

# Salivary and Plaque Fluoride Level after MU Caries Preventive Program in Daycare Centers

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## Abstract

This study aimed to investigate and compare fluoride levels in saliva and plaque between the MU caries preventive program and a standard program. A randomized controlled trial was conducted on 77 preschool children from five daycare centers in Pathum Thani, Thailand. Children were randomly arranged into 2 groups: 1) a control group was provided a standard program including oral examination, oral hygiene instruction, diet advice and a fluoride varnish application; 2) a treatment group was provided the MU caries preventive program, which added extra interventions, including Interim Therapeutic Restoration (ITR) and sealant on posterior teeth with glass-ionomer cement. Plaque and saliva samples were collected before and after the program implementation at 24 hours, 1 week, 1 and 3 months, respectively. Salivary fluoride level was measured by a fluoride electrode, while plaque fluoride level was analysed by micro-diffusion technique and using a fluoride electrode (Model 96-09 Orion). The difference of plaque and salivary fluoride levels between the two groups was analyzed by Repeated ANOVA and Mann-Whitney U test, respectively. The treatment group showed a significantly higher plaque fluoride level than the control group at 24 hours ( $p<0.001$ ), 1 week ( $p=0.018$ ), and 1 month ( $p=0.022$ ). However, no significant difference was observed between the two groups at 3 months ( $p=0.228$ ). The salivary fluoride levels showed the same tendency. The treatment group showed significantly higher salivary fluoride levels than the control group at 24 hours ( $p<0.001$ ), 1 week ( $p<0.001$ ), and 1 month ( $p=0.028$ ). However, no significant difference was observed between the two groups at 3 months ( $p=0.055$ ). This study was concluded that the plaque and salivary fluoride levels of children in MU caries preventive program were significantly higher when compared with the standard program at 24 hours, 1 week and 1 month.

**Keywords:** Daycare center, Fluoride, MU caries preventive program, Plaque, Saliva

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## Introduction

Salivary and plaque fluoride play an important role in caries prevention as enhancing remineralization and inhibiting demineralization. Thus, salivary fluoride concentration could be used as a predictor of caries risk.<sup>1</sup> Shields *et al.* (1987)<sup>2</sup> found that subjects with no caries experience had salivary fluoride levels of 0.04 ppm or higher, whereas high caries subjects had salivary fluoride levels of 0.02 ppm or less. Furthermore, maintaining the salivary and plaque fluoride level within an optimum therapeutic level could promote preventive effect and caries reduction. Evidences from *in vitro* studies showed that a concentration of fluoride as low as 0.03 ppm was able to enhance remineralization of demineralized enamel specimens. In addition, increasing fluoride levels up to 0.08 ppm could reach an optimal therapeutic effect for dental caries prevention.<sup>3,4</sup>

Because of this reliable evidence, fluoride releasing materials, especially glass ionomer cement are generally used in dentistry. The advantages of glass-ionomer cement include biological compatibility; chemical adhesion to the tooth structures, fluoride release, acceptable looks and less moisture sensitivity compared with resin composite.<sup>5</sup> For these properties, using this material under field condition or in remote area is possible.

Numerous studies have been on glass ionomer cement and its ability to release fluoride. The study of Koch *et al.* (1989)<sup>6</sup> showed fluoride concentrations in saliva increased immediately after being restored with glass ionomer cement. Three weeks later, the concentrations of fluoride decreased about 35 %. After that, it decreased by another 30 % within 6 weeks. The increasing of fluoride level during the entire observation period equaled 10-30 times greater than baseline levels. This concept results in embracing glass ionomer cement in a preventive program for daycare centers because preschool children have high risk dental caries. Moreover, most of them already had cavitated dental caries. Therefore, the MU

caries preventive program was developed from a standard program which included oral examination, oral hygiene instructions, diet advice and fluoride varnish application. The MU caries preventive program also consisted of the managements of occlusal caries, including Interim Therapeutic Restoration (ITR) for cavitated lesions and sealant for initial carious lesion or deep pits and fissures of posterior teeth. Because fluoride releasing material (glass ionomer cement) is added to this preventive program, the MU caries preventive program was hypothesized to improve oral environment by increasing salivary and plaque fluoride levels in higher amounts than the standard program.

## Materials and Methods

This study protocol was approved by the Committee on Human Rights Related to Human Experimentation, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Bangkok (MU-DT/PY-IRB 2015/ 043.0909).

### Sample size calculation

The sample size was based upon DenBesten and Ko, 1996<sup>7</sup>. The salivary fluoride level in the treatment group was  $0.33 \pm 0.13$  ppm while the control group was  $0.22 \pm 0.13$  ppm. Using a 2-sided,  $\alpha=0.05$ ,  $1-\beta = 0.8$ , a sample of 22 subjects was required for each group. To compensate for a 30 % dropout, at least 29 participants for each group were included.

### Inclusion and exclusion criteria

The subjects were healthy, co-operative and had at least one occlusal caries on posterior teeth, without pulpal exposure and any signs of irreversible pulpitis. The exclusion criteria were children with caries free or allergic to adhesives or colophony which is a component of fluoride varnish. Data of subjects, including medical history and all additional information were derived from a structured interview.

### Oral examination for selection

The oral examinations were performed in the daycare center rooms under fluorescent light. An explorer, a mouth mirror and a spoon excavator were used as examiner tools. Plaque accumulation was recorded using plaque index (PI) criteria of Silness and Loe, (1964)<sup>8</sup>. Dental caries was scored according to the criteria for classifying caries modified from Warren *et al*, (2002)<sup>9</sup> as follows; score 0 = Sound tooth, score 1 = Demineralization but no loss of enamel structure, score 2 = Lesions with loss of enamel structure that are confined to the enamel layer only, score 3 = Small lesions with loss of enamel structure that penetrate into dentine, score 4 = Moderate to large lesions that penetrate into dentine, score 5=Large lesion with pulpal involvement, and score 6=Lesion with pulpal involvement which can not restorable. The posterior teeth score 1 to 3 were treated with glass ionomer sealant, while score 4 were treated with Interim Therapeutic Restoration (ITR).

### Standardized examiners

Subjects were orally examined and followed up by two dentists. Intra-examiner productivity was assessed on ten subjects (13 % of subjects). The kappa values of plaque index and classifying dental caries record were 0.77 and 0.74, respectively. In addition the kappa value of sealant and ITR retention record at follow-up period was 0.83.

### Research procedure

The study was conducted at five daycare centers: Nong Suea, Nong Sam Wang, Watjaroenboon, Bueng Ba, and Srihakkanang in Nong Suea District, Pathum Thani Province.

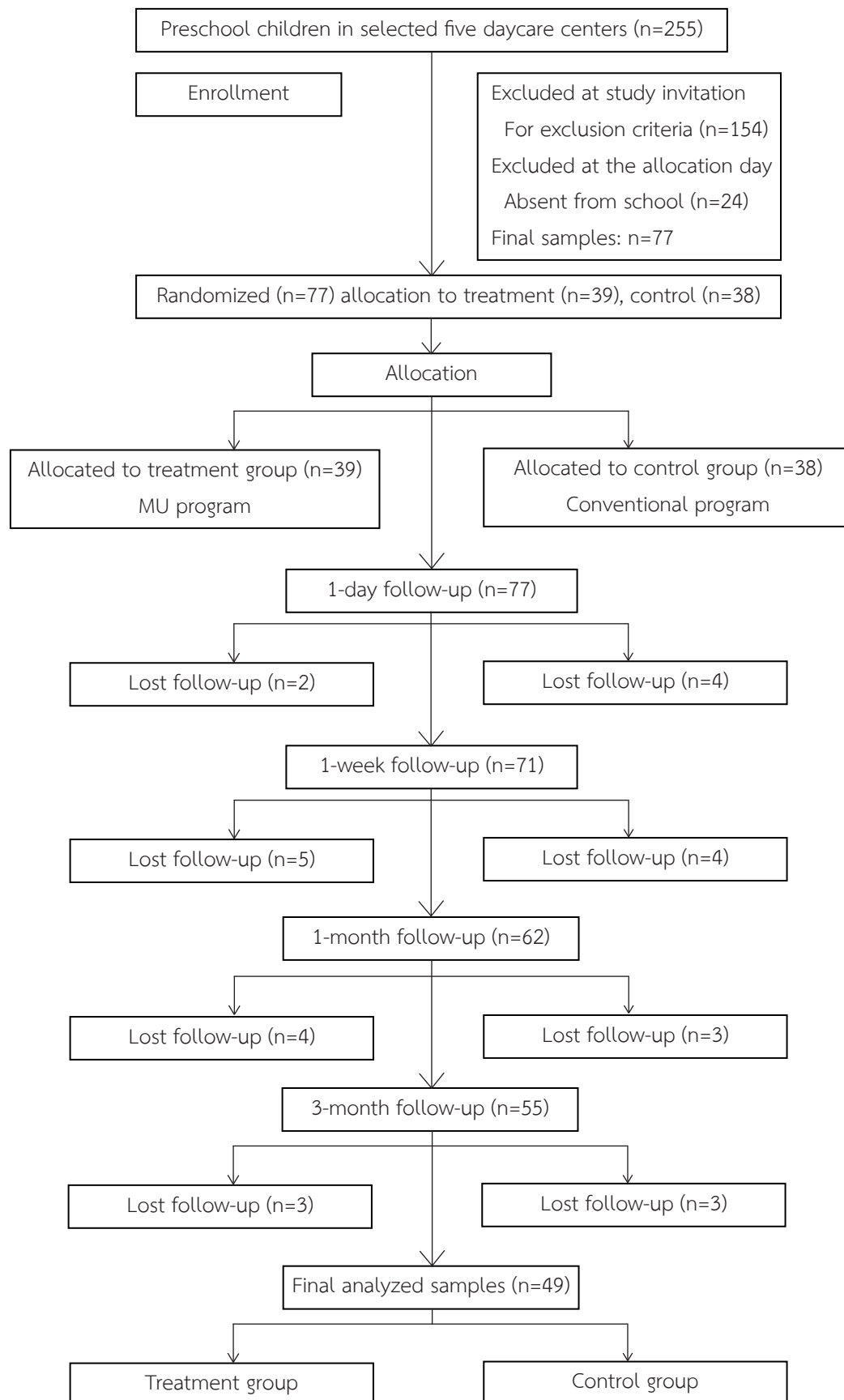
The subjects were randomized by drawing lots method. Due to avoiding the differing characteristics of subjects among five daycare centers, the subjects of each daycare center were randomized to treatment and control group. The total was 77 subjects who allocated into a control group (n=38) and a treatment group (n=39) then received the preventive program as described below; the control group was provided standard program, consisting of hands on oral hygiene instruction with fluoride toothpaste to parents,

diet advice and fluoride varnish (Duraphat<sup>®</sup>) application. The treatment group was provided the MU caries preventive program which, all interventions were similar to the standard program, but added ITR and/or sealant with glass ionomer cement (GC Fuji VII<sup>®</sup>) on posterior teeth. The study flow is shown in Figure 1.

### Treatment procedure

The treatment was performed in the daycare center by two dentists. The operators properly brushed children's teeth without toothpaste. Then all primary molars were isolated with cotton rolls. Then cotton pellets were used to dry the occlusal surfaces. When ITR was performed, soft caries was removed using a spoon excavator after that dentin conditioner (GC Corporation Tokyo, Japan) was applied with a small cotton pellet for 20 seconds. Then wet cotton pellet was used to wipe out the dentin conditioner followed by a dry cotton pellet. Next, glass ionomer cement (GC Fuji VII<sup>®</sup> pink GC Corporation Tokyo, Japan) was mixed in the ratio following the manufacturer's instructions. After that, the filling material was applied to the cavity using a plastic instrument filled with finger press technique and then coated with Vaseline. When sealant was indicated, the procedures were as similar as ITR except it was not needed to remove caries. The mixing ratio for sealant was strictly followed according to the manufacturer's instructions. Children were instructed not to eat for one hour after treatment. All children in both groups were applied fluoride Duraphat<sup>®</sup> varnish for full month after wiping the teeth with sterile gauzes and they were instructed not to brush their teeth that day.

After program implementation, plaque and saliva samples were collected under the time interval of 24 hours, 1 week, 1 and 3 months. For the treatment group, retention of ITR and sealant was recorded in each individual time interval using the criteria adapted from Pereira *et al*, 2001.<sup>10</sup> The plaque index was recorded at one and three months but decay-missing-filled teeth (dmft) and dmfs (decay-missing-filled surfaces) indexes were recorded only at three months.



Main reason of lost follow-up was school absence

Figure 1 Study flow diagram

### Sample collection

Samples were collected in the morning after subjects had breakfast and brushed their teeth for at least two hours.

**Plaque collection:** The subjects were instructed to swallow all remaining saliva then a spoon excavator was used to collect a pooled plaque sample from the buccal, palatal, lingual, and interproximal surfaces of all posterior teeth. Moreover, plaque was scraped gently without directly contacting the enamel surface. This scraping avoided food debris or calculus. The plaque sample was kept in a pre-weighed re-sealable plastic tube with a plastic strip placed inside.

**Saliva collection:** The unstimulated saliva sample was collected by asking subjects to spit saliva for 3 ml. in a re-sealable plastic bottle. During transportation, all samples were kept in a foam box containing ice then stored at -20°C.

### Sample analysis

Fluoride concentration in saliva was determined by direct analysis, while fluoride in plaque was determined using the microdiffusion method by Taves.<sup>11</sup> The fluoride measurement was performed by one examiner blinded as to which samples belonged to the treatment or control group as all samples were labelled by a number. Salivary and plaque fluoride levels were measured with a fluoride electrode (96-09 Orion, Thermo Electron, Beverly, MA, USA). Each sample was measured in duplicate. The accuracy of measurement was evaluated by reverse extraction of standard fluoride at the concentrations of 0.1 and 1 ppm.

## Statistical Analysis

The plaque fluoride level and plaque index between treatment and control group at different time intervals were compared using analysis of repeated

measures (repeated ANOVA) adjusted with the Bonferroni method. While the salivary fluoride level was analyzed by Mann-Whitney U tests. In addition, the dmft and dmfs were analyzed using the independent sample t-test with a level of significance set at 0.05.

## Results

After three month follow-up, forty-nine subjects could participate for all follow-up periods. Information of general characteristic and tooth brushing is shown in Table 1. At the baseline, no significant difference was observed in plaque index, dmft, dmfs, plaque, and salivary fluoride level between treatment and control group. No significant difference was observed in plaque index between the two groups at 1 month ( $p=0.404$ ). However, the plaque index of treatment group was significantly lower than in control group ( $p=0.018$ ) at 3 month follow-up (Table 2).

After program implementation, the plaque fluoride level in treatment group was significantly higher than in control group at 24 hours ( $p<0.001$ ), 1 week ( $p=0.018$ ) and 1 month ( $p=0.002$ ). However, no significant difference of plaque fluoride level was observed at 3 months ( $p=0.228$ ) (Table 3). In addition, salivary fluoride level in the treatment group was significantly higher than in the control group at 24 hours, 1 week ( $p<0.001$ ), and 1 month ( $p=0.028$ ). While at 3 months, the results showed no significant difference of salivary fluoride level ( $p=0.267$ ) (Table 4). In the control group, the pattern of fluoride release in both plaque and saliva illustrated peak fluoride level at 24 hours, and then continuously declined until reaching baseline level in 3 months. However, in the treatment group, the fluoride level in both plaque and saliva did not completely return to the baseline level.

**Table 1** General characteristic and tooth brushing of subjects in the control and treatment group

Group Factors		Treatment group (n=25)	Control group (n=24)
Gender	Boy	12	6
	Girl	13	18
Age (years, Mean±SD)		3.75 ± 0.24	4.08 ± 0.28
Daycare	Nong Suea	6	4
	Nong Sam Wang	8	7
	Watjaroenboon	3	4
	Bueng Ba	4	3
	Srikhakkanang	4	6
Type of toothpaste	Fluoride	25	24
	Non-fluoride	0	0
Frequency of toothbrushing	≤ 1 time/day	5	9
	2 times/day	18	13
	> 2 times/day	2	2
Tooth brushing	without supervision	13	12
	with supervision	7	7
	by caretaker	5	5

**Table 2** Mean±SD of plaque index (PI), dmft, and dmfs before and after the program

Group	n	0	1 month	3 months	p - value
PI					
Control	24	1.86±0.59 <sup>a</sup>	1.13±0.38 <sup>b</sup>	1.37±0.42 <sup>c</sup>	<0.001*, <0.001*, 0.018*
Treatment	25	1.8±0.52 <sup>a</sup>	1.05±0.32 <sup>b</sup>	1.06±0.44 <sup>b</sup>	<0.001*, <0.001*, 0.807
p - value		0.888	0.404	0.018*	
dmft					
Control	24	8.96±4.10	-	9.04±4.07	0.162
Treatment	25	8.72±3.53	-	8.8±3.54	0.161
p - value		0.828		0.825	
dmfs					
Control	24	14.04±9.97	-	14.25±10.23	0.022*
Treatment	25	13.28±9.30		13.4±9.52	0.083
p - value		1.00		0.765	

\*p< 0.05 is statistically significant difference.

Different superscript letters show significant difference

**Table 3** Mean±SD of plaque fluoride levels (ppm) at different time intervals

Time Group	n	Mean±SD of plaque fluoride levels (ppm)				
		Baseline	24 hours	1 week	1 month	3 months
Control	24	35.16±7.04	95.74±29.95	39.99±12.95	36.40±12.80	34.17±10.09
Treatment	25	34.85±5.86	151.04±43.9	56.50±30.55	56.48±27.28	38.46±14.04
p-value		0.866	<0.001*	0.018*	0.002*	0.228

\*p< 0.05 is statistically significant difference.

**Table 4** Mean±SD of saliva fluoride levels (ppm) at different time intervals

Time Group	n	Mean±SD of plaque fluoride levels (ppm)				
		Baseline	24 hours	1 week	1 month	3 months
Control	24	0.021±0.008	0.061±0.026	0.031±0.008	0.033±0.008	0.027±0.006
MU	25	0.019±0.006	0.163±0.110	0.067±0.031	0.042±0.018	0.032±0.010
p-value		0.401	<0.001*	<0.001*	0.026*	0.246

\*p< 0.05 is statistically significant difference.

The progression of dental caries at 3 month follow-up period was shown in Table 5. The subjects in the treatment group presented the progression of dental caries only in anterior teeth. The progression from decalcification (score 1) to enamel caries (score 2) was found the most (11 %). Meanwhile, for subjects in the control group, dental caries progression was found in

both anterior and posterior teeth. In addition, the progression from decalcification (score 1) to enamel caries (score 2) was mostly found in the anterior teeth (10 %). However, the progression from enamel caries (score 2) to small dentin caries (score 3) was found the most frequently in posterior teeth (16.7 %).

**Table 5** Dental caries progression at 3-month follow-up

Dental caries progression*	Treatment (9 teeth)	Control (25 teeth)	
	Anterior teeth (%)	Anterior teeth (%)	Posterior teeth (%)
Sound tooth (score 0) ↓			
Decalcification (score1)	3 (1.5%)	3 (1.6%)	3 (1.6%)
Decalcification (score1) ↓			
Enamel caries (score2)	2 (11%)	3 (10%)	-
Enamel caries (score2) ↓			
Small dentin caries (score3)	1 (1%)	1 (3%)	5 (16.7%)
Small dentin caries (score3) ↓			
Moderate dentin caries (score4)	2 (2.8%)	4 (6%)	2 (3%)
Moderate dentin caries (score4) ↓			
Dental caries involving pulp (score5)	1 (1.5%)	4 (6%)	-

\*Criteria classifying dental caries (Modified from Warren et al., 2002)<sup>9</sup>

The treatment group added ITR and sealant on posterior teeth, so the retention rate of both was shown in treatment group only. From twenty-five subjects in the treatment group, 48 teeth were treated with ITR and 152 teeth were treated with sealant. The retention rate of 48 ITR teeth after follow up at 24 hours, 1 week, 1 and 3 months were 100 %, 93.75 %, 93.75 % and 83.33 %, respectively. Additionally, the retention rate of 152 sealant teeth were 97.37 %, 94.74 %, 85.52 % and 72.37 %, respectively.

## Discussion

In this study, the average salivary fluoride level at baseline was  $0.02 \pm 0.008$  ppm, slightly lower than previous studies; that were  $0.29 \pm 1.7$  ppm<sup>7</sup> and  $0.26 \pm 0.2$  ppm.<sup>12</sup> Nevertheless, the level of salivary fluoride was similar to the study of Petersson *et al*, 2002<sup>13</sup>, that was 0.01-0.02 ppm. The possible reason might cause from the subjects living in low fluoridated area ( $\approx 0.1$  ppm).<sup>14</sup> The baseline of plaque fluoride level in this study was 34-35 ppm which was slightly higher than previous studies; those were 14-16 ppm<sup>15</sup> and 10.4-14.2 ppm.<sup>13</sup> In present study, the baseline fluoride level in saliva was low while fluoride level in plaque was high. The plaque and salivary fluoride levels were not parallel. It could have been caused from the study design that subjects were still using fluoridated toothpaste. Further, fluoride clearance from plaque took longer than from saliva. After being exposed to fluoride, plaque fluoride level took over six hours for clearance times.<sup>16</sup> However, salivary fluoride level took only 60 to 120 minutes to reach baseline level.<sup>7,17</sup> Hence, the subject's collection times after brushing teeth for at least two hours in the present study were not adequate to achieve plaque fluoride clearance times.

The patterns of fluoride release in both saliva and plaque were similar to numerous previous studies.<sup>16,17</sup> Fluoride level had "burst effect" at 24 hours. After that, it rapidly decreased. Then, it continuously declined until

back to baseline level. For the treatment group, the burst effect was a consequence of a majority of fluoride released within first 24 hours. It may be ascribed to an instability and erosion of glass ionomer cement during the early setting period<sup>18,19</sup> combined with a high amount fluoride release from fluoride varnish. However, the burst effect in the control group was only the consequence of fluoride release from fluoride varnish. It led to significantly higher salivary and plaque fluoride level in the treatment group than that in the control group at 24 hours.

Related *in vitro* studies<sup>3,4</sup> have shown salivary fluoride level as low as 0.03 ppm could slightly enhance the remineralization process. Moreover, the optimal therapeutic level for caries prevention was up to 0.08 ppm.<sup>3,4</sup> In the treatment group, the salivary fluoride level at 24 hours ( $0.163 \pm 0.11$  ppm) reached the optimal therapeutic level, exclusively. Regarding other periods of time in the treatment group; one week ( $0.067 \pm 0.03$  ppm), one month ( $0.042 \pm 0.018$  ppm) and three months ( $0.032 \pm 0.01$  ppm), salivary fluoride level merely enhanced the remineralization of tooth structures. Additionally, salivary fluoride level in the control group could not reach the optimal therapeutic level. However, at 24 hours ( $0.061 \pm 0.026$  ppm), 1 week ( $0.031 \pm 0.008$  ppm) and 1 month ( $0.033 \pm 0.008$  ppm) the salivary fluoride concentration was in the range of slightly enhanced remineralization levels.

In the previous studies<sup>15,16</sup> on plaque collection, subjects should refrain from brushing for a few days or in the morning of that day. However, the subjects of this study were allowed to brush their teeth as usual because these subjects had poor oral hygiene and most had moderate to high plaque deposit. Therefore, this study could obtain sufficient plaque without refraining from brushing. The average amount of plaque collection from treatment and control group were  $4.6 \pm 1.0$  mg and  $6.0 \pm 0.5$  mg; respectively. Furthermore, this parameter was considered as a plaque index (PI) which was measured at baseline, one and three month follow-up period.



After program implementation at three months, the dmfs in the control group was significantly increased when compare with baseline. However, both dmft and dmfs between the two groups did not significantly differ, due to the study design, which included ITR and sealant only on posterior teeth. Hence, the anterior teeth of both groups had an equal chance to initiate dental caries. However, the chance to develop dental caries in posterior teeth was greater only in the control group. Moreover, the dental caries process required more time to develop lesions.

The reducing of plaque index in both groups at one month follow-up period was caused from children in both groups, who also received oral hygiene instructions for the parents once. Moreover, one month was a short period of time so that parent could be enthusiastic to follow the dentist's advice. However, at three month follow-up period, plaque index was slightly higher than at one month in both groups. In contrast, the plaque index in the treatment group was lower than in the control group. Because the posterior teeth, treated with ITR, could better function, plaque accumulation was reduced. In addition, the oral hygiene instruction to parents should be stressed again after three months.

According to, the results of the plaque index, dmft, and dmfs corresponded to the results of plaque and salivary fluoride levels. Therefore, the fluoride level within the oral cavity could be another alternative outcome to evaluate the effectiveness of the recent program.

This study perceived a high number of missing subjects i.e., 36 %. However, the 28 children lost to follow-up presented no different characteristics from the remaining subjects, due to the comparison of dmft, dmfs, PI, and salivary fluoride level at baseline between final subjects and missing subjects. No significant difference was observed between both groups in dmft ( $p=0.617$ ), dmfs

( $p=0.688$ ), PI ( $p=0.210$ ), and salivary fluoride level ( $p=0.275$ ).

The estimated cost increase in the MU caries preventive program due to additional ITR and sealant with glass ionomer cement was 47.36 baht per child. Despite the increased cost, three months results showed that the MU caries preventive program could effectively counter the progression of dental caries in posterior teeth. Likewise, it could significantly reduce much more plaque index, compared with a standard program. As a result, the MU caries preventive program could be concluded to be effective.

The limitation of this study was that subjects were always absent from school because of fever and the common cold. Furthermore, time to conduct research was quite short in the daycare centers. Only two hours were available during the day to perform the whole procedures, because children must have lunch at 11.30 am before taking a nap. Moreover, children commonly stayed in the daycare centers for only one year before moving to kindergarten. Therefore, the only possible follow-up period for children in this age group was six months.

## Conclusion

The MU preventive program which added ITR and sealant with glass ionomer cement could elevate plaque and salivary fluoride level significantly when compared to standard program within 1 month.

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