Effect of Povidone-iodine on *Streptococcus Mutans* in Children With Extensive Dental Caries

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Abstract

Purpose: The purpose of this pilot project was to determine the effect of a 10% povidone-iodine solution on plaque *Streptococcus mutans* and on incidence of new caries in young children following dental rehabilitation under general anesthesia.

Methods: Twenty-five children ages 2 to 7 years, scheduled for dental treatment under general anesthesia, were enrolled. Children in the experimental group (N=13) had povidone-iodine applied 3 times at 2-month intervals. Control children (N=12) had no treatment. Plaque samples were taken from all children at baseline, 6 months and cultured for total bacteria and *S mutans*. Dental examinations were conducted at baseline, 6 months, and 1 year.

Results: Experimental and control children had similar dietary habits, caries experience, and *S mutans* levels at baseline. All children's *S mutans* counts decreased significantly at 6 months (P=.003). The difference between the 2 groups was not significant (P=.58). At 1 year, 5 of 8 children in the control group had new caries compared to 2 of 11 children in the experimental group (P=.06). Povidone-iodine was well accepted by participating families.

Conclusions: Extensive one-time restorative dental treatment resulted in a significant suppression *S mutans* levels at 6 months. Further exploration of the role of povidone-iodine in caries management is indicated. (*Pediatr Dent.* 2004;26:5-10)

KEYWORDS: *STREPTOCOCCUS MUTANS*, EARLY CHILDHOOD CARIES, CARIES PREVENTION, IODINE APPLICATION

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Dental caries in young children has a multifactorial etiology; therefore, preventive measures usually involve a combination of dietary counseling, oral hygiene, and fluoride application.[1] None of these interventions specifically targets *Streptococcus mutans*, the chief pathogen responsible for caries.[2] Furthermore, children who have had "one-time" restorative treatment completed under general anesthesia (GA) often quickly develop new caries following treatment.[3-5] Therefore, current methods of caries management that are limited to traditional preventive approaches in combination with restorative treatments have proved inadequate to control the disease.[3-8] New methods of managing dental decay in the primary dentition need to be developed.

An antibacterial agent that is effective and also acceptable to young children will be a useful supplement to current techniques for the prevention of caries. Chlorhexidine is the antimicrobial agent most familiar to dental professionals for prevention of dental caries in children.[9-12] It is delivered in mouth rinses and gels and, more recently, in varnishes. Chlorhexidine has been found to reduce *mutans streptococci* to low levels in saliva and dental plaque, although bacterial levels eventually increase to pretreatment levels.[9,10] The need for frequent application of chlorhexidine, plus other side effects such as unpleasant taste and staining,[11,12] has stimulated the search for alternatives that are more appropriate for young children.

Topical application of iodine solutions has also demonstrated suppression of oral *S mutans* populations. An early investigation indicated that a single application of 0.2% potassium iodine solution (KI) eliminated *mutans streptococci* from accessible human tooth sites for up to 13 weeks

following treatment.[13] A subsequent trial utilizing 2% iodine-potassium iodide solution (I_2-KI) reported similar findings, which persisted for 20 to 24 weeks after treatment.[14] Recently, investigators applied 10% povidone-iodine (PVP-I) at 2-month intervals to a group of children 12 to 19 months of age, identified to be at high risk for caries.[15] While none of the children in the PVP-I group developed caries, 5 of the 16 in the placebo group had caries in 8 months. Long-term follow-up corroborated the initial observations.[16] However, the response of oral bacteria to the 10% PVP-I was not measured.

PVP-I or betadine is a potent microbicidal agent with several advantages over other iodine solutions.[17] The 10% PVP-I solution contains 90% water, 8.5% polyvinylpyrrolidone (PVP), and only 1% available iodine and iodide.[18] Combining iodine with PVP increases its ability to dissolve in water and alcohol, reduces irritability, and decreases the staining caused by pure iodine.[19] PVP, the hydrophilic polymer that acts as a carrier in povidone-iodine, does not have any intrinsic antibacterial activity, but because of its affinity to the cell membrane, it delivers free iodine (I_2) directly to the bacterial cell surface.[19] Delivery of iodine to the sensitive elements of the cell membrane is a crucial event of antibacterial action. Iodine targets are located in the bacterial cytoplasm and cytoplasmic membrane, and its killing action takes place in a matter of seconds.[19]

The purpose of this pilot study was to investigate the effect of 10% PVP-I solution on *S mutans* levels and on incidence of new carious lesions in a group of young children, who had undergone oral rehabilitation under GA in a private practice setting.

Methods

Approval for the project was received from the Clinical Research Ethics Board of the University of British Columbia, Canada. This pilot study was designed as a randomized, single-blind, no-treatment control trial, and was conducted at Monarch Pediatric Dental Center, a pediatric dental practice in Burnaby, British Columbia. This private practice has 3 full-time pediatric dentists and an on-site general anesthetic suite. Twenty-seven children, ages 2 to 7 years, in need of GA as a consequence of caries were consecutively enrolled in the study over a 4-week period. All children resided in nonfluoridated communities. Criteria for a child to be included in the study were:

1. all treatment was to be performed under GA;
2. unremarkable medical history, particularly no history of thyroid disease;
3. at least 15 teeth remaining after completion of treatment.

Using a random number table, 15 subjects were randomly assigned to the experimental group and 12 to the control group (Figure 1). A survey instrument[20] obtained demographic and developmental data, including variables such as the child's age and gender, mother's age, and length of time the mother had been a resident of Canada. Data was also collected on feeding practices and oral hygiene. Height and weight of all participants were recorded at each study appointment.[20]

At the GA appointment following intubation, a dental examination, including the exposure of necessary radiographs, was conducted. The pediatric dentist treating the child performed the examination. However, the project investigator was always present to ensure consistency among the examiners. Tooth surfaces were scored for caries as 0=sound, 1=noncavitated, incipient lesion, or 2=cavitated lesion.

A pooled plaque sample was obtained by swabbing the gingival third of buccal surfaces of all the teeth with a sterile cotton swab (BBL CultureSwab System, Becton & Dickinson, Sparks, Md). The swabs were placed in transport media and shipped at room temperature to the authors' laboratory for processing within 24 hours of collection. The swab was placed into 1 ml of reduced saline (0.038 M NaCl, 1.073 mM KCl, 2.05 mM Na thiosulfate, 1 mM resazurin, and 23 mM L-cystine, PRAS) and vortexed for 1 minutes. The sample was serially diluted 1:10 in PRAS buffer, and 100 ml of the various dilutions was plated onto Brucella agar base (Becton Dickinson, Sparks, Md) and onto 5% blood supplement with vitamin K and hemin for total aerobic counts. Similarly, 100 ml of the various dilutions was plated onto Mitis Salivarius agar (Becton Dickinson, Sparks, Md) supplemented with 1% potassium tellurite and 2 mg/ml kanamycin (MSKB) to isolate *S mutans.*[21] All plates were incubated at 36.5°C for 72 hours. Only those colonies with typical *S mutans* were counted.

The active agent employed in this study was 10% povidone-iodine (Betadine solution, Purdue Frederick, Inc), applied 3 times at 2-month intervals to the teeth of children in the experimental group by dentists who had been standardized to the application technique. First, the teeth were wiped with a cotton roll and then betadine was applied by swabbing the dentition with a small sterile cotton ball that was saturated with the solution and held in locking cotton pliers. The teeth were wiped off immediately with gauze after application. Study dose for each application was approximately 0.20 ml—a dose that contains 2 mg iodine, which is nontoxic when applied bimonthly.[22]

After 6 months, all children had a follow-up dental examination by the available pediatric dentist with the project investigator in attendance. Plaque samples were taken from all subjects prior to tooth polishing and application of fluoride varnish. Parents were interviewed briefly to determine if their child had any problems or after effects related to betadine application. The study was originally funded to only include a dental examination and processing of plaque samples at a 6-month follow-up. However, while no funds were available for microbiology, a dental examination at 1 year was performed for all available subjects (N=19).

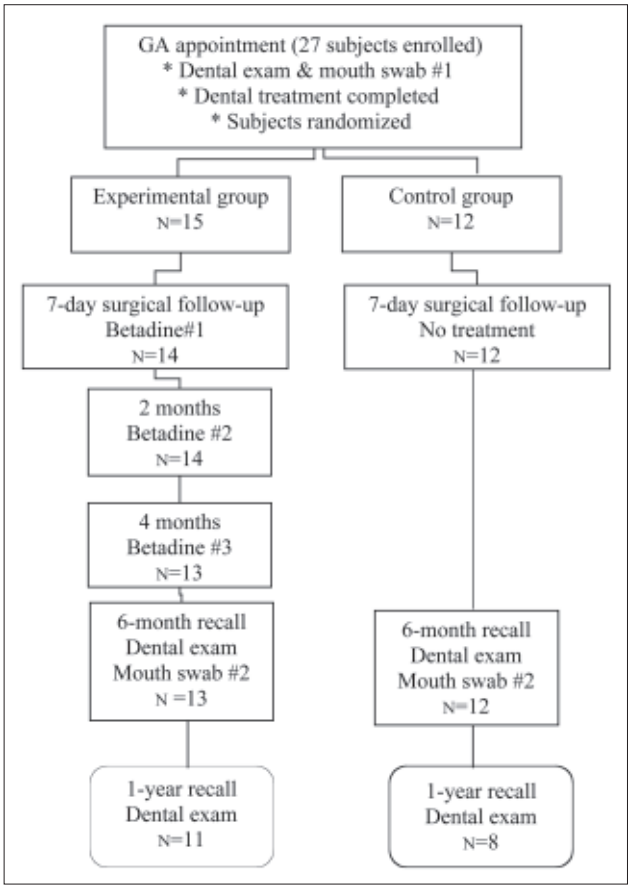

Figure 1. Overview of study design.

Baseline variables were compared by *t* test and Fisher exact test. Significant differences were defined as $P<.05$. CFUs per unit volume of *S mutans* counts were transformed to $\log_{10}$ values to control variance for the purpose of applying parametric statistical tests to the microbiological data.[14] Differences in $\log_{10}$ counts of *S mutans* at baseline and 6 months were compared for the experimental and control groups using a *t* test. One $\log_{10}$ reduction in the number of *S mutans* was considered to be a significant decrease. A Fisher exact test compared the number of children with new carious lesions at 6 months and 1 year in the experimental and control groups.

Results

A total of 25 of the original 27 children presented for 6-month follow-up (Figure 1). Two children in the experimental group withdrew for family reasons. Baseline comparisons demonstrated a significant difference in gender distribution between 2 groups ($P=.03$); however, gender was not significantly associated with reduction of *S mutans* levels. The experimental and control groups were similar for other demographic variables, feeding habits, caries status, and bacterial levels (Table 1). Height and weight measurements recorded regularly throughout the study revealed no adverse changes in physical growth and development for children who received betadine. Overall, parents and children reacted favorably to application of betadine and no untoward effect was reported.

Results demonstrated an overall reduction in total counts and *S mutans* for all children in the experimental and control groups, 6 months after GA dental treatment (Table 2). The reductions of total counts and *S mutans* were greater for the experimental group. However, the differences between the 2 groups were not statistically significant (P [total count]=.14, P [*S mutans*]=.58). Ten of 13 experimental children had a >1 $\log_{10}$ decrease in *S mutans* over 6 months, compared with 7 of 12 control children. Proportion of children with >1 $\log_{10}$ decrease was not significantly different between groups ($P=.21$).

At the 6-month recall dental examination, 3 children in the control group and 1 child in the experimental group demonstrated newly decalcified or carious surfaces ($P=.27$). At 1 year following GA, 19 of 25 subjects returned for follow-up. Two of 11 subjects from the experimental group and 5 of 8 subjects from the control group demonstrated dental caries. The difference was not significant ($P=.06$).

An unexpected number of subjects from both the experimental and control group demonstrated a 1 $\log_{10}$ or more decrease in *S mutans* levels 6 months following treatment. To determine if there were unforeseen factors responsible for this reduction in so many of the children, data from all the experimental and control subjects were pooled together and then separated into 2 groups:

1. children with ≥1 $\log_{10}$ reduction in *S mutans;*
2. children with <1 $\log_{10}$ reduction.

When comparisons were made between the 2 groups, the only variable that was significantly different was the time the mother had been in Canada ($P=.03$). Other variables—including age, gender, ethnicity, caries status, number of crowns placed for each child, and number of missing teeth at 6 months—were not significantly different between the 2 groups ($P>.05$).

Discussion

Positive outcomes have been demonstrated for PVP-I (betadine) in controlling the incidence of new carious lesions for children at risk of developing extensive caries.[15-16] The pilot study described in this paper was undertaken to explore the effect of betadine on *S mutans* and new caries in young children with a history of extensive caries. Pooled plaque samples were used to determine levels of *S mutans*. Plaque sampling was chosen because it is likely the most reliable method in children due to the higher odds ratio between caries and *S mutans* in plaque samples compared to saliva.[23] Collection of plaque also eliminated the difficulties associated with saliva collection in young children.[23-24] In addition, plaque sampling was much more practical and convenient for young children in this study who were likely to have had a dry mouth at the baseline visit because of fasting prior to GA and apprehension about the procedures.

Table 1. Baseline Demographics, Bottle-feeding History, Caries Status, and Bacterial Levels for Experimental and Control Children

Variables	All children	Experimental	Control	*P* value
Gender				
Female	13	4	9	.03
Male	12	9	3	
Child's age (years)	4.3 (1.1)*	4.2 (1.2)	4.3 (0.6)	.74
Mother's age (years)	33.5 (4.7)*	33.7 (5.1)	33.1 (4.3)	.75
Length of time mother in Canada (years)				.61
< 6	8	4	4	
> 6	17	9	8	
Still on bottle	7	5	2	.22
History of sleeping with bottle	15	10	5	.08
dmfs	27.9 (11.4) *	28.2 (14.3)	27.7 (7.8)	.92
***S mutans* $\log_{10}$**	3.9 (1.5)*	4.2 (1.4)	3.7 (1.5)	.38
Total count $\log_{10}$	7.8 (0.6)*	7.8 (0.6)	7.8 (0.7)	.79

*Mean (±SD).

Table 2. Comparisons of the Mean Log_{10} Total Counts and *Streptococcus mutans* at Baseline and 6 Months for All Subjects in Experimental and Control Groups

	Total count Mean $\log_{10}$(±SD)			*S mutans* Mean $\log_{10}$(±SD)		
	Baseline	6 months	*P* value	Baseline	6 months	*P* value
All children	7.8 (0.6)	7.0 (0.7)	.00006	3.9 (1.5)	2.0 (2)	.003
Experimental	7.8 (0.6)	6.8 (0.7)	.001	4.2 (1.4)	2.1(2.2)	.0005
Control	7.8 (0.7)	7.2 (0.5)	.03	3.7 (1.5)	1.9 (2.0)	.004

Conducting a prospective study in a private practice provides unique opportunities for a clinical trial, including access to a large number of potential subjects, but is not without challenges. In this study, language barriers and scheduling conflicts precluded the participation of many families. A secondary aim of the study was to determine the feasibility and acceptability of applying betadine at 2-month intervals to children in a private setting, and this aim was successfully fulfilled. All attempts were made to integrate the study as seamlessly as possible into the routine of the dental practice. Unfortunately, "routines" are not always as predictable in a private practice as in a more controlled clinical setting like a public health or hospital clinic. Despite the challenges, prospective clinical studies in the setting of a busy pediatric practice are to be encouraged.

A placebo group was not included in this pilot study because appointing both control and experimental children for appointments every 2 months may have substantially increased the number of dropouts. This study's primary focus was on the children in the experimental group, and great effort was expended to ensure that all experimental children were seen every 2 months. This effort was, for the most part, successful.

Results of this study demonstrated a significant decrease for *S mutans* counts, 6 months after restorative treatment, for all children. However, the decrease was not significantly greater for children who had received betadine than for the control children. A variety of reasons may explain why betadine did not demonstrate as significant an effect on *S mutans* at 6 months as had been anticipated. "One-time" restorative treatment performed for all subjects under GA probably made a major contribution to decreasing plaque *S mutans* at 6 months following treatment that may have overwhelmed the antibacterial effect of betadine. This reduction may be explained by the fact that, because complete dental rehabilitation eliminates the carious lesions, most of the *mutans streptococci* may be eliminated as well. However, previous studies on the effect of restorative treatment on levels of *S mutans* have produced equivocal results. Some investigators suggest that successful routine restorative treatment does not alter numbers of *S mutans*,[25] while others have demonstrated that extensive restorative dental treatment effectively reduces the level of caries-associated microorganisms for a period of at least 6 months.[5,26-27] In the present study, the extent or type of restoration was not related to *S mutans* levels. Furthermore, no significant association was found between the number of stainless steel or composite crowns and reduction in *S mutans*.

Time and circumstances of plaque collection differed between baseline and 6-month follow-up, and this difference may have affected bacterial levels. Baseline plaque samples were taken at the GA appointment after the children had been fasting and most likely had not brushed their teeth that morning. The 6-month plaque samples were taken at a routine recall appointment, when children were not fasting and might have brushed their teeth before the appointment. Altered plaque accumulations at baseline and 6-month visit related to overnight fasting before GA, and differences in tooth-brushing behavior at both time periods may also have had an unanticipated effect on *S mutans* counts.[28,29]

In addition, during the 6-month time period after surgery, families may also have been more attentive to preventive practices. Following their child's dental treatment under GA, parents have reported significant changes in their participation in tooth-brushing and reductions in their child's consumption of sweets.[30] Therefore, the effect of an additional chemotherapeutic intervention may not have been detectable. Unfortunately, follow-up information on oral hygiene and dietary practices at 6 months was not collected in the present study.

Betadine may have had a more pronounced effect if the levels of *S mutans* in the children had been higher at the beginning of the study. In spite of the elevated caries experience of these children (Table 1), baseline levels of *S mutans* were not as high as had been anticipated. In addition, a longer period of follow-up may have demonstrated greater differences as a result of betadine between the levels of *S mutans* in the experimental and control groups. Gradual increases have been reported in the number of *S mutans* at time periods greater than 6 months following restorative treatment.[26]

Further research on betadine, alone or in combination with other preventive strategies, should be explored. At 1-year follow-up, 5 of 8 control children had new decay, compared to only 2 of the 11 experimental children who presented for a recall appointment. This finding is promising and similar to results of previous studies.[15,16] While the number of subjects in this pilot study was small, the effectiveness of betadine cannot be rejected, and results of this study support further research. Furthermore, betadine was well-tolerated by the children, acceptable to parents, simple and quick to apply, and did not cause any staining of composite restorations. In fact, no negative side effects of betadine were noted.

Finally, it was an interesting but unexpected observation that the mean length of time that mothers had been in Canada for children with 1 $\log_{10}$ or more reduction in *S mutans* was twice as long as that for those whose levels did not change by this amount or increased. In other words, the longer a mother had lived in Canada, the more likely her child's *S mutans* counts had substantially decreased at 6-month recall. Perhaps families who been in Canada longer may have had increased knowledge of healthy dental practices and better home care. In addition, the oral hygiene and dietary practices of the mother, who is the main reservoir of cariogenic bacteria, may also have improved as a result of better access to oral health education, dental care products, and more availability of dental services in Canada than in her country of origin.[31]

Conclusions

1. Extensive one-time restorative dental treatment resulted in a significant reduction in plaque *S mutans* levels at 6 months.
2. Povidone-iodine did not significantly reduce plaque *S mutans* levels in children 6 months after dental treatment under GA.

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References

1. Tinanoff N, Kanellis MJ, Vargas CM. Current understanding of the epidemiology, mechanisms, and prevention of dental caries in preschool children. *Pediatr Dent*. 2002;24:543-551.
2. Loesche WJ. Role of Streptococcus mutans in human dental decay. *Microbiol Rev.* 1986;50:353-380.
3. O'Sullivan EA, Curzon MEJ. The efficacy of comprehensive dental care for children under general anesthesia. *Br Dent J.* 1991;171:56-58.
4. Berkowitz RJ, Moss M, Billings RJ, Weinstein P. Clinical outcomes for nursing caries treated using general anesthesia. *J Dent Child.* 1997;64:210-211.
5. Chase I, Berkowitz RJ, Mundorff S, Proskin H, Weinstein P, Billings R. Clinical outcomes for early childhood caries: The influence of salivary mutans streptococci levels. *Eur J Pediatr*. In press.
6. Johnsen DC, Gerstenmaier JH, DiSantis TA, Berkowitz RJ. Susceptibility of nursing-caries children to future approximal molar decay. *Pediatr Dent.* 1986;8:168-170.
7. Edelstein BL, Douglas CW. Dispelling the myth that 50 percent of US school children have never had a cavity. *Public Health Rep.* 1995;110:522-530.
8. Weinstein P. Public health issues in early childhood caries. *Community Dent Oral Epidemiol*. 1998;26 (suppl 1):84-91.
9. Twetman S, Petersson LG. Efficacy of a chlorhexidine and a chlorhexidine-fluoride varnish mixture to decrease interdental levels of mutans streptococci. *Caries Res.* 1997; 31(5): 361-365.

10. Van Lunsen DM, de Soet JJ, Weerheijam KL, Groen HJ, Veerkamp JSJ. Effect of dental treatment and single application of a 40% chlorhexidine varnish on mutans streptococci in young children under intravenous anesthesia. *Caries Res.* 2000;34:268-274.
11. Hoyos DF, Murray JJ, Show L. The effect of chlorhexidine gel on plaque and gingivitis in children. *Br Dent J.* 1977;142:366-369.
12. Addy M, Wade W, Goodfield S. Staining and antimicrobial properties in vitro of some chlorhexidine formulations. *Clin Prev Dent.* 1991;13:13-17.
13. Gibbons RJ, Depaola PF, Spinell DM, Skobe Z. Interdental localization of S mutans as related to dental caries experience. *Infect Immun*. 1974;9:481-488.
14. Caufield PW, Gibbons RJ. Suppression of S mutans in mouths of humans by a dental prophylaxis and topically-applied iodine. *J Dent Res.* 1979;58:1317-1326.
15. Lopez L, Berkowitz R, Zlotnik H, Mass M, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries. *Pediatr Dent.* 1999;21:9-11.
16. Lopez L, Berkowitz R, Spiekerman C, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries: A follow-up report. *Pediatr Dent.* 2002;24:204-206.
17. Schreier H, Erdos G, Reimer K, Konig B, Konig W, Fleischer W. Molecular effects of povidone-iodine on relevant microorganisms: An electron microscopic and biochemical study. *Dermatology*. 1997;195(suppl 2):111-117.
18. Zamora JL. Chemical and microbiologic characteristic and toxicity of povidone-iodine solutions. *Am J Surg.* 1986;151:400-406.
19. Vratsanos SM. On the structure and function of polyvinyl pyrrolidone-iodine complex. In: Degenes G, ed. *Proceedings of International Symposium on Povidone-iodine*. Lexington, KY: University of Kentucky; 1983:289-301.
20. Riedy CA, Weinstein P, Milgrom P, Bruss M. An ethnographic study for understanding children's oral health in a multicultural community. *Int Dent J.* 2001;51:305-312.
21. Kimmel L, Tinanoff N. A modified mitis salivarius medium for a caries diagnostic test. *Oral Microbiol Immunol*. 1991;6:275-279.
22. Pennington JAT. A review of iodine toxicity reports. *J Am Diet Assoc.* 1990;90:1571-1581.
23. Sanchez-Perez L, Acosta-Gio AE. Caries risk assessment from dental plaque and salivary S mutans counts on two culture media. *Archs Oral Biol.* 2001; 46:49-55.
24. Dasanayake AP, Caufield PW, Cutter GR, Roseman JM, Kohler B. Differences in the detection and enumeration of mutans streptococci due to differences in methods. *Arch Oral Biol.* 1995;40:345-351.
25. Gregory RL, El-Rahman AMA, Avery DR. Effect of restorative treatment on mutans streptococci and IgA antibodies. *Pediatr Dent.* 1998;20:273-277.
26. Twetman S, Fritzon B, Jensen B, Hallberg U, Stahl B. Pre- and post-treatment levels of salivary mutans streptococci and lactobacilli in pre-school children. *Inter J Paediatr Dent*. 1999;9:93-98.
27. Van Lunsen DM, de Soet JJ, Weerheijam KL, Groen HJ, Veerkamp JSJ. Effect of dental treatment and single application of a 40% chlorhexidine varnish on mutans streptococci in young children under intravenous anesthesia. *Caries Res.* 2000;34:268-274.
28. Birkhed D, Heintze U, Edwardsson S, Aly KO. Short-term fasting and lacto-vegetarian diet does not affect human saliva. *Scand J Dent Res*. 1984;92:408-411.
29. Warren PR, Jacobs D, Low MA, Chater BV, King DW. A clinical investigation into the effect of toothbrush wear on efficacy. *J Clin Dent.* 2002;13:119-124.
30. Peretz B, Faibis S, Ever-Hadani P, Eidleman E. Dental health behaviour of children with BBTD treated using general anesthesia or sedation, and of their parents in a recall examination. *J Dent Child.* 2000;67:50-54.
31. Reisine S, Douglas JM. Psychological and behavioural issues in early childhood caries. *Community Dent Oral Epidemiol.* 1998;26(suppl 1):32-44.