# Plaque Removal Efficacy of Triple-Headed Toothbrush in 4-6-Year-Old **Children: A Randomized Crossover Study**

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Objectives: The objective of this randomized crossover designed study was to compare the efficacy of plaque removal between a triple-headed toothbrush and a conventional single-headed toothbrush in 4–6-year-old children. Materials and Methods: A total of 73 children, aged 4–6 years, were randomly divided into two groups (triple-headed toothbrush and conventional single-headed toothbrush). The children were instructed on how to use the toothbrush by watching a video clip for each toothbrush type and then brushed their teeth for 2 min. The remained plaque was recorded by Greene and Vermillion plaque index (PI). After a 3-week washout period, the toothbrush type was switched, the children brushed their teeth with the alternate toothbrush, and the PI scores were re-recorded. **Results:** Plaque scores in the triple-headed toothbrush group were statistically significantly lower than those in the single-headed group in almost all (10 out or 12) surfaces measured (P < 0.05). Plaque scores were not significantly different on the buccal surfaces of the upper posterior teeth. Conclusion: The triple-headed toothbrush showed better efficacy in plaque removal and could potentially be a good alternative to the conventional single-headed toothbrush in young children with limited hand skill.

**KEYWORDS:** Plaque, single-headed toothbrush, triple-headed toothbrush

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### Introduction

ental caries has been a significant health problem in Thailand. According to the report of the 8th Thailand oral health survey in 2017, the prevalence of dental caries in 3- and 5-year-old children was as high as 52.9% and 95.6%, respectively.[1]

The relationship between accumulation of dental plaque and dental caries in young children was demonstrated in several studies.<sup>[2-6]</sup> The removal of dental plaque by tooth brushing with fluoride toothpaste is one of the most efficient measures for prevention of dental caries. The effective plaque removal depends on appropriate tooth brushing technique, patient compliance, and patient's fine motor skill. Sarvia et al.[7] reported that children under 7 years of age performed significantly lesser in brushing skill. In this age group, children

Access this article online Quick Response Code: Website: www.jispcd.org DOI: 10.4103/jispcd.JISPCD 93 21 were more likely to miss brushing areas and more plague was left on tooth surfaces after brushing. The brushing time for unsupervised children was less than 60 s per brushing in young children. [8,9] Lingual surfaces were the surfaces that children most often failed to brush.[10,11] Due to the lack of motor skills, parents and caregivers are recommended to brush their children's teeth during the first few years of life.[12] However, many Thai preschool children have to brush their teeth by themselves. Over 40% of 3-year-old Thai children were

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reported to perform their own tooth brushing without parental assistance and the number was increased to 80% in 5-year-old children. From this survey, 78.2% and 77.1% of 3- and 5-year-old children had plaque deposit on their tooth surfaces. For this reason, we were interested in finding ways to improve tooth brushing efficacy in these young children.

Various designs of toothbrushes have been recommended to enhance the effectiveness of plaque removal.<sup>[13-17]</sup> One interesting design is the triple-headed toothbrush which was designed to clean buccal, lingual, and occlusal surfaces at the same time.<sup>[18,19]</sup> The handling of the triple-headed toothbrush is easier and more convenient for small children.<sup>[18]</sup> The studies of the effectiveness of triple-headed toothbrush were conducted in several age groups, but only few studies were conducted in preschool children.<sup>[20-22]</sup>

The objective of this study was to compare the efficacy of plaque removal between a triple-headed toothbrush and a conventional single-headed toothbrush in 4–6-year-old children.

### MATERIALS AND METHODS

#### STUDY POPULATION

The sample size was calculated by G\*POWER program version 3.1.9.2, with the Cohen's measures for the effect size at 0.5, the error probability at 0.05, and the power of analysis at 0.95. After the 20% dropout compensation rate, a total of 73 healthy children were recruited in this study. All the participants studied at Wat Thatthong Bangkok Metropolitan administration nursery school. Parental written consent must be provided prior to children's participation in the study.

The participants inclusion criteria included: (1) 4–6-yearold of age, (2) no disability that would affect tooth brushing skill, and (3) have adequate numbers of teeth for plaque scoring. The exclusion criteria included: (1) absence from the school on the first or second day of trial and (2) presence of soft tissue ulcers or gingival abscess.

Ethical approval of this study was obtained from the Institution Review Board of Faculty of Dentistry, Srinakharinwirot University (IRB DENTSWU-EC-20/2560).

### QUESTIONNAIRE

The questionnaires were sent to the parents 1 week prior and returned on the day of the trial.

The content of the questionnaires included: (1) How often your child brushes his/her teeth per day (once a day, twice a day, more than twice)?

(2) Who brush your child's teeth (child, parent)?

(3) Do you think your child can perform tooth brushing (yes, no, not sure)?

### **GROUP ALLOCATION**

This trial was a randomized crossover design study. At the first session, the participants were randomly divided into two groups: a triple-headed toothbrush (Dr Barman's Superbrush (child size for 0–6 years) group or a conventional single-headed toothbrush (Berman, regular soft bristle, small size) group. The children were instructed to brush by watching the VDO clip for each type of toothbrush (as shown in the link https://drive.google. com/drive/folders/1xqyZUyZg4DO6DWUNGXyvtq\_ dc9U1VkzH?usp=sharing). They then brushed their teeth themselves with assigned toothbrush for 2 min. In the triple-headed toothbrush group, the children were instructed to brush all of their teeth by placing the toothbrush on occlusal surfaces and moved the brush in a short back and forth movement for 10 strokes in each area. In the conventional single-headed toothbrush group, the children were instructed to brush with horizontal scrub technique for 10 strokes in each area. They were taught to place the bristles 90° to buccal, lingual, and occlusal surfaces. After brushing, erythrosine solution was applied to the teeth with the cotton swab to visualize remaining plaque.

# **EVALUATION OF PLAQUE REMOVAL EFFICACY**

Plaque scores of all participants were recorded by two examiners who were blinded from the type of toothbrush. Plaque scores were measured in 12 areas from buccal and lingual surfaces of 6 teeth (55B, 55L, 51B, 51L, 65B, 65L, 75B, 75L, 71B, 71L, 85B, 85L), according to Greene and Vermillion with scale 0–3. [23]

During the 3-week washout period, the participants performed tooth brushing their usual ways. After that, in the second session, the children were instructed to brush by watching the VDO clip for alternate type of toothbrush and then brushed for 2 min. The remaining plaque scores were recorded by the two examiners by the same protocol as the first session.

### STATISTICAL ANALYSIS

Inter-examiner reliability was determined using Cohen's kappa score. Statistical analysis was performed using SPSS 16.0. A *P*-value less than 0.05 was considered statistically significant. The differences in plaque scores between single- and triple-headed toothbrush groups were analyzed using paired *t*-test.

### RESULTS

### **SUBJECTS' CHARACTERISTICS**

The participants consisted of 73 children, age 4–6 years old from kindergarten 1 and 2. The mean age of the

children was 5 years 2 months. The characteristics of the participants and the information about tooth brushing practice were shown in Table 1.

The majority of children 78% brushed their teeth by themselves, two times per day (76.7%). Most parents (98.6%) reported that their children have the ability to brush their teeth by themselves.

### PLAQUE REMOVAL EFFICACY

The kappa inter-examiner reliability was 0.89. Mean plaque scores from the triple-headed toothbrush group were statistically lower than those from the single-headed group in 10 out of 12 surfaces measured (*P*<0.05) [Figure 1A and B]. Only at the buccal surfaces of upper molars (55B and 65B) that the plaque scores of the two groups were not significantly different. From 12 areas, the highest plaque scores were found at the buccal surfaces of the upper posterior teeth (55B and 65B) in both groups.

A comparison of the effectiveness of the two groups in different areas of the mouth was shown in Table 2. In seven out of eight areas tested (exception for buccal surface of maxillary posterior teeth), the plaque scores of the triple-headed toothbrushes were significantly lower than those of the single-headed ones.

When mean plaque accumulation scores were assessed [Table 3], the triple-headed toothbrush group showed statistically significant lower scores on lingual surfaces, buccal surfaces, and total surfaces when compared with the single-headed group.

Table 1: Characteristics of the participants and the information about tooth brushing practice

Gender	N (%)
Boys	41 (56.2)
Girls	32
	(43.80)
Brushing frequency	
None	0 (0)
1 time/day	13 (17.8)
2 times/day	56 (76.7)
More than 2 times/day	4 (5.5)
Person responsible for your child's brushing	
Children	57
	(78.08)
Parents	16
	(21.92)
Your child has ability to brush the teeth by	
themselves	
Yes	72 (98.6)
No	0(0)
Not sure	1 (1.4)

Plaque index (PI) values were calculated according to Greene and Vermillion.<sup>[23]</sup> PI in the triple-headed toothbrush group was significantly lower than that of the single-headed toothbrush group [Table 3].

### **DISCUSSION**

Plaque accumulation is one of the etiologic factors for dental caries, especially in young children.<sup>[2,3]</sup> Data from our cross-over design study revealed that almost 80% of children performed tooth brushing by themselves and the use of the triple-headed toothbrush significantly increased the efficacy of plaque removal in almost all the surfaces when compared with the conventional toothbrush.

As recommended, [24,25] preschoolers (3–6-year-old children) required parental assistance to brush their teeth as they lack the adequate motor skill. [7,26] However, our result is in agreement with the previous report from 2018 National Oral Health Survey of Thailand that only 20% of 5-year-old children have their parents brushed their teeth. [1] This fact is possibly due to various life limitations in each family, and it emphasizes the needs to search for additional tools to improve the children's brushing ability.

A triple-headed toothbrush is intended to clean buccal, lingual, and occlusal surfaces at the same time. The two heads are designed with 45° bristles for cleaning buccal and lingual surfaces and the third head is designed for occlusal surfaces. It is suitable for people who have limited manual skills such as physical and mental handicaps or young children.<sup>[15,22]</sup>

When a conventional single-headed toothbrush was used, lingual surfaces were often missed.[10,11] The study of tooth brushing behavior in 5-year-old children revealed that they brushed only 25% of the tooth surfaces and only 10 s was used to brush lingual surfaces.[27] Our results confirmed the previous findings that showed the greater efficacy of a tripleheaded toothbrush in plaque removal from lingual surfaces.[19,26] This may be attributed to easier handling of the triple-headed toothbrush which clean all three surfaces simultaneously. Our study also found that the most difficult area to clean was buccal surfaces of upper molars (55B and 65B) in both groups, which was similar to the study by Mahmoodi et al. [26] in 2014. The difficulty in cleaning this area might due to the anatomy of buccal oral mucosa that hinders the placement of the toothbrush against the cervical region of the teeth.

Most previous studies of triple-headed toothbrush measured long-term efficacy with home-based use of the brush. Those studies could not strictly control the

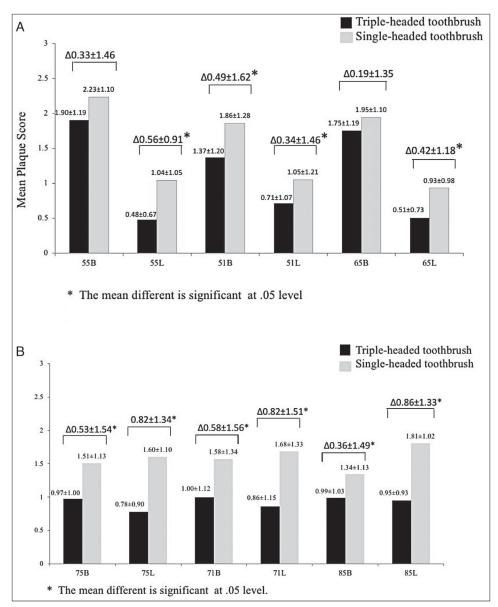


Figure 1: (A) Comparisons of mean plaque score in the maxillary arch. (B) Comparisons of mean plaque score in the mandibular arch

brushing time<sup>[18,22,28]</sup> except the study by Oliveira *et al*.<sup>[21]</sup> In our study, the children were taught how to brush and then brushed themselves for a controlled period of 2 min. As recommended by the American Academy of Pediatric Dentistry and American Dental Association, in order to obtain the good oral hygiene, the brushing time should be at least 2 min.<sup>[24,29]</sup> The study from Creeth *et al*.<sup>[30]</sup> found that brushing for 2 min removed 26% more plaque than brushing for 45 s. However, many studies reported that in practice the average brushing time for children under the age of 6 was less than 60 s.<sup>[8,9]</sup> Mentes and Atukeren<sup>[8]</sup> found that the mean brushing time in 3–5-year-old Turkish children was 28 s. Similarly, the study by Das and Singhal<sup>[9]</sup> in Indian children found that the mean brushing time for

this age group was 45 s. Brushing time is one of the determinants of plaque removal. In order to reduce this confounding factor, we controlled the 2-min brushing time to ensure that the efficacy of the toothbrush was not reduced by short brushing time. The future application of the superiority of the triple-headed over the single-headed toothbrush reported here must also emphasize the 2-min brushing time.

The results of our study confirmed that unsupervised self-performed brushing with a triple-headed toothbrush for 2 min showed significantly better reduction in plaque scores. Azrak *et al.*<sup>[20]</sup> studied the self-performed effectiveness of a triple-headed toothbrush in 4–5-year-old preschool children and found that the numbers of surfaces with plaque as well

Table 2: Mean plaque score in upper and lower arch								
			Single-headed	Triple-headed	Mean difference	<i>P</i> -value		
Maxillar	Anterior teeth	Buccal	1.86	1.37	0.49	0.011*		
		Lingual	1.05	0.71	0.34	0.05*		
	Posterior teeth	Buccal	2.09	1.83	0.26	0.07		
		Lingual	1.00	0.49	0.51	<0.001*		
Mandibular	Anterior teeth	Buccal	1.58	1.00	0.58	0.002*		
		Lingual	1.68	0.86	0.82	<0.001*		
	Posterior teeth	Buccal	1.42	0.98	0.44	0.008*		
		Lingual	1.71	0.86	0.85	<0.001*		

Table 3: Mean plaque accumulation scores and PI for toothbrush groups

Surfaces	Single-headed	Triple-headed	T-test	P-value					
	mean (SD)	mean (SD)							
Mean plaque accumulation scores									
Buccal	10.47 (3.86)	7.98 (4.07)	-6.8	<0.001*					
Lingual	8.12 (4.03)	4.29 (3.26)	-3.8	<0.001*					
Buccal	18.59 (7.15)	12.27 (6.68)	-5.76	<0.001*					
and									
lingual									
Plaque index score									
Plaque	3.10 (1.19)	2.05 (1.11)	-5.711	<0.001*					
index									

as whole mouth plaque scores were significantly reduced in the three-headed toothbrush group. Similarly, the studies in the older age group found that the PI in the triple-headed toothbrush group was significantly lower than that of the conventional single-headed and electric toothbrush groups.[18,31] In contrast, Kiche et al.[28] reported no significant difference in plaque removal efficacy between triple- and single-headed toothbrush in 7-10-year-old children with mixed dentition. Their study was crossover design similar to our present study; however, the brushing time was not controlled. The insignificant results reported might possibly due to inadequate brushing time. Another factor was the height of the permanent teeth which might not be thoroughly cleaned by the triple-headed toothbrush. It has been suggested that triple-headed toothbrush may not be suitable for periodontitis patients with elongated clinical crown.[31]

A significantly higher plaque removal efficacy of a triple-headed toothbrush in our study may be due to its advantage, which is that three surfaces of the tooth were cleaned simultaneous in one stroke. As a result, the brushing time of a triple-headed toothbrush was effectively triple than that of a single-headed toothbrush.

When parents performed brushing, the previous study also showed better performance when the triple-headed toothbrush was used than the conventional one. [21,22] Interestingly, the tooth brushing performance index, in terms of correct placement of toothbrush on the tooth and adequate numbers of stokes, was higher in the triple-headed toothbrush group. This result demonstrated that even when parents performed the brushing for their children, a triple-headed toothbrush was more convenient and easier. Moreover, when 7–10-year-old children were asked for the preference between two types of toothbrush, 83% of them preferred the triple-headed toothbrush. The reasons were that they felt it cleaned better, faster, more comfortable, and easier to use. [18]

In our study, in order to control the brushing time, we measured the plaque score immediately after single brushing. For this reason, we were unable to assess the long-term efficacy of the triple-headed toothbrush. By using cross-over design, the confounding factors are reduced because each crossover subject serves as its own control. For the future research, long-term efficacy of plaque removal and gingival status needs to be investigated.

All in all, preschool children should be toothbrushed by their parents. The use of a triple-headed toothbrush is not a replacement for parental care but a helper for children to brush their own teeth more effectively. Moreover, the 2-min brushing time should be emphasized.

#### Conclusions

The triple-headed toothbrush showed better efficacy in plaque removal in 4–6-year-old children. It could be an option to the conventional single-headed toothbrush in children with limited hand skills who have to perform brushing by themselves.

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### **C**ONFLICTS OF INTEREST

There are no conflicts of interest.

#### **A**UTHORS CONTRIBUTION

K. Y. and N. K. contributed to the development of the study design, data interpretation, and manuscript preparation. K. Y., N. T., N. A., Y. W., and M. R. participate in data collection and data analysis.

### ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical approval of this study was obtained from the Institution Review Board of Faculty of Dentistry, Srinakharinwirot University (IRB DENTSWU-EC-20/2560).

### PATIENTS DECLARATION OF CONSENT

Not applicable.

### **D**ATA AVAILABILITY STATEMENT

The data set used in the current study is available upon request at nathawut@g.swu.ac.th.

# REFERENCES

- Dental Health Bureau. Report on the Seventh National Oral Health Survey of Thailand 2017. Ministry of Public Health; 2018.
- Featherstone JDB, Chaffee BW. The evidence for caries management by risk assessment (CAMBRA®). Adv Dent Res 2018;29:9-14.
- Chaffee BW, Featherstone JDB, Zhan L. Pediatric caries risk assessment as a predictor of caries outcomes. Pediatr Dent 2017;39:219-32.
- Bashirian S, Shirahmadi S, Seyedzadeh-Sabounchi S, Soltanian AR, Karimi-Shahanjarini A, Vahdatinia F. Association of caries experience and dental plaque with sociodemographic characteristics in elementary school-aged children: A cross-sectional study. BMC Oral Health 2018;18:7.
- Khalid T, Mahdi SS, Khawaja M, Allana R, Amenta F. Relationship between socioeconomic inequalities and oral hygiene indicators in private and public schools in Karachi: An observational study. Int J Environ Res Public Health 2020:17:8893.
- Tinanoff N, Baez RJ, Diaz Guillory C, Donly KJ, Feldens CA, McGrath C, et al. Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective. Int J Paediatr Dent 2019;29:238-48.

- Sarvia ME, Bush JP, Mourino AP. Psychomotor skills and incentive as predictors in a children's toothbrushing program. J Pedod 1989:14:31-5.
- Mentes A, Atukeren J. A study of manual toothbrushing skills in children aged 3 to 11 years. J Clin Pediatr Dent 2002;27: 91-4.
- 9. Das UM, Singhal P. Tooth brushing skills for the children aged 3-11 years. J Indian Soc Pedod Prev Dent 2009;27:104-7.
- Deinzer R, Cordes O, Weber J, Hassebrauck L, Weik U, Krämer N, et al. Toothbrushing behavior in children—An observational study of toothbrushing performance in 12 year olds. BMC Oral Health 2019;19:68.
- Kayalvizhi G, Radha S, Prathima GS, Mohandoss S, Ramesh V, Arumugam SB. Comparative evaluation of plaque removal effectiveness of manual and chewable toothbrushes in children: A randomized clinical trial. Int J Clin Pediatr Dent 2019;12:107-10.
- American Academy of Pediatric Dentistry. Policy on Early Childhood Caries (ECC): Classifications, Consequences, and Preventive Strategies. American Academy of Pediatric Dentistry; 2016. Available from: https://www.aapd.org/ globalassets/media/policies\_guidelines/p\_eccclassifications.pdf (accessed March 10, 2021).
- Davidovich E, Shafir S, Shay B, Zini A. Plaque removal by a powered toothbrush versus a manual toothbrush in children: A systematic review and meta-analysis. Pediatr Dent 2020;42:280-7.
- Gallie A. Should five to eleven-year-olds use manual or electric toothbrushes to clean their teeth? Evid Based Dent 2019:20:76.
- Doğan MC, Alaçam A, Aşici N, Odabaş M, Seydaoğlu G. Clinical evaluation of the plaque-removing ability of three different toothbrushes in a mentally disabled group. Acta Odontol Scand 2004;62:350-4.
- 16. Kerr R, Claman D, Amini H, Alexy E, Kumar A, Casamassimo PS. Evaluation of the ability of five- to 11-year-olds to brush their teeth effectively with manual and electric toothbrushing. Pediatr Dent 2019;41:20-4.
- Warren DP, Rice HC, Turner S. Comparison of plaque removing ability of one standard and two flexible-head toothbrushes. J Dent Hyg 2004;78:340-2.
- Zimmer S, Didner B, Roulet JF. Clinical study on the plaqueremoving ability of a new triple-headed toothbrush. J Clin Periodontol 1999;26:281-5.
- Kalf-Scholte SM, Van der Weijden GA, Bakker E, Slot DE. Plaque removal with triple-headed vs single-headed manual toothbrushes—A systematic review. Int J Dent Hyg 2018:16:13-23.
- Azrak B, Barfaraz B, Krieter G, Willershausen B. Effectiveness of a three-headed toothbrush in pre-school children. Oral Health Prev Dent 2004;2:103-9.
- Oliveira LB, Zardetto CG, Rocha Rd, Rodrigues CR, Wanderley MT. Effectiveness of triple-headed toothbrushes and the influence of the person who performs the toothbrushing on biofilm removal. Oral Health Prev Dent 2011;9:137-41.
- 22. Telishevesky YS, Levin L, Ashkenazi M. Assessment of parental tooth-brushing following instruction with single-headed and triple-headed toothbrushes. Pediatr Dent 2012;34:331-6.
- 23. Greene JC, Vermillion JR. The oral hygiene index: A method for classifying oral hygiene status. J Am Dent Assoc 1960;61:172-9.
- 24. American Academy of Pediatric Dentistry. Fast facts. Dental care for your preschooler; 2013. Available from: https://www.

- aapd.org/assets/1/7/HealthySmilesGuidebook.pdf (accessed March 10, 2021).
- Jeffrey AD, Christopher VH. Mechanical and chemotherapeutic home oral hygiene. In: Dean JA, Avery DR, McDonald RE, editors. McDonald and Avery's Dentistry for the Child and Adolescent. 10th ed. Maryland, MO: Mosby–Elsevier; 2016. p. 120-37.
- Mahmoodi P, Salimi P, Ashtiyani RD, Valaii N, Azarshab M, Shafizadeh N. Assessment of fine motor skills and tooth brushing skills in 5–6 year olds in Tehran. J Res Dent Sci 2014;11:176-81.
- Rugg-Gunn AJ, Macgregor ID. A survey of toothbrushing behaviour in children and young adults. J Periodontal Res 1978:13:382-9
- Kiche MS, Fayle SA, Curzon ME. A clinical trial comparing the effectiveness of a three-headed versus a conventional toothbrush for oral hygiene in children. Eur J Paediatr Dent 2002;3:33-8.
- 29. American Dental Association. Mouth Healthy: Brushing Your Teeth. Available from: https://www.mouthhealthy.org/en/az-topics/b/brushing-your-teeth (accessed March 10, 2021).
- Creeth JE, Gallagher A, Sowinski J, Bowman J, Barrett K, Lowe S, et al. The effect of brushing time and dentifrice on dental plaque removal in vivo. J Dent Hyg 2009;83: 111-6.
- Levin L, Marom Y, Ashkenazi M. Brushing skills and plaque reduction using single- and triple-headed toothbrushes. Quintessence Int 2012;43:525-31.