ESTIMATION OF URINARY FLUORIDE EXCRETION IN INFANTS AND TODDLERS AFTER BRUSHING WITH FLUORIDE TOOTHPASTE

Siriruk Nakornchai[1], Sivaporn Horsophonphong[1], Sirima Sanguansin[2] and Rudee Surarit[2]

[1]Department of Pediatric Dentistry, [2]Department of Oral Biology,
Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Abstract. Excessive ingestion of fluoride during tooth formation can cause dental fluorosis. In young children, fluoride toothpaste represents a major proportion of the total fluoride intake. A recommended method to detect daily fluoride intake is urinary fluoride excretion. The aim of this study was to determine excessive fluoride ingestion during tooth brushing by comparing the estimated urinary fluoride excretion values among infants and toddlers before and after brushing with fluoride toothpaste. Thirty-four healthy Thai children participated in this study. Following the European Academy of Paediatric Dentistry (EAPD) guidelines, the children studied belonged to two age ranges: less than 2-year-olds (n=20) and 2-2.5 year-olds (n=14). Their parents were trained in tooth brushing technique using horizontal scrub and wiping out bubbles while brushing twice a day. The concentrations and amounts of fluoride toothpaste used also followed EAPD guidelines: a pea size of 500 ppm fluoride toothpaste for children less than 2-year-olds, a pea size of 1,000 ppm fluoride toothpaste for 2-2.5 year-old children. For the first two days, their teeth were brushed without any toothpaste and urine samples were analyzed for baseline fluoride and creatinine ratios. Teeth were brushed with fluoride toothpaste for 6 days and morning spot urine samples were collected after brushing 3 days and every day for 3 days. The samples were analyzed for fluoride and creatinine concentrations. Fluoride/creatinine (F/Cr) ratio was used to calculate 24-hour urinary fluoride excretion from the standard creatinine value of 15 mg/kg bw/day. The urinary fluoride excretions before and after brushing were compared using paired sample t-test at the significance level of 0.05. For children less than 2-year-olds, the mean (SD) of estimated fluoride excretions before and after brushing were 0.11 (0.07) and 0.12 (0.08) mg, respectively. For 2-2.5 year-old children, the estimated fluoride excretions before and after brushing were 0.13 (0.06) and 0.14 (0.07) mg, respectively. A significant difference was found in less than 2-year-old children (p=0.04). However, none was found in 2-2.5 year-old children (p=0.20). In conclusion, children aged <2 years had a significant increase in estimated urinary fluoride excretion after brushing with a pea-sized amount of 500 ppm fluoride toothpaste, but children aged 2-2.5 years did not after brushing with a pea-sized amount of 1,000 ppm fluoride toothpaste.

Keywords: creatinine, fluoride, infant, toddler, toothpaste, urine

Correspondence: Dr Siriruk Nakornchai, Department of Pediatric Dentistry, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand.
Tel: +66 (0) 89 7723011; E-mail: siriruk.nak@mahidol.ac.th

INTRODUCTION

The effectiveness of fluoride has been well established in caries prevention (Petersen and Lennon, 2004). The most widely used method to deliver fluoride is toothpaste, which can reduce caries incidence approximately 25% (Clarkson *et al*, 1993). Caries preventive effects of fluoride toothpaste increase with higher fluoride concentrations (Walsh *et al*, 2010). However, excessive fluoride ingestion can cause dental fluorosis, which is a hypomineralization of enamel as a result of excess fluoride intake during the period of enamel formation (Ishii and Suckling, 1991). The use of fluoride toothpaste before age 2 years increases the odds for developing dental fluorosis 11 times (Osuji *et al*, 1988).

To maximize the caries preventive effects of fluoride toothpaste and prevent the occurrence of dental fluorosis, some recommendations have been made regarding the use of fluoride toothpaste in young children. The Dental Association of Thailand suggested a smear or slight layer of 1,000 ppm fluoride toothpaste be used to brush the teeth of Thai children aged <3 years (The Dental Association of Thailand, 2017). The American Academy of Pediatric Dentistry recommended using a smear layer of 1,000 ppm fluoride toothpaste for brushing the teeth of American children aged <2 years and a pea-size amount of 1,000 ppm fluoride for ages 2-5 years (American Academy of Pediatric Dentistry Liaison with Other Groups Committee, 2008-2009). The European Academy of Pediatric Dentistry (EAPD) recommended using a pea-size amount of 500 ppm fluoride toothpaste for brushing the teeth of European children aged <2 years and a pea-size amount of 1,000 ppm fluoride toothpaste for ages 2-6 years (European Academy of Pediatric Dentistry, 2009).

Fluoride toothpaste represents a major proportion of total fluoride intake among young children (Pessan *et al*, 2003). Urinary fluoride excretion is a non-invasive method recommended to estimate daily fluoride intake (Marthaler, 1999; Villa *et al*, 2000; Villa *et al*, 2010). Fluoride/creatinine (F/Cr) ratio obtained from a spot urine sample has been suggested as the method for estimating daily urinary fluoride excretion among infants and young children (Marthaler, 1999; Zohouri *et al*, 2006). Creatinine is a by-product of muscle metabolism that has low variation in 24-h urinary excretion (Narayanan and Appleton, 1980). Due to the fact that urinary fluoride concentration may vary throughout the day but urinary creatinine output is relatively constant. Therefore, creatinine output can be used to calculate 24-h urinary fluoride excretion by multiplying F/Cr ratios to creatinine reference value (Remer *et al*, 2002).

Among guidelines on toothpaste for young children, EAPD recommended higher amount of toothpaste and lower fluoride concentration compared to the other recommendations. Only a few studies have estimated urinary fluoride excretion among infants and toddlers (Ketley *et al*, 2002). The aim of this study was to determine excessive fluoride ingestion during tooth brushing by comparing the urinary fluoride excretion among infants and toddlers before and after brushing with fluoride toothpaste.

MATERIALS AND METHODS

The study was approved by the Committee on Human Rights Related to Human Experimentation, Mahidol University, Thailand (COA. No. MU-IRB

2012/076.2404) and written informed consent was obtained from the parents of all the study subjects.

Samples enrollment and data record

Thirty-four healthy Thai children, aged between 9 and 30 months, who were not taking any dietary fluoride supplements or allergic to fluoride, were enrolled in the study by convenience sampling. The age, weight, height, sex, and type of milk consumed were recorded for each study subject. The samples of their drinking water were obtained. The workflow of study method was described in Fig 1.

Instructions for parents or caregivers

During the eight day period of the study, the subjects avoided dental treatment involving fluoride therapy, refrained from eating seafood or marine fish and abstained from drinking fluoridated milk or taking fluoride tablets. Parents recorded their child's health status, food consumed and medications. The parents or caregivers brushed child teeth twice a day, in the morning and before bedtime using horizontal scrub brushing technique.

For the first 2 days, the child teeth were brushed without toothpaste. After that, the teeth were brushed with fluoride toothpaste for 6 days following the EAPD recommendation: a pea-sized amount of 500 ppm fluoride toothpaste [Kodomo®, Lion Corporation (Thailand)] for children less than 2 years old and a pea-sized amount of 1,000 ppm fluoride toothpaste (Macleans® Little Teeth; GlaxoSmithKline, Brentford, UK) for children 2 years old and above.

Urine sample collection

The first void of urine was collected in the morning of the first two days and after tooth brushing with fluoride toothpaste for 3 days and then every day for 3 days as illustrated in Fig1. For toilet-trained children, the urine samples were collected using a screw top plastic urine bottle. However, for a child wearing nappy, a

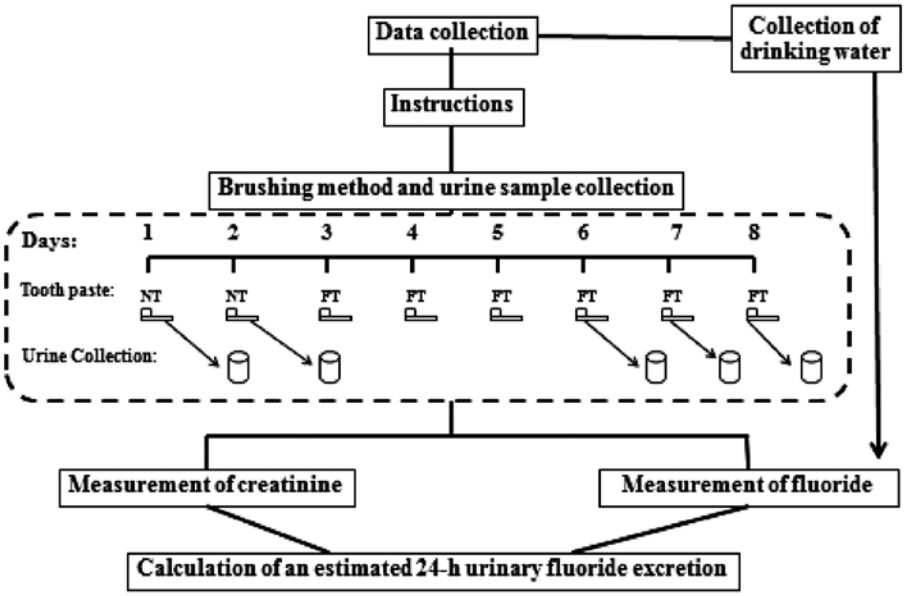

Fig 1-Workflow diagram of the study. NT, no toothpaste; FT, fluoride toothpaste.

pediatric urine collector (M-niles®, T. Cheaw Charn Plastic, Bangkok, Thailand) was used to collect the urine by attaching the urine bag to the child. Soon after urine collection, the urine samples were immediately stored in a freezer (- 20°C).

Measurement of fluoride and creatinine concentration

The concentration of fluoride in urine and drinking water were examined by a fluoride-specific electrode (model 9609 BN; Orion Research, Cambridge, MA) and fluoride-ISE meter (model EA 940, Orion Research, Cambridge, MA). The samples were mixed with TISAB III and placed under the electrode. Creatinine concentration was investigated using an automated analyzer: (Cobas 6000 analyzer series module c501; Roche, Manhein, Germany).

Calculation of an estimated 24-h urinary fluoride excretion

The estimated 24-h urinary fluoride excretion was calculated by multiplying F/Cr ratios (Q) to an estimated 24-h urinary creatinine excretion, which was calculated from a standard creatinine value (15 mg/kg bw/day) (Remer *et al*, 2002).

$$\text{Estimated 24-h urinary fluoride excretion (mg)} = \frac{\text{Q (mg/g)} \times \text{estimated 24-h urinary creatinine excretion (mg)}}{1{,}000}$$

Estimated 24-h urinary creatinine excretion (mg) = 15 (mg/kg) × body weight (kg), Q: Fluoride/creatinine ratio of spot urine sample (mg/g).

Statistical analysis

The estimated urinary fluoride excretion values before and after brushing with fluoride toothpaste were compared by paired sample *t*-test. A *p*-value<0.05 was considered statistically significant.

RESULTS

A total of 34 subjects (18 males) were included in the study; 20 aged <2 years, 14 aged 2-2.5 years. The mean age of subject was 21 (range: 9-29) months. The mean fluoride concentration in the examined drinking water was 0.13 (range: 0.02-0.42) mg/l (Table 1). The mean (SD) of estimated urinary fluoride excretion in 24 hours before and after brushing with fluoride toothpaste among subjects aged <2 years were 0.11 (0.07) mg and 0.12 (0.08) mg and subjects aged 2-2.5 years were 0.13 (0.06) mg and 0.14 (0.07) mg, respectively (Table 2).

Comparison of calculated urinary fluoride excretion before and after fluoride toothpaste brushing.

The calculated estimate of urinary fluoride excretion in 24 hours increased 9.1% after fluoride toothpaste brushing compared to baseline among subjects aged <2 years and increased 7.7% among subjects aged 2-2.5 years. We found a significant (p=0.04) increase in the calculated urinary fluoride excretion after fluoride toothpaste brushing compared to baseline among subjects aged <2 years but not among subjects aged 2-2.5 years (p=0.2).

DISCUSSION

The mean (SD) estimated 24-hour urinary fluoride excretion among all our study subjects before and after brushing with fluoride toothpaste were 0.11 (±0.06) and 0.13 (±0.08) mg, respectively, indicating low fluoride excretion. The fluoride concentration in the children's drinking water was in the range 0.02-0.42 mg/l with a mean fluoride concentration of 0.13 mg/l indicating low fluoride consumption from drinking water (Fawell *et al*, 2006). Dietary records of foods and drinks

Table1
Study subject characteristics and their drinking water fluoride concentrations.

Characteristics and drinking water fluoride concentrations	< 2 years, *n*=20 Mean (SD)	2-2.5 years, *n*=14 Mean (SD)	Total, *n*=34 Mean (SD)
Age in months	17 (4.7)	26.3 (1.6)	20.8 (5.9)
Body weight in kg	10.7 (1.4)	13.1 (2.4)	11.7 (2.2)
Height in cm	80.3 (4.8)	88.6 (5.6)	83.7 (6.6)
Fluoride concentration in drinking water in mg/l	0.13 (0.12)	0.13 (0.10)	0.13 (0.11)

Table 2
Measured and calculated laboratory results among study subjects.

Variables	Age groups				Total *n*=34	
	< 2 years *n*=20		2-2.5 years *n*=14			
	Before Mean (SD)	After Mean (SD)	Before Mean (SD)	After Mean (SD)	Before Mean (SD)	After Mean (SD)
F	0.28 (0.17)	0.29 (0.17)	0.4 (0.24)	0.37 (0.26)	0.33 (0.21)	0.32 (0.21)
Cr	0.58 (0.46)	0.53 (0.39)	0.58 (0.27)	0.56 (0.18)	0.58 (0.39)	0.54 (0.32)
Q	0.66 (0.44)	0.73 (0.52)	0.63 (0.26)	0.73 (0.35)	0.65 (0.37)	0.73 (0.45)
Est.ex.F	0.11 (0.07)	0.12 (0.08)	0.13 (0.06)	0.14 (0.07)	0.11 (0.06)	0.13 (0.08)

F, spot urinary fluoride concentration in mg/l; Cr, spot urinary creatinine concentration in g/l; Q, ratio of F/Cr in mg/g; Est.ex.F, calculated urinary fluoride excretion in 24 hours.

consumed during the study indicated no fluoride-rich products such as: marine fish, seafood, mineral water, fluoridated milk or tea.

During the study period, 7 study subjects took medications, which were roxithomycin, amoxicillin, paracetamol, actifed, and chlorpheniramine. None of these medications have an impact on urinary excretion of creatinine (Andreev *et al*, 1999).

After brushing with fluoride toothpaste, the mean fluoride/creatinine ratio among all study subjects was 0.73 mg F/g Cr and the mean estimated 24-hour urinary fluoride excretion was 0.13 mg F. Our results were lower than the mean fluoride/creatinine ratio of 1.49 mg F/g Cr and the mean estimated 24-hour urinary fluoride excretion of 0.33 mg F reported in a study of 12- to 36-month-old English children, living in an area with moderately fluoridated water (0.81 mg F/l) (Zohouri *et al*, 2006).

The estimated 24-hour urinary fluoride excretion found in our study is similar to that found in a study from Finland among children aged 2.3-3.6 years (0.16±0.08 mg

F) living in a low fluoride area (<0.15 mg F/l) (Ketley *et al*, 2004). Our study findings were lower than those reported from Europe among children aged 1.5-3.5 years (0.23±0.19 mg F) living in non-fluoridated areas (Ketley *et al*, 2004). Higher daily urinary fluoride excretion is usually reported among children living in areas with fluoridated water. For example, the mean 24-hour urinary fluoride excretion reported in a study from Chili among children aged 3-5 years (0.36±0.76 mg F) living in an area with fluoridated water (0.5-0.6 mg F/l) (Villa *et al*, 2000), was higher than that found in our study, as was that reported from Iran among children aged 4 years (0.34±0.1 mg F) whose water contained 0.33-0.39 mg F/l (Zohouri and Rugg-Gunn, 2000), from Ireland (0.37 $\pm$ 0.11 mg F) among children aged 3 years living in an area where the water contained 0.8-1.0 mg F/l (Ketley *et al*, 2004), from England (0.33 mg F) among children aged 12-36 months living in an area where the water contained 0.81 mg F/l (Zohouri *et al*, 2006) and from Germany (0.48 mg F) among children aged 3-6 years living in an area where the water was not fluoridated but the subjects were taking either fluoridated salt or fluoride tablet (Haftenberger *et al*, 2001).

In our study, to reduce toothpaste ingestion by study subjects we had the parents or caregivers used gauze or a cloth to wipe out the remaining toothpaste from the mouth after brushing, as recommended by the Dental Association of Thailand (2017). In spite of this, there was a significant increase in estimated 24-hour urinary fluoride excretion after brushing in subjects aged <2 years but not those aged 2-2.5 years. This suggests subjects aged <2 years swallowed the fluoride toothpaste and those aged 2-2.5 years did not. This result is similar to other reports suggesting ingestion of toothpaste decreases with increasing age of the study subject (Burt, 1992; Stookey, 1994). Our findings suggest using a pea-size amount of 1,000 ppm fluoride toothpaste for children aged ≥ 2 years is safe.

In this study, fluoride toothpaste was the only source of fluoride intake. However, other sources of fluoride may be considered for children at moderate to high risk for developing dental caries. Further studies are needed among subjects receiving fluoride supplementation such as fluoride tablets or fluoridated milk to determine their effect. Even more importantly, further studies are needed to determine these various levels of fluoride intake on dental caries formation and dental fluorosis.

In conclusion, children aged <2 years had a significant increase in estimated urinary fluoride excretion after brushing with a pea-sized amount of 500 ppm fluoride toothpaste, but children aged ≥2 years did not after brushing with a pea-sized amount of 1,000 ppm fluoride toothpaste. From the results of our study, it is recommended that the amount of fluoride toothpaste for children aged <2 years should be decreased.

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