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Research Article

Salivary Fluoride and pH Levels After Using Toothbrush With 2-Minute Timer (Prototype Trial)

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Abstract

The recommended tooth brushing duration is 2 minutes to obtain optimal fluoride levels in the mouth. Unfortunately, many people, especially teenagers and children, struggled to comply with the minimum duration. This study developed and tested the effectiveness of a toothbrush with a 2-minute timer prototype. This study determined the gaps and the mean scores of salivary pH, fluoride levels, plaque scores, and the number of *Streptococcus mutants* between the control group that used manual toothbrushes and the experimental group that used the prototype. A crossover and single-blind method was employed, involving 40 second-grade junior high school students selected using a simple random sampling technique. Data was collected by measuring the pH, fluoride levels, and the number of *Streptococcus mutants* bacteria in unstimulated saliva. The plaque score was also calculated using the Quigley-Hein modified Turesky plaque index (TMQHPI). The average fluoride level obtained by the experimental group was higher (8.86 ± 1.29) than the one of the control group (1.46 ± 0.44). The Independent T-test showed a significant difference in fluoride levels ($p = 0.0001$), while no significant difference in salivary was found in pH ($p = 0.58$) between both groups. The toothbrush with a 2-minute timer prototype developed in this study increased the fluoride levels, preventing dental caries and restoring salivary pH levels.

Keywords: child; electric toothbrush; fluorides; saliva; toothbrush

INTRODUCTION

The condition of children's dental caries in Indonesia is quite worrying as the dental caries suffered by most children in Indonesia usually have reached the pulp. If plaque control can be carried out optimally, this problem can be prevented from an early age.[1,2] Tooth brushing is one of the mechanical plaque controls that is effective in preventing dental caries. Plaque control can be done by brushing teeth at least twice daily, i.e., after breakfast and right before bed at night, for 2-3 minutes each.[2] Tooth brushing duration is a factor that affects oral hygiene. The recommended tooth brushing duration varies between 120 seconds (US) and 180 seconds (Europe).[3]

A study conducted by Rahardjo et al. 2015 on 120 families in Jakarta, Indonesia, showed that only 2% brushed their teeth for more than 2 minutes, and the mean duration of brushing teeth was 57.29 seconds.[4] JE Creeth, 2016 determined the effect of tooth brushing duration and toothpaste quantity on enamel remineralization by grouping people into two groups with different tooth brushing durations; 45 seconds and 120 seconds. People who brushed their teeth for 2 minutes were found to have significant fluoride levels compared to those who

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brushed their teeth for 45 seconds.[5] The results of previous studies also indicated the importance of brushing duration as the fluoride uptake supports the remineralization process in enamel lesions, and also brushing teeth for 2 minutes will help maintain the fluoride to last longer in saliva.[6-8]

Tooth brushing can also increase the fluoride level and bring the saliva's pH to neutral.[9] Neutral salivary pH can be a protective factor against dental caries because it prevents the pH level from dropping when glucose is converted to acid by bacteria.[10] The normal degree of saliva acidity (pH) ranges between 6,7 and 7,3, and the critical pH value for demineralization is usually between 5,2 and 5,5.[11,12] Studies analyzing the impacts of tooth brushing duration on changes in pH level in the mouth cavity are still limited.

Toothbrushes with a 2-minute timer feature have not yet been produced by manufacturers in the Indonesian market, and most of the toothbrushes sold are imported goods. A toothbrush with a timer can be a solution to increase the duration of tooth brushing as it has a timer alarm. In this research, a toothbrush with a timer prototype that has an alarm function and vibration feature was developed. Furthermore, its effectiveness was also analyzed to maintain the fluoride levels and pH in saliva, preventing plaque and Streptococcus mutant bacteria. This research aims to determine the mean difference in salivary pH, fluoride levels, plaque scores, and the number of Streptococcus mutants between the control group that used manual toothbrushes and the experimental group that used the prototype toothbrushes with a 2-minute timer.

MATERIALS AND METHODS

The development of a toothbrush with a 2-minute timer prototype

The process of prototype-making started with designing the hardware by creating an electronic circuit that connects all hardware into a system consisting of input, processing unit, and output. The input consists of a micro switch on the brush and keypad. The processing unit was built using a microcontroller, while the output consisted of an LED beam and a speaker. After the device was built, the software was developed using a programming language that would later be transferred to the microcontroller. The software was designed using the CodeVision AVR compiler, C programming language, and ATMega16 microcontroller. After the algorithm was completed, it was installed on the computer's microcontroller using the AVR Downloader USB cable. The prototype toothbrush has some parts as follows: a) Connector: a device to connect the handlebar and the head of the brush; b) Start button: to switch the toothbrush on and off; c) Sound/ alarm: as a timer/sign when the toothbrush is being used; d) Head brush: the head of the brush is separated from the handlebar with a total length of 7 cm and 1.3 width

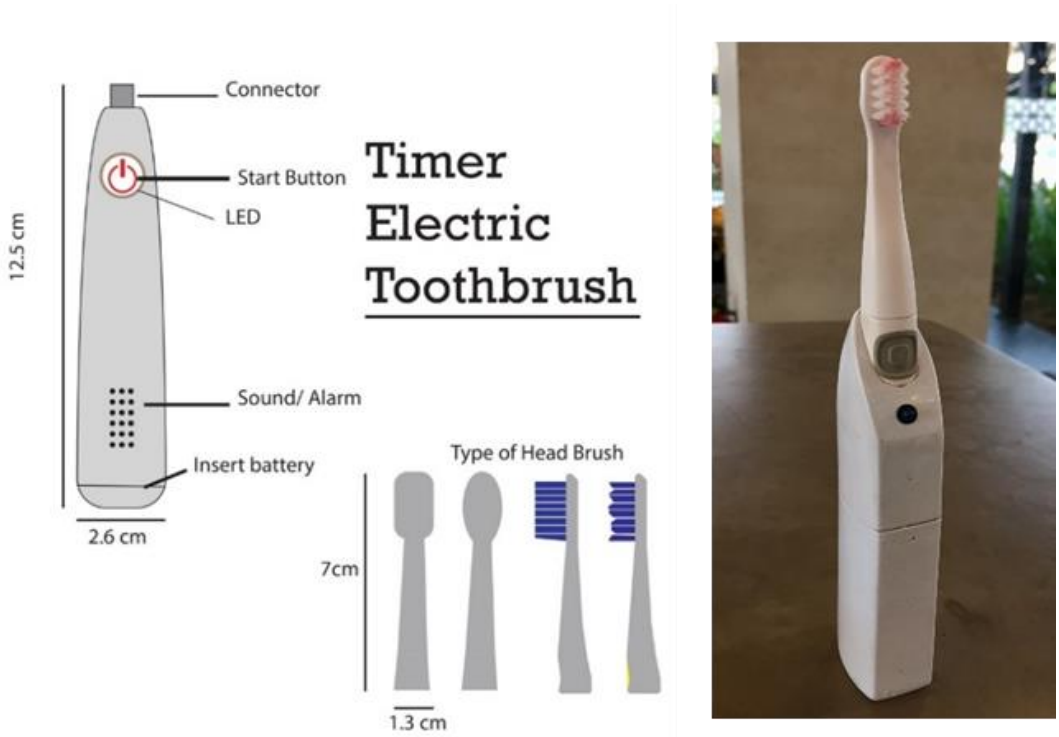

Figure 1. The design of the toothbrush prototype with a 2-minute timer (original picture from authors)

Ethical eligibility and research samples

This research fulfilled the ethical issue of the Ethics Institute of YARSI University, Jakarta. Parents of students were given an explanation sheet and research consent without any coercive action. This research is an experimental field research done using single-blind and crossover. The research setting was determined using a purposive sampling technique, while samples were determined using simple random sampling. The subjects of this research were 40 students of SMPN 137 Jakarta who met the inclusion criteria. Most of them were 14 years old and had no systemic diseases, were not under orthodontic treatment, or were using prosthodontics (fixed or removable), and their oral hygiene was considered good. The exclusion criteria were students who had a habit of gargling with tea or mouthwash in the last two weeks, had a history of taking antibiotics for three months or were on routine medication, were not cooperative during sampling, those whose parents did not give permission, and students who have lost more than 4 posterior teeth.

The initial subjects who were willing to participate were 60 students. However, only 40 students were involved after screening and scaling, as they met the inclusion criteria. This number has met the minimum sample size. One subject was excluded for being non-cooperative, and 2 subjects were absent. Hence, the total sample was 37 respondents, consisting of 17 boys and 20 girls. All research subjects were subjected to crossover, but one child was not present at the crossover. Thus, the final number of samples was 72.

The implementation of the research and measurement

This research was conducted from October 2020- February 2021. Students were given counseling or education in advance about the correct technique of tooth brushing that should be applied during the research, namely the Stillman modification technique. Researchers also used the same toothpaste with fluoride

during the study to control confounding. The research subjects were divided into 2 comparison groups. Twenty students were assigned to the conventional (control) group, and 20 were assigned to use the prototype timer toothbrush. The remaining 20 students were divided into 2 comparison groups: the electric toothbrush group without a timer and the prototype timer toothbrush.

Subjects in each group were assigned to brush their teeth using the correct technique as taught. The duration of brushing was calculated using a stopwatch. Respondents' saliva was collected using chewing sterile corks in a centrifuge tube. The pH of saliva was calculated using a calibrated digital pH meter (Metrohm AG). Plaque examination was done by applying the disclosing solution to the entire tooth surface. The plaque score was obtained using the modified Turesky Quigley Hein plaque index, using a mouth mirror with a score range of 0-5 on each buccal/labial and lingual/palatal surface of all teeth except the third molar.

The microbiology laboratory procedure was carried out at Yarsi University. First, a serial dilution was performed using 9 ml of liquid broth, added with 1 ml of saliva, then shaken to homogeneous. Dilution was carried out to obtain a concentration of 10^{-5}. Furthermore, planting was carried out on Tryptone Yeast Extract Cystine w/ Sucrose and w/o Bacitracin Agar (TYCSB) selective media, with 0.05 ml of the bacterial suspension placed on the surface of the media, being spread using a spatula L and incubated in the facultative and anaerobic way using an excavator. On top of the agar pile, TYCSB, which has been planted with bacteria, is given a lit candle and cotton soaked in water to create an environmental atmosphere facultative anaerobe. The dead candle in the desiccator shows that oxygen has been lost, and there is CO_2 produced from wet cotton. Furthermore, the excicator containing TYCSB media was incubated at 37^oC for 24 hours. After 24 hours of staining and imaging bacteria under a scanning electron microscope with gram staining, the number of *Streptococcus mutants* was counted using a colony counter.

Fluoride level measurement

The research sample was examined for dental and oral hygiene using the OHIS index. Dental scaling was given to samples that have poor oral hygiene. The subjects' saliva was collected in 5 ml test tubes before and after brushing their teeth to calculate the fluoride levels in the saliva. The subject was previously instructed to chew the wax and then was asked to spit to collect the saliva into the test tube. The measurement of fluoride levels was conducted at the laboratory using a UVVIS Spectrophotometer TECAN Infinite M200 Pro® (Männedorf, Switzerland). The absorption of the solution was measured by calculating the maximum wavelength produced by sodium fluoride (NaF) solution and SPADNS reagent $\lambda = 510$ nm. SPADNS reagent that reacts with fluoride ions can cause a color change reaction, which its UV-Vis Spectrophotometer then detects. Color change shows the function of the fluoride ion and is directly proportional to fluoride concentration, used to measure internal fluoride water (saliva).

RESULT

The age varied (table 1), showing that the average and median age of children in this study were 13.9 years and 13.88 years. Most of the samples were 14.08 years old. The youngest age in this study was 13.58 years old, and the oldest was 14.26 years. The mean and median scores for the salivary fluoride level were 4.78 ppm and 1.99 ppm, respectively. The average conventional tooth brushing duration was 1.16 minutes, and most students spent 1 minute.

Table 2 compares the independent t-test for plaque index and pH between the prototype and conventional toothbrush groups. The result showed a significant difference between both groups with p-value = 0.005 ($p<0.05$). Meanwhile, the pH comparison test between the 2 groups showed p-value = 0.580 ($p>0.05$), indicating no significant difference in pH between the prototype and conventional toothbrushes. The results of the Independent T-test for plaque index and pH between the prototype toothbrush group and the electric group showed p-value = 0.326 ($p>0.05$) and p-value. = 0.456 ($p>0.05$), indicating no significant difference.

Table 3 shows the results of the Independent T-Test for fluoride levels between groups that used conventional toothbrushes and the one that used an electric timer toothbrush prototype. The mean salivary fluoride level in the group using the electric timer toothbrush prototype was higher at 7.91 ppm, while those who brushed their teeth using conventional toothbrushes obtained 1.46 ppm. The results of the Independent T-test for salivary fluoride levels between the groups that used prototype toothbrushes and electric toothbrushes showed no significant difference at p-value = 0.425 ($p> 0.05$).

The significance value of the difference in the number of *Streptococcus mutants* between the two groups (prototype and conventional) was 0.452 ($p> 0.05$), indicating no significant difference. Likewise, T-Test showed results between the prototype timer groups and electrically non-timer ($p = 0.589$, $p> 0.05$). The mean score of the number of *Streptococcus mutants* in saliva in samples that brushed their teeth in less than 2 minutes using an electric toothbrush without a timer was higher at 728,25 x 10^{-5} CFU/ml, while in samples that brushed their teeth for 2 minutes using a brush electric timer prototype gear was 552,20 x 10^{-5} CFU / ml.

Table 1. The Distribution of Research Subject and Variables

Variable	Mean	Median	Modes	Minimum	Maximum
Age	13.90	13.88	14.08	13.58	14.26
Fluoride Level	4.78	1.99	0.92	0.92	18.46
Duration	1.16	1.15	1.00	0.53	1.59

Table 2. The results of the *independent t-test* on plaque index and pH level between the prototype group, conventional and non-timer electric toothbrush group

Variable	Group (Mean±SD)		P	95% CI
	Prototype	Conventional		
Plaque	0.44±0.27	0.78±0.37	0.005	(-0.58)-(-0.11)
pH	7.90±0.31	7.95±0.12	0.580	-0.22-0.13
	Prototype	Electric	P	95% CI
Plaque	0.53±0.17	0.46±0.28	0.326	(-0.08)-0.23
pH	7.75±0.45	7.66±0.26	0.456	(-0.15)-0.33

Table 3. The results of Independent T-Test on Fluoride levels

Variable	Group (Mean±SD)		P	95% CI
	Prototype	Conventional		
Fluoride levels	(8.86±1.29)	(1.46±0.44)	0.0001	6.73-8.06
	Prototype	Electric	P	95% CI
	8.43±2.11	7.95±2.87	0.853	(-2.10)-1.74

Table 4. The result of Independent T-Test Number of Streptococcus Mutans between groups

Variable	Group (Mean±SD)		P	95% CI
	Prototype	Conventional		
*Streptococcus mutans**	552.20±282.47	728.25±383.65	0.452	(-698.96)- 346.87
	Prototype	Electric	P	95% CI
*Streptococcus mutans**	768.20± 221.24	909.25± 506.67	0.589	(-730.29)- 448.19

*Calculation of Streptococcus mutans with 10^{-5} dilution and units of CFU/ml

Figure 2. *Streptococcus mutant* bacteria from timer-equipped electric toothbrush group (a); (b) non-timer electric toothbrush group; (b) conventional toothbrush group (c) microscopic images at 1000x magnification

DISCUSSION

The tooth brushing duration of subjects who used conventional toothbrushes in this study was less than 2 minutes. Similar results were also found in a study conducted in Jakarta, Indonesia in 2015 that the average tooth brushing duration was only 57.29 seconds.[3] This problem requires special attention since most people do not brush their teeth for at least 2 minutes. This current study also proved that using an electric toothbrush with a timer might optimize tooth brushing rather than using a conventional one to reduce plaque. It is in line with research conducted by Erbe et al. In 2018, there was a decrease in plaque by 6.2% in the group using conventional toothbrushes and 38.1% in the group using electric toothbrushes.

The vibrations in the filament or bristles of electric toothbrushes have been proven effective in removing bacterial adhesions on the mouth's surface. In addition, the hydrodynamic pressure in the bristles was able to remove plaque faster, down to the interproximal parts of the teeth.[13,14] Two main mechanisms are involved: the movement of the bristle filaments on an electric toothbrush, namely mechanical and sonic. Mechanical brushes use a motor that drives the bristles or brush heads; sonic brush uses high-frequency acoustic energy. Other studies have shown that both mechanisms were clinically effective in removing plaque.[15,16]

In this case, using the prototype toothbrush was assumed to show equal ability as an electric toothbrush in increasing the pH of saliva. Some factors contribute to the changes in salivary pH, namely salivary flow rate, oral microorganisms, and salivary buffer capacity. The faster the saliva flow is, the more alkaline the pH of the saliva will be, leading to higher salivary pH. Increasing the flow rate of saliva will also increase saliva production, enhancing the salivary secretion. One of the factors that can increase the salivary flow rate or the rate of saliva secretion is mechanical stimulation. In this case, mechanical stimulation was obtained from brushing teeth.[17] The mechanical stimulation is produced from the repeated brushing movements, stimulating higher salivary secretion and, at the same time, increasing the bicarbonate ion concentration. The higher the bicarbonate ion concentration is, the higher the pH will be. [18]

This study stated that people who brush their teeth using the electric toothbrush prototype equipped with a timer developed in this study had higher fluoride levels than those who used conventional toothbrushes. The average salivary fluoride level in the prototype group was 8.86 ppm, while the conventional group was only 1.46 ppm, indicating that the use of the prototype increased the salivary fluoride level. It is in line with research conducted in 2016 by JECreeth, who wanted to determine the effect of brushing duration and toothpaste quantity on enamel remineralization by grouping people into two groups: a group that performed tooth brushing within 45 seconds and 120 seconds or 2 minutes. The results showed significantly higher fluoride levels in those who brushed their teeth for 2 minutes than in 45 seconds. Higher fluoride depends on the duration of people brushing their teeth. The longer people brush their teeth, the longer their oral cavity will be exposed to fluoride from toothpaste, increasing the amount of fluoride in saliva than those exposed in a shorter time.[4] Meanwhile, the comparison of fluoride levels shows that salivary fluoride levels between groups of prototype toothbrushes and electric brushes showed no significant difference ($p > 0.05$). Therefore, tooth brushing using a 2-minute timer electric toothbrush prototype worked better as an electric toothbrush without a timer in increasing salivary fluoride levels. It is in contrast to previous research, which showed that the duration of tooth brushing did not make a difference in the amount of fluoride in saliva.[19]

The number of *Streptococcus mutants* bacteria was not significantly different between the group who brushed their teeth for 2 minutes using a timer electric prototype toothbrush and those who brushed theirs for less than 2 minutes using a non-timer electric toothbrush or conventional toothbrushes. This result supported those of other studies that compared bacteria to toothbrushes done by Haffajee et al., whose results showed equal effectiveness in reducing the number of bacteria in the group after 6 months of use.[20] Limitation of this study is the absence of baseline measurements to determine changes after brushing teeth using various types of toothbrushes and the limited number of samples in each group.

CONCLUSION

This study concluded that using an electric toothbrush with a 2-minute timer has better results in reducing plaque, increasing fluoride levels in saliva, and decreasing the number of *Streptococcus mutans.*

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