was a single-blind, stratified comparison of three parallel groups of children who performed either tongue scraping or tongue brushing along with tooth brushing or only tooth brushing twice daily under professional supervision for a 21-day period. A total of three saliva samples were taken from each individual, at baseline, on day 10 and on day 21, and colony counts of mutans streptococci were determined. All data were subjected to statistical analysis using Wilcoxon's signed-ranks sum test and Mann–Whitney *U*-test. *Results and conclusions:* The results of the present study show that tongue scraping and tongue brushing demonstrated statistically significant reductions in salivary mutans streptococci counts after 10 days and also after 21 days. It was also noted that tongue scraping and tongue brushing were equally effective in reducing colony counts.

Key words: mutans streptococci; tongue brushing; tongue scraping

Introduction

Tooth brushing is the most frequently practiced oral hygiene procedure, but it cleans only selected areas of the tooth and the gingiva. Even adjunctive methods of oral hygiene such as water jet devices, dental floss and toothpicks, when successfully employed, clean only tooth surfaces and gingiva (1).

The tongue is a haven for the growth of microorganisms, the papillary nature of the tongue dorsum creating a unique ecological site that provides an extremely large surface area, favouring the accumulation of oral bacteria (2). The oral surfaces are colonized by over 500 bacterial species, and tongue has the largest bacterial load of any oral tissue and makes the greatest contribution to the bacteria found in saliva (3). More than 100 bacteria may be attached to a single epithelial cell on top of the tongue, whereas only about 25 bacteria are attached to each cell in other areas of the oral cavity (4).

Tongue cleaning and scraping have been used since antiquity, and many ancient religions emphasized cleanliness of the entire mouth including the tongue. The 'Datana' or Indian toothbrush made of a tree twig from an aromatic plant was about 8 inches long and equal in circumference to the smallest finger. This green twig was crushed and chewed

at the end until it became a soft brush. After 20–30 min of up and down brushing of the teeth, the twig was split, bent in an inverted 'V' shape and used as a tongue scraper. This procedure was used twice a day. The ancient Hindus also used tongue scrapers with sharp curved edges made of silver, gold, copper, tin or brass. Tongue scraping is still practiced in India and the Far East by the use of flat plastic strips (5).

Few references appear in the early 20th century dental literature about tongue scraping or cleaning. The Army and Navy Cooperative Society's 1907 catalogue listed bent and straight tortoise shell and ivory tongue scrapers. Only a brief reference to tongue brushing was made by one participant in the celebrated National Preventive Symposium of 1915 (5). The last few years however have brought forth scores of devices and gadgets to clean the tongue (6).

Because dental caries and gingival diseases are both believed to be produced by microorganisms, it appears obvious that the function of oral hygiene procedures should be to reduce the number of microorganisms in all areas of the oral cavity including the tongue (1). Although tongue brushing and tongue scraping have been practiced for hundreds of years, they are still not completely understood by the public. The effects of these simple indigenous adjunctive oral hygiene procedures in children have not been documented, and there is a relative paucity of studies in the dental literature in this regard. Hence, the present study compared and evaluated the effects of tongue scraping and tongue brushing on salivary mutans streptococci levels in children.

Materials and methods

The investigation was a single-blind, stratified comparison of three parallel groups of children who performed either tongue scraping, tongue brushing or only tooth brushing (placebo group) twice daily under professional supervision for a 21-day period. All the school children in the age group of 9–12 years from a residential school (boarding school) for boys were examined using oral health survey forms, and 45 children were selected based on the following selection criteria:

1 Subjects in the age group of 9–12 years.
2 At least four restored, decayed and/or missing teeth (DMFS/dmfs ≥4).
3 Subjects adhering to twice-daily tooth brushing routine (using toothbrush and non-fluoridated toothpaste) and practicing no other oral hygiene measures, either professional or home based, other than the requisites of the research project.
4 No history of antibiotic usage during the past 1 month.
5 No orthodontic appliance worn.
6 No abscess, draining sinus, cellulitis or other conditions requiring emergency dental treatment.
7 Participant cooperation and acceptance of the treatment regimen.
8 No medical/hereditary condition or long-term/recent/current regimen of medication that can affect salivary flow or necessitate diet modification.

Verbal consent from children and signed consent forms from parents or guardians were obtained after the nature of the study and the possible risks were fully explained. The study project was approved by the concerned ethics committee. The allocation of subjects resulted in three balanced treatment groups of 15 subjects each that were comparable in terms of gender (only male subjects could be chosen as the study was carried out at a boarding school for boys), age, number of teeth and average dmfs/DMFS. The oral hygiene measures assigned to each group were as follows:

Group I: This group involves 15 participants who constituted the tongue-scraping group. Participants were given a metal tongue scraper and asked to scrape the dorsum of the tongue twice daily. The tongue scraper used in the present study was an inverted 'V' shaped stainless steel scraper with plastic handles at the ends. The scraper was grasped by the handles and the apex of the inverted 'V' made of a flattened stainless steel strip was placed on the dorsum of the tongue. The following instructions were given:

1 Place the tongue as far out of the mouth as possible and place the tongue scraper as far posterior as possible on the tongue (comfortable enough to avoid gagging).
2 Apply force on the scraper to flatten the tongue, making it conform to the surface of the tongue and pull the scraper forward slowly but firmly up to the tip of the tongue.
3 Spit out the excess saliva and/or debris that accumulates on the tongue and remove the debris from the tongue scraper by placing it under a stream of running water.
4 Repeat the procedure five times.

Group II: This group involves 15 participants who constituted the tongue-brushing group. Participants were given a soft multi-tufted nylon toothbrush with a mini head and asked to brush the dorsum of the tongue twice daily. The following instructions were given:

1 Place the tongue as far out of the mouth as possible. Place the brush as far posterior as possible on the tongue in the midline (comfortable enough to avoid gagging) and give five firm forward and backward strokes (moving the brush till the tip of the tongue and back).
2 Spit out the excess saliva and debris that accumulates on the tongue and clean the brush by placing it under a stream of running water.
3 Repeat the procedure on either side of the midline of the tongue.

Group III: This group involves 15 participants who continued with their regular tooth brushing regimen twice daily.

A monitor trained to instruct the subjects and to assist them to perform the various oral hygiene procedures directly supervised the treatment regimen at the residential school hostel where the participants were staying. The procedures were performed twice daily (once in the morning after breakfast and once after the evening meal).

Clinical procedure

Clinical assessments were performed at the residential school by a single examiner using portable dental operatories and accepted methods of infection control. The monitor coded the study subjects from 1 to 45 before clinical examination and saliva collection by the examiner to ensure that at no time was the examiner aware of the group assignment of any subject. The data were later decoded at the end of the investigation. At each examination, paraffin-stimulated whole saliva samples were collected in sterile bottles in the mid-morning with no eating/drinking for 2 h prior to the sampling. The child was asked to chew a piece of paraffin wax for 2 min after which the child expectorated the accumulated saliva into the sterile bottle. No transport medium was used, as culturing was performed within half an hour of collection of the samples (7). The samples were then subjected to microbiological analysis. Saliva samples were obtained from each individual initially, prior to the start of the experiment, to establish baseline mutans streptococci levels. Subsequent samples were obtained 10 and 21 days after the start of the experiment. Thus, a total of three saliva samples were taken from each individual.

At each visit, subjects were questioned, and an intraoral examination was performed to detect adverse or unusual reactions such as desquamation, owing to improper use of the tongue scraper or tooth brush. The size, site and severity of any lesions or aberrations and tentative diagnosis, if possible, were recorded. A judgement was made as to whether or not any findings were attributable to any of the methods used.

Microbiological method

Each saliva sample was vortexed vigorously for 30 s to ensure a representative mixture throughout the sample prior to the preparation of dilutions and plating. The medium used in this study for culturing salivary mutans streptococci was mitis salivarius bacitracin (MSB) agar (8). Hundred microlitres of the vortexed salivary sample was pipetted out using a standard 100-μl pipette, and serial dilutions were prepared. A 100 μl volume from each of the dilutions was pipetted onto separate agar plates and evenly spread onto the agar surface using sterile spreaders. The preparation of dilutions and agar plating were carried out within an inoculating hood. The plates were then incubated at 37°C for 48 h under 5–10% CO2. To avoid bias, all plates were processed and examined by the same investigator who was unaware of the group assignments of the samples. Colonies of mutans streptococci were identified as round or spherical, raised, convex, black in colour, ranging from a pinpoint to pinhead size with a rough surface. The colony count of each plate was recorded, and the mean colony-forming units (CFU ml^{-1}) was determined after multiplying the colony count of each plate with its respective dilution factor (9).

Statistical evaluation

All the data were entered into a database on Microsoft Excel and analysed using SPSS software (version 11; SPSS Inc., Chicago, IL, USA) with two-way ANOVA (for overall group mean comparisons), Wilcoxon's signed-rank sum test (for intra-group comparison of differences between baseline, day 10 and day 21 examinations of salivary mutans streptococci counts) and Mann–Whitney *U* test (for inter-group comparisons of salivary mutans streptococci counts).

Results

The sample characteristics of the study population are presented in Table 1. The mean salivary mutans streptococci counts of all the groups at baseline, day 10 and day 21 are shown in Table 2.

Intra-group comparison

Comparison of the differences in salivary mutans streptococci levels between baseline and day 10, between baseline and day 21 and between day 10 and day 21 are presented in Table 3. Children who performed tongue scraping and tongue brushing showed statistically significant reductions in salivary mutans streptococci counts after 10 days and also after 21 days. Statistically significant results were also obtained when comparing the results between day 10 and day 21. However, children who continued with tooth brushing alone exhibited no statistically significant reductions in salivary mutans streptococci counts after 10 days and even after 21 days when compared with the baseline. No statistically significant difference was found between the day 10 and the day 21.

Inter-group comparison

The inter-group comparisons of the salivary mutans streptococci counts at baseline, day 10 and day 21 are presented in Table 4. No statistically significant difference in salivary

Table 1. **Sample characteristics of the study population**

	Mean and standard deviation values			
Groups	Age	No. of teeth	dmft/DMFS	dmfs/DMFS
Group I *n* = 15	10.50 ± 1.09 (9–12)	23.42 ± 0.9	7.00 ± 2.37	11.50 ± 6.7
Group II *n* = 15	10.50 ± 1.00 (9–12)	23.42 ± 0.9	6.92 ± 2.88	11.42 ± 7.29
Group III *n* = 15	10.53 ± 1.16 (9–12)	23.42 ± 0.79	7.67 ± 3.75	11.33 ± 8.04

P value: 0.414.

Table 2. **The mean and standard deviation values of salivary mutans streptococci counts (log values) at baseline, day 10 and day 21 of all groups**

	Mean and standard deviation values at		
Groups	Baseline	Day 10	Day 21
I	5.94 ± 0.71	5.27 ± 0.55	4.58 ± 0.49
II	6.01 ± 0.74	5.18 ± 0.79	4.47 ± 0.65
III	5.60 ± 0.94	5.35 ± 0.91	5.45 ± 0.99

Table 3. **Comparison of differences between baseline, day 10 and day 21 examinations for salivary mutans streptococci counts (log values) using Wilcoxon's signed-rank sum test**

	Baseline versus day 10		Baseline versus day 21		Day 10 versus day 21	
Groups	*Z*	*P*	*Z*	*P*	*Z*	*P*
I	3.061	0.002*	3.059	0.002*	3.061	0.002*
II	3.064	0.002*	3.062	0.002*	3.059	0.002*
III	2.142	0.062†	1.471	0.141†	1.172	0.241†

*Highly significant.
†Not significant.

Table 4. **Inter-group comparison of salivary mutans streptococci counts (log values) at baseline, day 10 and day 21 using Mann–Whitney *U*-test**

	Baseline		Day 10		Day 21	
Groups	*Z*	*P*	*Z*	*P*	*Z*	*P*
I versus II	0.319	0.750*	0.000	1.000*	0.405	0.685*
I versus III	1.216	0.224*	0.203	0.839*	2.113	0.035†
II versus III	1.012	0.311*	0.087	0.931*	2.140	0.032†

*Not significant
†Significant.

mutans streptococci levels was observed between groups I, II and III at baseline and after 10 days. After 21 days, groups I and II showed statistically significant differences over group III ($P = 0.035$ and 0.032, respectively). No statistically significant differences were observed between groups I and II ($P = 0.685$).

Discussion

For several decades, the principal cariogenic microorganisms were the lactobacilli, which were the focus of caries studies. More recently, it was found that the DMF index is only weakly linked to salivary lactobacilli and is independent of plaque lactobacilli, that carious dentin is responsible for the salivary hyper-contamination with lactobacilli and that the lactobacillus count could hardly be a predictive test (10). Because of their relationship to the disease, the evaluation of mutans streptococci concentrations in saliva aids the diagnosis of caries activity (7).

Saliva enters the mouth essentially sterile from the salivary glands, but expectorated saliva contains over 100 000 000 cultivable CFU per ml. This means that large numbers of bacteria are constantly being shed into the saliva from the oral surfaces. The contributions of various surfaces would approximate the size of their surface areas with tongue making the greatest contribution and teeth perhaps only a 5% contribution. It has also been well established that increased bacterial growth on the tongue is the reason why there are increased numbers of bacteria in the saliva (1, 3, 6).

Tongue microflora projects a bacterial population that contains on average 29% streptococci, 48% gram-negative anaerobes and 2.5% with an H_2S-producing phenotype (11). Only one study so far has focused on Streptococcus mutans levels inhabiting the tongue. In this study, high numbers of Streptococcus mutans were repeatedly found on the dorsum of the tongue after thorough scrapings (12). Significant immediate reduction in salivary Streptococcus mutans after professional tooth cleaning and tongue scraping was also noted (13). This indicated that dorsum of the tongue was an important reservoir for Streptococcus mutans. Furthermore, another study found a significant correlation between the prevalence of Streptococcus mutans in saliva and its prevalence on the dorsum of the tongue (14). These studies suggest that oral hygiene measures should include the dorsum of the tongue, especially in high-risk patients, who have endogenously high levels of Streptococcus mutans residing in the oral cavity.

In the present study, salivary mutans streptococci counts were determined at baseline, day 10 and day 21. Because saliva samples collected immediately after diet may interrupt the microbial level of the oral environment, sampling was performed with no eating or drinking for 2 h prior (15). Because the results of the tests performed on unstimulated saliva are less reliable than those performed on stimulated secretion, paraffin-stimulated whole saliva samples were collected. Chewing helps to washout bacteria from the tooth surfaces and mix them with the secretion. The stimulation of flow rate also minimizes intra individual variations of secretion flow (7).

The collected saliva samples were then incubated in MSB agar, which is the most commonly used selective medium for mutans streptococci. In contrast to most other bacteria, mutans streptococci can grow in an environment with a high sucrose concentration and are resistant to a particular antibiotic, bacitracin (8). The technique adopted for agar plating and colony counting was similar to that suggested by Wan *et al.* (9).

Age is a critical factor in subject selection for many reasons, of which the most important is the number of tooth surfaces at risk. Subjects with a mean age of approximately 11 years were chosen because they were entering a period of high caries activity, with many permanent teeth erupting (16). Subjects with either rampant tooth decay or very poor oral hygiene were also included in the study as it was important to determine whether the protocol remained effective for all ranges of hygiene with different baselines for salivary mutans streptococci.

Because our study was conducted in a residential school for boys, female subjects could not be selected. However, epidemiological studies in caries prevalence have not shown any significant difference in the caries susceptibility of boys and girls at an average age (17).

As the present study was conducted in a residential school, all the subjects consumed the same diet during the period of investigation. Dental caries is a dietary carbohydrate–modified infectious disease, because the major causative factors are believed to be local in nature (18). The frequency of exposure to a cariogenic diet and the form of intake of cariogenic food substances appear to be important factors in the development of dental caries (19). As diet was controlled in our study, the different tongue cleaning procedures were possibly given the best chance of demonstrating their efficacy against salivary mutans streptococci.

The results of this clinical trial showed that both the tongue-scraping and the tongue-brushing groups exhibited statistically significant reductions in salivary mutans streptococci counts when baseline values were compared with post-treatment values after day 10 and day 21. These results showed that there is a definite decrease in the salivary mutans streptococci levels even within 10 days of regular tongue cleaning. The control group, however, did not show any statistically significant change, when the day 10 and the day 21 values were compared with baseline values.

Intergroup comparisons revealed that there were no statistically significant differences in salivary mutans streptococci levels between the three groups at baseline. This implies that all groups were statistically equivalent before the start of treatment. It was observed that after 21 days, both the tongue-scraping and tongue-brushing groups showed statistically significant differences over the control group.

Gilmore and Bhaskar (1) conducted one of the first studies involving tongue cleaning. They found that tongue brushing on a daily basis decreased the bacterial populations on the tongue (1). The study however focused on Streptococcus salivarius levels, and not Streptococcus mutans.

Gross *et al.* (20) found dramatic reduction in bacterial counts in the mouth when tongue brushing was combined with tooth brushing. DeBoever and Loesche (21) found 74% bacterial reduction on the tongue after tongue cleaning. However, in their study, tongue cleaning was combined with the use of chlorhexidine (both as rinse and as paste), which by itself might explain the tremendous bacterial reduction.

Menon and Coykendall (12) reported small changes in bacterial load (Streptococcal counts) after tongue scraping which were neither statistically nor bacteriologically significant. But their observations were based on a one-time tongue scraping by the volunteers with samplings done before and after the tongue-scraping procedure. The effects of tongue cleaning practiced as part of routine oral hygiene protocol and over a longer period of time were not evaluated. Quirynen *et al.* (22) evaluated the impact of tongue scraping and tongue brushing on microbial load and found that tongue cleaning did not significantly reduce the bacterial load. The study, however, did not focus on mutans streptococci; rather, the focus was on overall microbial load of the oral cavity, recovered with non-selective blood agar plates.

Our data are in agreement with White and Armaleh (23) who compared the efficiency of tongue scraping, saturated saline rinse and Listerine strips in reducing salivary mutans streptococci levels. They found that all the treatment groups showed a significant reduction in colony counts from baseline to one or more post-treatment periods. Tongue scraping was found to be more effective than saturated saline rinse and Listerine strips in reducing colony counts (23). However, their study was carried out on adult subjects who practiced the oral hygiene measures only once daily for seven days. The results of the present study, which was carried out on subjects in their late mixed dentition period, who practiced the oral hygiene measures twice daily, revealed that at the end of 21 days, tongue scraping and tongue brushing were equally effective in reducing colony counts.

The oral hygiene measures used in our study were simple, could be carried out fast and the benefits for most children far outweighed the small investment and time required to accomplish the procedure. Also, none of the participants had any compliance problems, aberrations, lesions, etc. any time during the study after using either the scraper or the brush. Our study was a novel attempt designed to simulate a realistic home regimen in which the subjects either performed tongue scraping or tongue brushing daily while continuing their normal twice-daily tooth brushing routine. In this context, it is noteworthy that the reductions in salivary mutans streptococci in our study occurred in addition to the effects of twice-daily tooth brushing.

The effect of mechanical oral hygiene techniques on salivary levels of microorganisms, especially mutans streptococci, is of great interest to dentists and dental hygienists focused on preventive care. Tongue cleaning seems to have a more dramatic effect on the salivary levels of mutans streptococci as compared to tooth brushing alone (20). Moreover, the concept of tongue cleaning is so logical and so simple that prevention-oriented people should need only minimal encouragement to incorporate tongue cleaning into their oral hygiene routine (6). Thus, in this new era of Dentistry, it is important that research prove the need to include the tongue in all oral hygiene measures. With tongue scraping/brushing becoming established as excellent tools for reducing the levels of mutans streptococci in the oral cavity, it would be of great interest to compare their efficacy with other more mainstream methods.

Conclusion

The tongue-scraping and tongue-brushing groups showed statistically significant reductions in salivary mutans streptococci counts after 10 days and also after 21 days when performed along with tooth brushing. Thus, in the present study, these simple tongue cleaning procedures emerged as effective adjunctive oral hygiene measures.

Acknowledgements

Ms Arularasi Aparna (Microbiologist, Rajah Muthiah Dental College & Hospital) is thanked for the expertise rendered in the microbiological procedures.

Conflict of interest

The authors declare that they have no conflict of interests.

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