Community Dental Health (2023) **40,** 92–96
Received 25 October 2022; Accepted 4 January 2023

doi:10.1922/CDH_00243Thilakarathne05

Toothbrushing practices as risk factors for dental fluorosis in an area with varying fluoride levels in drinking water

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Background: Despite contributing to a reduction in dental caries, improper use of fluoridated toothpaste could add to the burden of dental fluorosis in children. ***Aim***: To assess the association between tooth-brushing practices such as the type and amount of toothpaste used, frequency of tooth brushing, parental assistance in tooth brushing, timing of tooth brushing and dental fluorosis in school children in Kurunegala district, an endemic area for dental fluorosis in Sri Lanka. ***Methods***: For this case-control study, a sex-matched sample of 15-year-old school children attending government schools in Kurunegala district and who were lifetime residents of the district was selected. Dental fluorosis was measured using the Thylstrup and Ferjeskov (TF) Index. Those children with a TF>1 were considered as cases and those with a TF score of 0 or 1 served as controls. An interview of parents/caregivers of the participants was used to assess risk factors for dental fluorosis. The fluoride concentration in drinking water was measured using spectrophotometry. Data analysis used chi-square tests and conditional logistic regression. ***Results***: Tooth brushing ≥ twice/day, brushing after breakfast and parent/care giver brushing the child's teeth reduced the likelihood of developing fluorosis. ***Conclusion***: Use of fluoridated toothpaste adhering to the recommended guidelines could prevent dental fluorosis in children in this endemic area.

Keywords*: Risk factors, dental fluorosis, tooth brushing, water fluoride*

Introduction

Dental fluorosis is a public health problem in areas with high levels of fluoride in the drinking water (World Health Organization, 2019). Dental fluorosis may present as white patches, brown discolorations or pitting of the tooth surface (Thylstrup and Fejerskov, 1978). The pathophysiology is characterized as an enamel defect that results from excessive intake of fluoride during the developmental stages of teeth. Excessive fluoride intake can affect the transition and maturation stages of tooth formation, giving rise to a hypo-mineralized enamel. The window of susceptibility for dental fluorosis is between birth and eight years of age (Aoba and Fejerskov, 2002).

Dental fluorosis is endemic to certain areas of the dry zone of Sri Lanka. High temperatures in these areas cause extensive evaporation which tend to concentrate substances such as fluoride in water (Chandrajith *et al*., 2012). In Udawalawe, Sri Lanka consuming water with a fluoride level of 1 mg or higher and consumption of tea before the age of seven are associated with the presence of diffused opacities due to dental fluorosis (Van Der Hoek *et al*., 2003).

The main risk factors for dental fluorosis are consumption of water containing high levels of fluoride and infant formula, use of fluoride supplements and fluoridated toothpastes (Mascarenhas, 2000). In addition professionally applied topical fluorides, including gels, varnishes, and mouth rinses (Wong *et al.*, 2010), high altitude, (Akosu *et al*., 2009) and factors such as age, sex, nutritional status, place of residence, educational level of parents, and sibling status are risk factors (Zhou *et al.*, 2018). Recent evidence also suggests a genetic predilection (Tremillo-Maldonado *et al.*, 2020).

Use of fluoridated toothpaste has contributed to the decline in dental caries in recent times. However, according to some studies it has led to an increase in the burden of dental fluorosis in children (Mascarenhas, 1995). Lack of parental supervision during toothbrushing, which can lead to inadvertent ingestion of fluoridated toothpaste in children who are younger than 8 years, could cause dental fluorosis (Wright *et al.,* 2014). In addition, the type and amount of toothpaste used, frequency of toothbrushing, timing and duration of toothbrushing, method of toothbrushing and whether toothpaste is spat out or swallowed are also factors contributing to dental fluorosis in children (Do and Spencer, 2007). Celeste and Luz (2016) have shown that the amount of toothpaste in the brush, eating toothpaste and using an adult toothbrush to be responsible singly or in combination for dental fluorosis.

Therefore, the aim of this study was to determine the association between toothbrushing practices such as type and amount of toothpaste used, frequency and timing of tooth brushing at age 5 and dental fluorosis in an endemic area for dental fluorosis in Sri Lanka.

Methods

Cases and controls for this sex-matched study were selected from a survey to determine the prevalence of dental fluorosis among 15-year-old school children attending government

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schools in Kurunegala district, an area with high levels of fluoride in ground water in the North-Western province of Sri Lanka (Thilakarathne and Ekanayake, 2022). The sample of that study was 989 lifetime residents of the area. The Thylstrup and Fejerskov Index (TF Index, Thylstrup and Fejerskov, 1978) was used to record dental fluorosis. The TF index assesses the fluorosis status objectively on an ordinal scale from 0 to 9, does not have an ambiguous category such as 'questionable' and has good validity (Mabelya *et al.*, 1994). A participant with a TF>1 was considered as a case while those with scores of 0 or 1 were considered as controls. This cut-off was selected as TF scores of 1 represent very mild dental fluorosis.

The survey used two- stage cluster sampling among Grade 10 students. Students with learning difficulties, wearing fixed orthodontic appliances and who were absent on the day of the examination were excluded. Although the initial plan was to exclude students who had taken treatments for dental fluorosis, no students had received such treatments.

The Ethics Review Committee, Faculty of Medicine, University of Colombo (Ref no EC-17-016) granted ethical clearance to conduct the survey. Permission was obtained from the Provincial Director of Education of the North-Western Province, the relevant zonal directors of education, and principals of selected schools. Written assent was obtained from the participants and written consent was obtained from the parents of each participant. A detailed description of the survey method is given elsewhere (Thilakarathne and Ekanayake, 2022).

Cases and controls were considered exposed if the fluoride levels in their drinking water source were > 0.7 mg/l. (U.S. Public Health Service, 2015). The formula for individually matched case-control studies with 1:1 matching was used to calculate the number of participants required (Schlesselman, 1982). A pilot study estimated the proportion of exposed controls and cases in the target population which was 0.08 (2/25) and 0.2 (5/25) respectively. Considering these rates in cases and controls and to detect an Odds Ratio (OR) for a risk factor of 4 (calculated from the pilot study) with a power of 0.9, at α=0.05, it was necessary to recruit a minimum of 101 cases and 101 controls.

The first author requested the school principal of each school inform parents/caregivers to accompany the child to school on the day of data collection. Only 481 did so, all of whom were invited for the interviews. Study participants were selected from the children of these 481 parent/caregivers.

The first author examined participants in the schools' Home Science or Science laboratories under natural light with participants seated on a mobile dental chair. The examiner had been calibrated against a specialist in Restorative Dentistry to record the TF index. Inter-rater reliability when assessed using Kappa statistic was 0.71. During data collection one student from each school was re-examined, yielding a Kappa statistic for intra-rater reliability of 0.91.

After the oral examination the parents/caregivers were interviewed. The interview schedule was developed in Sinhala and validated to assess historic risk factors for dental fluorosis. The face and content validity of the schedule were assessed based on expert opinion and it was pretested with 5 mothers of 15- year students from a school not included in this study.

The interviewer was masked to the fluorosis status of the students during the interviews. The students were sent to their classes beforehand so that their presence would not influence data collection. Nor did the assistant or interviewer have access to the clinical data during the interviews.

The interviews enquired about the following tooth-brushing practices at age 5: trade name of the toothpaste used (later categorized as fluoridated or non-fluoridated), type of toothbrush (adult or child), amount of toothpaste taken onto the toothbrush (> half or ≤ half), frequency of daily toothbrushing (≤once or ≥ twice), whether the parent/caregiver brushed the child's teeth, whether the child spat out or swallowed the toothpaste and time of tooth brushing (before or after breakfast).

Parents' level of education and paternal occupation (based on the Sri Lanka standard classification, Department of Census and Statistics, 2008) were recorded. The occupation status was later categorized into high, middle, and low: high included professionals, legislatures, technicians and associated professions, middle included clerks, service workers, shop owners/keepers and those in the armed forces, low included skilled agricultural and fisheries workers, craft and related workers, plant and machine operators and elementary occupations.

Based on the TF scores of the 481 participants whose parents/caregivers were interviewed, it was possible to select 168 cases with TF>1 and 170 sex-matched controls with TF = 0 or 1. The controls were selected from the same schools as the cases. When it was not possible to select an sufficient cases to controls from one school, the nearest equivalent type of school was considered to recruit sex-matched participants. Although the power calculation required 110 cases and controls, it was possible to identify 168 and 170 respectively, data from all of whom were used to enhance power.

Students were asked to bring a sample from their household drinking water source on the day of data collection. The fluoride content of the samples was assessed using spectrophotometry at the National Water Supply and Drainage Board Regional Laboratory, Kurunegala.

Data were analysed using STATA (version 17) software (Stata Corp, Texas, USA). Bivariate associations between risk factors and dental fluorosis were assessed using chi-square tests. As cases and controls were matched for sex, conditional logistic regression analysis was used to identify risk factors for dental fluorosis. Those factors significant at $p < 0.05$ in bivariate analysis were included in the regression model. As there could be an interaction between the type of toothpaste and frequency of toothbrushing, a stratified analysis was conducted to determine whether it was appropriate to add this interaction to the model. However, too few children brushed their teeth once a day or less to lend sufficient power to this analysis and interactions could not be considered further.

Results

The severity of dental fluorosis among participants varied from TF 0 - 6 and drinking water fluoride levels varied from 0 to 1.6 mg/l. TF scores of 0 and 1 were found in 86% and 14% of control group participants respectively. TF scores of 2, 3, 4, 5 and 6 were found in 53%, 27%, 15%, 4% and 1% of the cases respectively.

Table 1 compares demographic factors among those with and without fluorosis. More mothers of students without fluorosis had more than 10 years of education than the mothers of children with fluorosis.

Table 1. Demographic factors among 5-year-olds with and without fluorosis.

	Controls %	*Cases* %	*p (Chi sq.)*
Mother's education	(n=169)	(n=167)	
≤ 5 years	4.73	8.98	0.038
6-10 years	62.72	69.46	
> 10 years	32.54	21.55	
Father's education	(n=163)	(n=161)	
≤ 5 years	5.52	8.69	0.053
6-10 years	65.64	73.29	
>10 years	28.83	18.01	
Father's occupation	(n=148)	(n=150)	
High	11.48	7.33	0.548
Middle	42.56	39.33	
Low	45.94	53.33	

Sources of fluoride at age 5 are compared across groups in Table 2. Counter-intuitively, participants with fluorosis were less likely to have used fluoridated toothpaste and less likely to brush their teeth ≥ twice/day than those without. They were also more likely to brush their teeth before breakfast, more likely to have drinking water with > 0.5 mg/l fluoride and were less likely to have had their teeth brushed by their parents/caregivers at the age of five.

In the adjusted conditional logistic regression model, water fluoride level remained a risk factor for dental fluorosis with odds of having dental fluorosis three times higher when the fluoride level in drinking water was >0.5 mg/l. The type of toothpaste was not a risk factor, but children who brushed ≥ twice/day had lower odds of having dental fluorosis than those who brushed ≤once /day. Dental fluorosis was also more common among children who brushed their own teeth and children who brushed before breakfast.

Table 2. Sources of fluoride among 5-year-olds with and without fluorosis.

	Controls %	*Cases* %	*p (Chi sq.)*
Type of toothpaste used	(n=169)	(n=166)	
Non fluoridated/both types	8.88	18.07	0.014
Fluoridated	91.12	81.93	
Frequency of toothbrushing/ day	(n=170)	(n=167)	
≤Once	7.65	17.37	0.007
≥Twice	92.35	82.63	
Amount of toothpaste used on brush	(n=169)	(n=166)	
> half	8.28	10.85	0.426
≤ half	91.72	89.15	
Toothbrushing carried out by	(n=170)	(n=167)	
Parent	58.24	41.32	0.002
Child	41.76	58.68	
Swallowed toothpaste when brushing	(n=168)	(n=162)	
Never	76.19	80.86	0.302
Sometimes	23.81	19.14	
Swallowed or spat out after brushing	(n=169)	(n=165)	
Swallowed	11.83	9.10	0.413
Spat out	88.17	90.90	
Time of brushing	(n=170)	(n=165)	
Before breakfast	76.47	87.27	0.01
After breakfast	23.53	12.73	
Type of toothbrush used	(n=169)	(n=167)	
Children's	95.27	97.01	0.408
Adult's	4.73	2.99	
Consumption of powdered milk	(n=169)	(n=168)	
Consumed before age 5	33.53	27.38	0.206
Consumed after age 5	65.88	72.62	
Consumption of cow's milk	(n=169)	(n=168)	
Consumed before age 5	2.35	4.17	0.353
Consumed after age 5	97.06	95.83	
Fluoride levels in drinking water/ mg/l	(n=169)	(n=167)	
≤ 0.50	84.71	66.67	<0.01
> 0.50	14.71	32.74	

Discussion

The counter-intuitive finding that 15 year-olds who brushed their teeth more frequently were less likely to have dental fluorosis might be explained by post eruptive changes that can exaggerate or reduce the signs of dental fluorosis, with tooth brushing wearing off fluorotic porous surfaces (Do *et al*., 2016). Cohort studies have shown that very mild to moderate dental fluorosis diminish with time (Do *et al*., 2016; Curtis *et al*., 2020). As most participants of this study had mild to moderate dental fluorosis, it is possible that these opacities may have worn off when the tooth brushing frequency was high.

The higher frequency of dental fluorosis among children who brushed their own teeth is likely due to the supporting role played by parents in toothbrushing (Saied-Moallemi *et al.*, 2008). Parents who brushed their children's teeth may have instructed them not to swallow toothpaste but to spit it out. do Nascimento et al. (2013) also reported that the risk of dental fluorosis increased when the child brushed his/her teeth compared to a guardian doing it.

Use of fluoridated toothpaste, amount of toothpaste used and swallowing of toothpaste were not associated with dental fluorosis. Similar findings have been reported in Malaysian 9- and 12-year-olds living in areas with

different levels of fluoride in drinking water (Mohd Nor *et al*., 2021). In contrast, using 1000 mg/l fluoride toothpaste compared with 400- to 550- mg/l fluoride toothpaste and eating/licking toothpaste were associated with dental fluorosis in Australian children living in areas with community water fluoridation (Do and Spencer, 2007). A study conducted in Brazil among 12-year-old school children in a community with water fluoridation found that the amount of toothpaste on the brush, eating toothpaste and using an adult toothbrush to be significantly associated with dental fluorosis. But the authors had failed to control for the effect of fluoride in drinking water in regression analysis (Celeste and Luz, 2016).

Time of tooth brushing was also associated with dental fluorosis, with those brushing after breakfast less likely to have the condition. A cross-over double blind study has shown that brushing after a meal reduces the risk of dental fluorosis which could be due to lower gastro-intestinal absorption of fluoride (Cury *et al*., 2005).

Table 3. Conditional logistic regression for risk factors for dental fluorosis.

Independent variables	*Odds ratio*	*95% CI*
Mother's education		
≤ 5 years	1	
6-10 years	0.42	0.13, 1.37
> 10 years	0.26	0.07, 0.91
Fluoride level in drinking water (mg/l)		
≤ 0.50	1	
> 0.50	2.91	1.46, 5.79
Type of toothpaste used		
Non fluoridated/both	1	
Fluoridated only	0.53	0.23, 1.22
Frequency of toothbrushing/day		
≤Once	1	
≥Twice	0.32	0.13, 0.80
Toothbrushing was carried out by		
Parent	1	
Child	2.41	1.39, 4.2
Time of brushing		
Before breakfast	1	
After breakfast	0.401	0.29, 0.81
Pseudo $R^2 = 0.217$		

Like all research there are limitations to this study. Recall bias is a weakness in case-control studies. Although 5 years; a milestone in a child's life (formal schooling commences at that age in Sri Lanka) was considered to minimize recall bias, there could be errors when recalling past events, particularly when there are several children in the family. Also, social desirability bias may have influenced the tooth brushing frequency and parental assistance data. As these behaviours are known to be socially acceptable, there may have been a tendency for over-reporting. However, recall and social desirability biases would affect both cases and controls. Evidence indicates that fluoride levels in drinking water do not change over time (Wickramarathna *et al*., 2017). Therefore, it was assumed that the current fluoride levels would correspond to those in the same source a decade ago (when the participant was 5 years old). This may be a limitation of the study.

In conclusion, tooth brushing twice daily or more may reduce the visibility of fluorotic porous surfaces. Tooth brushing after breakfast and parental brushing were also associated with less likelihood of dental fluorosis. The use of fluoridated toothpaste was not associated with the development of dental fluorosis in this endemic area for dental fluorosis. Therefore, use of fluoride toothpaste under parental supervision could be recommended for young children living in endemic areas for dental fluorosis in the North-Western province of Sri Lanka.

Acknowledgements

The Research Grant (RG/2016/84/D) received from University of Peradeniya; Sri Lanka supported this study.

Conflict of interest

Authors declare no conflict of interest.

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