Comparison between Silver Diamine Fluoride and Fluoride Varnish on their Ability to Reduce *Streptococcus mutans* Levels and Caries Progression among Thai Preschool Children

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Abstract

Objective: To compare the effectiveness of silver diamine fluoride (SDF) and sodium fluoride (NaF) varnish in stopping caries progression and effects on *S. mutans* level.

Materials and Methods: Ninety-one children aged 2-5 years old were selected and divided into 3 groups: 29 in Group 1-treated with 38% SDF, 31 in Group 2-treated with 5% NaF varnish and 31 in Group 3-control group. Dental caries was assessed using ICDAS scoring system. Supra gingiva plaque was collected at 1, 3 and 6 months visits. DNA was extracted and performed real-time PCR to quantify amount of *S. mutans*.

Results: At 6 months, 69 subjects were remained, 22 in Group 1 and 3, 25 in Group 2. Mean age was 4.2 ± 0.96 years. Only in Group 1, *S. mutans* levels were different between baseline to 3 months ($p = 0.031$), and 3 months to 6 months ($p = 0.035$). At 1-, 3- and 6-month examination, total number of arrested surface scores in Group 1 was higher than Group 2 and 3 at all follow-up visits ($p < 0.001$). Arrested surface scores at anterior and posterior teeth in Group 1 were higher than in Group 2 and 3 at 3- and 6-month follow-up visits ($p < 0.001$). The progression rate of current caries lesions in Group 1 was lower than in Group 2 and 3 at the 6 months examination ($p < 0.001$).

Conclusion: *S. mutans* levels in SDF group were different between baseline to 3 months, and 3 to 6 months. SDF was more effective than NaF in stop caries progression.

Keywords: Early Childhood Caries, *S. mutans,* Silver Diamine Fluoride, Fluoride Varnish, Arrested Caries, ICDAS System

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Introduction

Early childhood caries (ECC) is one of common childhood diseases. In Southeast Asia and Thailand, the prevalence of ECC among children aged 2 years and 3-5 years were 47% and 50%, respectively (1, 2). Untreated dental caries and poor access to dental care are major public health problems, especially in Thailand.

ECC is a multifactorial disease resulting from an interaction between acidogenic bacteria, sucrose and host susceptibility (3). In the oral cavity there exist biofilm or dental plaque. It is a dynamic environment, and the microorganism population in biofilm can shift between healthy and pathological stages when a factor such as sugar is enhanced (3, 4). Other contributing factors including the parent's demographic, feedings habits, oral hygiene care and sugar containing snacks consumption were also associated with ECC (5-7).

S. mutans is a causative pathogen of dental caries and commonly isolated from dental plaque (3, 5-8). Important products of *S. mutans* are water-insoluble polymers or glucan which serve as glue in the adhesion and colonization process on teeth surfaces. The important virulence factors of *S. mutans* that enhance its cariogenic property is its extracellular glucosyl transferases (Gtfs) expression which can synthesize intracellular polysaccharide (IPS) that supports acid production continuously in the presence of a low exogenous substrate (3,7). Recent studies in Thai children found that *S. mutans* in plaque were detected higher in children with ECC (5,6). For more effective prevention or stop the deeper of dental caries lesions, it is worth to inhibit *S. mutans* ability mentioned above.

Fluoride varnish is a non-aqueous form of topical fluoride which is composed of an active ingredient - 5% sodium fluoride (NaF) containing 22,600 ppm fluoride (9). Systematic review of randomized controlled clinical trials over 2 years duration evaluated the effect of caries prevention of fluoride varnish when applied by dental professional and reported a preventive effect of 30% in permanent teeth that recently erupted when compared to untreated controls (9). The meta-analysis of the 13 clinical trials showed that when treat permanent teeth with fluoride varnish had an average 43% reduction in dmft scores (9). The American Dental Association (ADA) recommends using fluoride varnish for dental caries prevention in moderate and high-risk patients of any age group (10).

Recently, Silver diamine fluoride (SDF) has been used to stop caries progression of current lesions in ECC. This intervention is non-invasive and easy to perform (11). The fluoride in SDF enhances remineralization. Moreover, the silver ions are antibacterial which can inhibit the growth of biofilms (11,12). However, the study comparing the effectiveness between fluoride varnish and SDF in both reducing *S. mutans* and arrested caries is still limited.

This study aims to compare the level of *S. mutans* quantitatively in plaque samples using real-time PCR between groups applied with 5% NaF and 38% SDF in preschool children at 1-, 3-, 6- month follow-ups, and the caries arrested rate between the two groups. The hypothesis is that the levels of *S. mutans* and caries arrested rates between these two groups should be different.

Materials and Methods

This cross-sectional study was approved by the Human Institutional Review Board of the Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2019/047. 3107). Based on Sample size calculations from previous study using the software package Primer of Biostatistics (McGraw-Hill, NY, USA). With type I error =5%, Type II error =20%, power= 80%, the minimum of 20 children in each group was enough for statistical achievement (8).

Subject selection

Subjects consisted of Thai children aged 2 to 5 years old. They were recruited from child development centers or schools in Prachuap Khiri khan province, Thailand. Total subjects were 91 children. Inclusion criteria were: Healthy children with no allergy to silver particles. If they had a history of systemic disease or used antibiotics within 1 month prior to examination, had a history of allergy to fluoride or silver, or colophony agents, or received topical fluoride 3 months prior to the enrollment and children with presence of spontaneous pain from dental caries or signs of pulpal infection, were excluded. Study subjects were divided into three groups using block randomization: 29 subjects in Group 1-treated with 38% SDF (Topamine, Dentalife, Australia), 31 subjects in Group 2-treated with 5% NaF varnish (Duraphat, Colgate Palmolive, USA) and 31 subjects in Group 3-the control group received no treatment.

At the beginning of the study, parents/ caregivers filled in a questionnaire about demographic data and oral health related behavior and attended an oral health instruction session. All children received supervised oral hygiene instruction, a toothbrush and dentifrice for home use (1,000 ppm fluoridated toothpaste). Standardized oral hygiene practice was explained to all children and reinforced at every visit.

Clinical examination

One examiner who is in a residency training program in pediatric dentistry performed a clinical examination at public schools using World Health Organization CPI periodontal probes, a dental mouth mirror with adequate illumination in a dental mobile unit using ICDAS II criteria. Dental caries status was recorded on a 0 and 2–5 scale by surface (five surfaces were buccal, lingual, mesial, distal and occlusal in each posterior tooth, and four surfaces were labial, lingual, mesial and distal in each anterior tooth). The ICDAS system based on ICDAS II criteria for caries status was assessed by recording all surfaces of primary teeth (score 0-5)(13). The score 1 was not recorded because this code is only detected after prolonged air drying. As air drying was not available because clinical examination were performed at public schools. The caries activity status was diagnosed as active lesions if the lesion presented with the tactile sensation of a rough surface while gently drawing the probe across the lesion, with or without plaque stagnation. Smooth and hard surfaces that could not be penetrated easily were classified as arrested lesions. Participants were examined at their time of entry into the study (baseline) and at follow-up visits at 1-, 3-and 6 months. No radiographs were taken. Approximately 5% of study participants were reexamined to determine inter-examiner reproducibility. Oral hygiene status was measured using a modified debris index for primary dentition (13-16).

Plaque sample collection

All children were instructed to brush their teeth under parental supervision at 8.00 PM the night before plaque collection day. No food or drink before sample collection. Collected pooled overnight supra gingiva plaque using a sterile toothpick and released in 1 ml of TE buffer and immediately transported on ice to the Laboratory and stored at -20°C until DNA extraction process.

DNA extraction

DNA was extracted based on enzymatic lysis using a commercial kit (Flavogen, Taiwan) as previously described (5). In brief, 20 µl of Proteinase K was added, 400 µl of FABG buffer and 20 µl of a lysozyme mixture (lysozyme 20 mg/ml and mutanolysin (Sigma Aldrich, USA) in 1:10 proteinase K) and vortex. Incubated at 60 °C for 1 h.; 200 µl ethanol was added and centrifuged at 11,000 rpm for 30 s. The solution was transferred into a spin column and centrifuged for 1 min. The supernatant was discarded, 500 µl of W1 buffer was added and centrifuged for 1 min. The supernatant was discarded. Then 750 µl of wash buffer was added and centrifuged for 1 min. The next step was adding 50 µl of elution buffer, left at room temperature for 3 min, before a final centrifuge for 2 min. The extracted DNA concentration and purity was measured using a spectrophotometer at 260 nm/280 nm (Nanodrop 2000C Thermo Scientific, Delaware, USA).

Culture condition and standard strain

S. mutans ATCC 25175 was used as the standard strain. It was grown anaerobically (5% CO_2) in BHI (Brain Heart Infusion) broth at 37°C for 24-48 hrs. Extracted genomic DNA and ten-fold serial dilution starting from 10^8-10^2 CFU/ml was done.

Quantitative Real-time PCR

Using specific primers for detecting *S. mutans* (forward primer SM 1: 5'-GGTCAGGAAAGTCTG-GAGTAAAAGGCTA-3' and reverse primer SM 2: 5'-GCGTTAGCTCCGGCACTAAGCC-3'), the reaction mixture (total volume of 20 µl) contained (varied from 2 to 9.1 µl) of water, 10 µl of 2X KAPA SYBRR FAST qPCR Master Mix, 0.4 µl of 10 µM forward and reverse primer, and (varied from 0.1 to 7.2) µl of bacteria DNA. The thermocycler (C1000™ Thermal cycler and CFX 96 Real-time System) was set for 40 cycles. Each cycle consisted of enzyme activation at 95 °C for 3 min, denaturing at 95 °C for 3 seconds, annealing at 60 °C for 20 seconds. Melting curves were generated from 60 °C to 95°C and read every 0.5 °C for 5 seconds (5). The amount of *S. mutans* from plaque sample was compared with the standard curve generated from the standard strain (*S. mutans* ATCC 25175).

Agarose gel electrophoresis

Stained 2% agarose gel (UltraPure Agarose, ThermoFisher Scientific, USA) with ethidium bromide and direct visualized PCR products.

Treatment procedure

Treatment procedures were conducted by a dentist who was not involved in the examination process. In Group 1, steps of 38% SDF application: Any food debris and plaque were removed from the cavities lesions by wiping with gauze, no caries was removed. Isolated the decayed teeth and kept dry with cotton rolls and gauze. Placed one drop of SDF in a plastic dappen dish and applied onto the carious tooth surfaces with a micro-applicator for 1 minute. Removed excess by gentle blotting using a cotton pellet. Instructed

children to avoid drinking, eating or rinsing in the next 30 minutes after the application. In group 2, steps of 5% NaF varnish application: Any food debris and plaque were removed from the tooth surfaces thoroughly by wiping with gauze. Isolated the decayed teeth and kept dry with cotton rolls or gauze. A thin coat of 5% NaF varnish using a suitable brush to all surfaces of the teeth (buccal, lingual, occlusal and proximal) was applied. The children were instructed to do not rinse, drink or eat for 2 hours and refrain from brushing in the same day.

Follow-up periods

Follow-up examinations were performed at 1, 3 and 6 months by the same examiner. These parameters were recorded at each follow-up visit: Plaque index, carious lesion activity (deeper or wider) and status of caries (new carious lesions presence or not). Observed black stain on each lesion clinically. If there was an illness associated with SDF treatment (nausea, vomiting, generalized discomfort) or an adverse effect of SDF application such as tooth or gingival pain, gingival swelling or gingival bleaching, informed parents to contact the class teacher immediately. The applications were repeated at 3 months of follow-up for both groups in the same manner. Plaque sample collections using the above-mentioned techniques were repeated at 1, 3 and 6 months of follow-up in all study groups.

Statistical analysis

Intra-examiner reproducibility in dental caries detection was measured by Cohen's Kappa statistic. Descriptive statistics were used to summarize the demographics data, oral hygiene habits, fluoride toothpaste usage, sugary snack habits, parents' education and family income and socioeconomic status. Chi-square test was used to test the differences between the treatment groups. The pattern evaluation of the sample distribution was tested by Shapiro-wink test showing the data were nonparametric, a Kruskal-Wallis test was performed to assess the differences among the three treatment groups including the children's mean age, dmfs, dmft scores and *S. mutans* levels. This was followed by a Friedman test to assess the differences among the follow-up periods. According to the numbers of surfaces of carious lesion from the baseline data compared after 1-, 3- and 6 months follow–up, they were tested by McNemar's test. The level of statistical significance was set at 95% confidence level ($p < 0.05$). Performed all statistical analysis with SPSS 18.0 software (Microsoft Corporation. CA, USA).

Results

Three hundred and forty-eight children were screened and 91 children fulfilled the inclusion criteria. Mean age of all participants was 4.2 ± 0.96 years. The number of children in Group 1 (38% SDF), Group 2 (5% NaF), and Group 3 (control group) were 29, 31 and 31, respectively. One dentist examined all the children (intra-examiner reproducibility level = very good) (Cohen's Kappa statistic > 0.9). At 6 months follow up visit, 69 subjects were remained in the study, 22 subjects in Group 1 and 3, 25 subjects in Group 2. Thirty-nine and 30 subjects were male and female, respectively. Mean (+ standard deviation) age of study subjects in Group 1, 2, and 3 were 4.18 (± 0.91), 4.08 (± 0.9), and 4.36 (± 1.1) years, respectively. An overall dropout rate of 24.2% due to loss of follow-up and received dental treatment during the follow-up period (Fig 1).

Gender, age, plaque index, decayed missing and filled teeth (dmft) scores were not different among the three groups (Table 1). Demographic background and oral health habits were not different among three groups. Most of the children received prolonged milk feeding for over 18 months (68%) and brushed their teeth by themselves (38%) with fluoride toothpaste (94%) twice a day (87%). Most of them had cariogenic snacks between meals more than 2 times/day (86%).

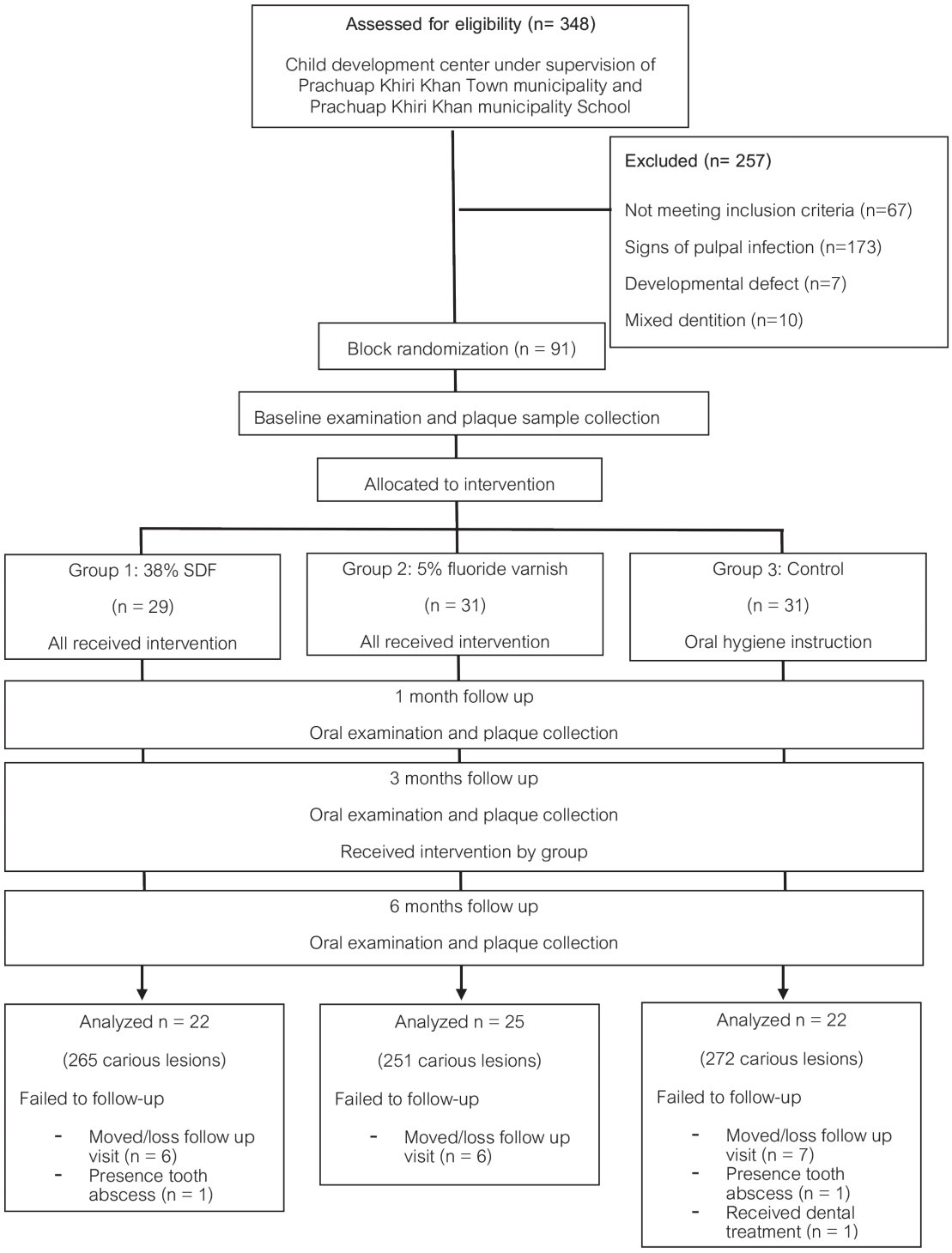

Fig 1. Flowchart of the study.

Table 1. Characteristics of the study subjects (n = 69).

Variables	Group 1: 38% SDF	Group 2: 5% NaF	Group 3: Control	p-value
Participants, n (%)	22 (31.9)	25 (36.2)	22 (31.9)	
Gender, n (%)				
Boy	14 (63.6)	13 (52)	12 (54.5)	0.706[b]
Girl	8 (36.4)	12 (48)	10 (45.5)	
Age*, n (%)	4.18 ± 0.91	4.08 ± 0.90	4.36 ± 1.1	0.600[a]
Clinical parameter, Mean ± SD				
[1]dmfs	6.09 ± 5.63	5.44 ± 5.25	6.05 ± 5.08	0.781[a]
[2]dmft	4.14 ± 2.98	3.16 ± 2.48	3.64 ± 2.34	0.560[a]
[3]Plaque index	1.23 ± 0.41	1.09 ± 0.31	1.10 ± 0.35	0.537[a]

[a]Kruskal-Wallis test; [b]Pearson chi square test

[1]Mean dmfs ± SD = 5.84 ± 5.25, 2Mean dmft ± SD = 3.62 ± 2.60,

[3]Mean plaque index ± SD = 1.14 ± 0.36

dmfs: total number of surfaces that are decayed (D), missing (M), or filled (F) in an individual; dmft: total number of teeth that are decayed (D), missing (M), or filled (F) in an individual; NaF: fluoride varnish; SD: standard deviation; SDF: silver diamine fluoride

Quantitative Real-time PCR

The specificity and sensitivity of *S. mutans* primers were tested and reported in a previous study (16). At baseline, *S. mutans* levels were not different among the three groups. After the interventions, there were no significant differences among the treatment groups at the 1-, 3- and 6-month follow-up visits. *S. mutans* level was highest in group 3 (control group) and lowest in group 1 treated with SDF, with no statistically significant differences (Table 2). Table 3 shows the difference in mean level of *S. mutans* between each follow-up period. No significant differences were showed among the three groups. However, in Group 1 the difference of *S. mutans* levels between baseline and 1 month follow-up indicated a decrease to 6.30 x 10^3 DNA copies; as well, the difference of *S. mutans* levels between 3 months and 6 months was a decrease to 3.03 x 10^4 DNA copies. From baseline to 3 months and 6 months, the *S. mutans* level had increased. The different levels of *S. mutans* in the SDF group were significantly different between baseline to 3 months, and 3 months to 6 months ($p = 0.035$). In Group 2 and Group 3, the different levels of *S. mutans* in each follow-up period showed an increase, but with no significant differences.

Table 2. Quantitative levels of *S. mutans* at baseline, 1-, 3- and 6-month follow-up examinations.

Sample collecting time	Group 1: 38% SDF Median (min,max)	Group 2: 5% NaF Median (min,max)	Group 3: control Median (min,max)	p-value
Baseline (T0)	2.83×10^4 (9.37×10^3,1.95×10^5)	3.21×10^4 (7.04×10^3,3.03×10^5)	3.78×10^4 (4.29×10^3,5.09×10^5)	0.898[a]
Follow up 1 month (T1)	2.77×10^4 (6.36×10^3,6.04×10^4)	2.77×10^4 (7.04×10^3,4.48×10^5)	4.03×10^4 (1.02×10^4,8.24×10^5)	0.329[a]
Follow up 3 months (T2)	4.73×10^4 (4.69×103,4.99×10^5)	3.95×10^4 (6.40×103,8.29×10^5)	4.09×10^4 (5.36×103,2.20×10^6)	0.875[a]
Follow up 6 months (T3)	3.72×10^4 (5.79×103,2.88×10^5)	4.11×10^4 (1.29×104,5.52×10^6)	4.21×10^4 (7.89×103,4.13×10^6)	0.506[a]
p-value	0.173[b]	0.206[b]	0.585[b]	

[a]Kruskal-Wallis test; [b]Friedman's two way analysis; SDF: silver diamine fluoride; NaF: fluoride varnish min: the minimum amount of *S. mutans* at the sample collection time; max: the maximum amount of *S. mutans* at the sample collection time

Table 3. Difference of quantitative levels of *S. mutans* between baseline, 1-, 3- and 6-month follow-up examinations.

Sample collecting time	**Group 1: 38% SDF Mean (SD)**	**Group 2: 5% NaF Mean (SD)**	**Group 3: control Mean (SD)**	**p-value[a]**
Baseline –1 month (T1-T0)	$-6.30\times10^3(2.72\times10^4)$	$2.05\times10^3(4.13\times10^4)$	$2.41\times10^4(1.76\times10^5)$	0.798
Baseline – 3 months (T2-T0)	$3.74\times10^4(1.04\times10^5)$	$4.64\times10^4(1.22\times10^5)$	$7.82\times10^4(3.65\times10^5)$	0.566
Baseline – 6 months (T3-T0)	$7.10\times10^3(6.10\times10^4)$	$2.21\times10^5(1.10\times10^6)$	$2.83\times10^5(8.93\times10^5)$	0.330
3 months - 6 months (T3-T2)	$-3.03\times10^4(6.20\times10^4)$	$1.74\times10^5(1.12\times10^6)$	$2.05\times10^5(8.30\times10^5)$	0.141
p-value[b]	Amount of *S. mutans* betweenT2-T0 was significant (p = 0.031) Amount of *S. mutans* between T3-T2 was significant (p = 0.035)	0.150[b]	0.990[b]	

[a]Kruskal-Wallis test; [b]Friedman's two way analysis; SDF: silver diamine fluoride; NaF: fluoride varnish; SD: standard deviation

Table 4 shows that the overall arrested carious surfaces of Groups 1, 2 and 3 were 71.3%, 26.4% and 16.7% at 3 months and 78.5%, 25.2% and 26.6% at 6 months, respectively. At the 1-, 3- and 6-month examination, the mean number of arrested carious tooth surfaces among the three groups were significant different ($p < 0.001$). The total number of arrested carious surfaces in Group 1 was higher than in Group 2 and 3 significantly at all follow-up visits. In Group 1, the caries arrest rate at 6 months (78.5%) was significantly higher than at 3 months. The proportion of caries arresting rates at anterior teeth and posterior teeth in Groups 1 were higher than in Group 2 and 3 at the 3- and 6-month follow-ups ($p < 0.001$) significantly. In addition, the proportion of caries arresting rates of the anterior teeth were higher than the posterior teeth in the SDF group at the 3- and 6-month follow-ups ($p < 0.001$) (Table 5). As shown in Table 6, the progression rates of current caries lesions of Groups 1, 2 and 3 were 1.3%, 1.9% and 3.4% at 3 months; and 2.1%, 3.5% and 5.1% at 6 months, respectively. At the 6-month examination, the progression rates of current caries lesions among the three groups were statistically significant different ($p < 0.001$). The progression rate of current caries lesions in Group 1 was lower than in Group 2 and Group 3 at the 6 months examination significantly.

Table 4. Caries arrest rates of active carious tooth surfaces at baseline,1-, 3- and 6-month follow-up examinations.

Sample collecting time	Group 1: 38% SDF		Group 2: 5% NaF		Group 3: control		p-value	
	Active surface(%)	Arrested surface(%)	Active surface(%)	Arrested surface(%)	Active surface(%)	Arrested surface(%)		
1 month	51 (23.6)	165 (76.4)	196 (79.7)	50 (20.3)	200 (80.6)	48 (19.4)	< 0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.787 Gp1-Gp2:<0.001*
3 months	75 (28.7)	186 (71.3)	181 (73.6)	65 (26.4)	219 (83.3)	44 (16.7)	< 0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.008 Gp1-Gp2:<0.001*
6 months	56 (21.5)	205 (78.5)	184 (74.8)	62 (25.2)	193 (73.4)	70 (26.6)	< 0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.716 Gp1-Gp2:<0.001*
p-value	< 0.001*	< 0.001*	< 0.001*					

Pearson chi square; All each follow-up examination, *significantly different at ($p < 0.05$)

Gp: group; SDF: silver diamine fluoride; NaF: fluoride varnish

Table 5. Caries arrest rates by tooth position at baseline ,1-, 3- and 6-month follow-up.

Examination time/ teeth position		Group 1: 38% SDF		Group 2: 5% NaF		Group 3: control		p-value	
		Active surface (%)	Arrested surface (%)	Active surface (%)	Arrested surface (%)	Active surface (%)	Arrested surface (%)		
1 month	Anterior teeth	18(15.7)	97(84.3)	116(87.9)	16(12.1)	88(81.5)	20(18.5)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.167 Gp1-Gp2:<0.001*
	Posterior teeth	33(32.7)	68(67.3)	80(70.2)	34(29.8)	112(80)	28(20)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.070 Gp1-Gp2:<0.001*
3 months	Anterior teeth	17(12.2)	122(87.8)	105(79.5)	27(20.5)	95(81.9)	21(18.1)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.640 Gp1-Gp2:<0.001*
	Posterior teeth	58(47.5)	64(52.5)	76(66.7)	38(33.3)	124(84.4)	23(15.6)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.001* Gp1-Gp2:0.003
6 months	Anterior teeth	6(4.3)	133(95.7)	109(82.6)	23(17.4)	80(69)	36(31)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.012 Gp1-Gp2:<0.001*
	Posterior teeth	50(41)	72(59)	75(65.8)	39(34.2)	113(76.9)	34(23.1)	<0.001*	Gp1-Gp3:<0.001* Gp2-Gp3:0.048 Gp1-Gp2:<0.001*
p-value	Anterior teeth	<0.001*		<0.001*		<0.001*			
	Posterior teeth	<0.001*		<0.001*		<0.001*			

Pearson chi square test, *significantly different at ($p < 0.05$)

Gp=group, SDF=Silver Diamine Fluoride, NaF=Fluoride varnish

Table 6. Progression rates at baseline,1-, 3- and 6-month follow-up.

Examnination time	Progression surfaces, n/N (%)			p-value*	
	Group 1:38% SDF	Group 2:5% NaF	Group 3:control		
1 month	7/1760 (0.4)	17/2200 (0.8)	25/1848 (1.4)	0.007	Gp1-Gp3: 0.002
					Gp2-Gp3: 0.070
					Gp1-Gp2: 0.131
3 months	26/1936 (1.3)	42/2200 (1.9)	65/1936 (3.4)	< 0.001*	Gp1-Gp3: < 0.001*
					Gp2-Gp3: 0.003
					Gp1-Gp2: 0.153
6 months	41/1936 (2.1)	78/2200 (3.5)	99/1936 (5.1)	< 0.001*	Gp1-Gp3: < 0.001*
					Gp2-Gp3: 0.013
					Gp1-Gp2: 0.006
p-value	< 0.001*	< 0.001*	< 0.001*		

Pearson chi square test, *significantly different at ($p < 0.05$)

Gp: group; SDF: silver diamine fluoride; NaF: fluoride varnish

Discussion

SDF and fluoride varnish are one of non-invasive method for caries management, especially in young children because of their limited cooperation. Data from *in vitro* studies has shown good evidence of the microbicidal efficacy of SDF on dentin caries lesions resulting in stopping the caries progression of current lesions (7,17,18). However, *in vivo* studies published on the antimicrobial efficacy of SDF were found limited. In this study, microbial quantification data were collected from supra gingival plaque, which is different from previous studies that collected plaque from carious dentin (18). Previous results showed that there was no difference in *S. mutans* levels between saliva and dental plaque (5,19). Our results showed that *S. mutans* levels were not significantly different among three treatment groups. Despite *S. mutans* levels in all three groups not significantly different in all the follow-up periods, it was interesting that *S. mutans* levels was decreased after the first and second applications of the SDF in this study. Likewise, in previous study, *S. mutans* levels decreased after the application of SDF (20). However, their results found that even though *S. mutans* levels had decreased but other bacteria such as Veillonella sp., *Lactobacillus sp., Rothia sp.,* and *Streptococcus sobrinus* tended to increased (20). SDF has shown intense antibacterial effects on certain cariogenic biofilms in mono-cariogenic species biofilm or multi-cariogenic species biofilm (20). However, those studies was evaluated the amount of *S. mutans* from dentine caries not supra gingiva plaque. As far as recent knowledge, *S. mutans* played a crucial role in caries initiation and progression but caries process is dynamic and some studies suggested that other species were found associated with initial and deep caries and might be able to detect while *S. mutans* was

absence (21). Also, the area of sample collection might affect the results. Further studies in quantitatively measure other cariogenic bacteria levels are recommended in the future. However, in this study the effect of SDF in lowering *S. mutans* was not found but its effect on caries progression inhibition was obvious. Various mechanisms of action have been proposed to explain SDF's caries-arresting efficiency. Some researchers suggested that SDF hardens a caries lesion by regaining calcium and phosphate from saliva, and it reduces dentine collagen degradation by inhibiting collagenases like MMPs and cathepsins (20).

It was previously found that *S. mutans* levels in active caries were higher than those in arrested caries (20). Previous clinical study showed that SDF had higher antibacterial activity in infected soft dentin than chlorhexidine (22). Conversely, after fluoride varnish application, *S. mutans* levels decreased only after the first application, and had risen up at the 3- and 6 month follow-ups. There was no difference in *S. mutans* levels between each follow-up visit between the fluoride varnish application group and the control group. This was similar to previous studies that showed no reduction of *S. mutans* in dental plaque after fluoride varnish application (23-25).

Systematic review shows that there is a high level of evidences for the potential of SDF for arresting carious lesion or stopping the caries lesions progression (16,26-28). This study's report includes interventions for arresting either non-cavitated or cavitated caries lesions in the primary teeth. In this study, in the group receiving 38% SDF application, subjects demonstrated significantly higher caries arresting rates than the other two groups. After 6 months, the caries arresting rate of those receiving the application of SDF was significantly higher (78.5%) than those receiving the application of 5% NaF varnish or the control group (25.2% and 26.6%, respectively). The effectiveness at arresting dental caries by SDF application after 6 months, in this study, was similar to previous studies results (23,27,29,30). In this study, when increased the frequency of fluoride application to every 3 months, the results showed an increase in the proportion of active caries that had become arrested or stop progression, increasing from 71.3% to 78.5% in the SDF treatment group, and from 20.3% to 25.3% in the 5% NaF varnish group. This is in the same direction with the recommendation, that high caries risk children should receive higher frequencies of the topical fluoride application (31,32). Although, there is no recommendation for the SDF application, in order to achieve the highest efficacy, more frequent applications of SDF have been reported (33). When SDF was applied every 6 months, the caries arresting rate was 91% but it dropped down to 79% when it was applied every 12 months (34). Moreover, Fung and colleagues reported a 67% arrest rate when applied annually and 76% with biannual application (33).

In addition, this study demonstrated the variability in caries arresting rates depending on tooth position. In previous studies, they reported that lesions at anterior teeth had a higher chance of becoming arrested or stopped progression compared to posterior teeth (28,34). Similarly, in this study most of the anterior teeth with treated caries lesions that were active at baseline became arrested (95.7%) more than the posterior teeth

(59%) at 6 months follow-up. The arrested rates of anterior teeth in this study were higher than previously reported (33). Their study was done in deciduous incisors at 24 months and reported a percentage of efficacy of 73.8 and 86.1% when applied annually and semiannually, respectively. The finding is consistent with several previous studies (28, 34). The caries arrest outcome in this study was evaluated earlier than other clinical studies of SDF to treat dental caries. Even so, children who received SDF treatment showed significantly high surfaces of arrested caries at 6 months.

None of the children in this study reported adverse side effects during the study period. However, SDF failure cases were found in one posterior tooth with cavitated lesions (ICDAS 5) with the abscess on the buccal gingiva without any symptom. Posterior teeth seem to be difficult to brush and clean, especially in a deep cavitated lesion that is easily accumulated by food debris (33). The most notable undesirable side-effect after SDF treatment is the black discoloration of carious dentin, but no complaints came from the parents concerning the color of the arrested lesions. Black discoloration and hardening of caries lesions were found not only in children receiving SDF but also in children receiving NaF varnish and in the control group. Fluoride varnish is safe, with a reasonably effective rate of 30-40% for preventing carious lesions development and non-cavitated lesions progression on both coronal and root surfaces (37). There is no evidence indicating that fluoride varnish is an effective treatment for cavitated lesions passing the dentin passing dentin layer of teeth. While fluoride varnish is effective on non-cavitated lesions, SDF is an effective tool against cavitated lesions that pass the dentin layer of teeth, with a success rate of 60-80% after one time application. Fluoride varnish is recommended for new carious lesions prevention when used on sound tooth surfaces and for the management of white-spot lesions which still non-cavitated lesions on the enamel layer of teeth (37). However, non-cavitated lesions may progress to cavitated lesions. At this stage, the use of SDF is more appropriate than fluoride varnish to stop caries progression before the presence of irreversible pulpitis.

Even though the outcome in reducing the level of *S. mutans* in this study was not clear at showing the antimicrobial effect of fluoride agents as a definitive conclusion, the clinical values from this study show the short-term effectiveness of SDF in arresting or stop the progression of caries (36). SDF was more effective in arrest or stop the progression of caries than the fluoride varnish and the control group. From our study, we suggest SDF for uncooperative children with high caries risk, existing multiple cavitated carious lesions which may not all be treated in one visit, or experiencing difficulty accessing dental care. In addition, SDF can be applied in a community setting, which is a simple method to stop the progression of cavitated dental caries in outreach community health services (36).

The limitation of the study was no radiographs were obtained because the study was done in a community setting. Teeth were selected based on clinical examination using a visual-tactile examination, which may have failed to assess the proximal caries or cavities between two teeth to the pulp in some cases. This might explain one child who presented an abscess in an SDF-treated tooth. A larger sample size and

longer follow-up period are recommended to clearly show the efficacy of antimicrobial fluoride application. Further research continues to be undertaken on SDF with regards to developing an optimal treatment strategy of SDF to arrest caries, and especially to establish evidence-based guidelines of SDF in high risk caries. Moreover, it would be more value to follow up the new caries lesions between SDF and fluoride varnish groups in future study.

In conclusion, the present study shows that different levels of *S. mutans* in the SDF group were significantly different between baseline to 3 months, and 3 months to 6 months. SDF was more effective than the fluoride varnish or control groups at 6 months in arresting or stop the progression of current dental carious lesions. With 2 applications within 6 months, 38% SDF can arrest almost 80% of dental caries in preschool children.

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