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Urinary and Water Fluoride Concentrations in Dental Fluorosis Patients in Thailand

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ABSTRACT

Background and Objective: Excessive fluoride intake during tooth development may result in dental fluorosis which is one of the most common signs of fluoride exposure in humans. Water fluoride concentration is a common indicator for fluoride measurement in a national survey. Currently, there is no updated studies on urinary and water fluoride concentrations in dental fluorosis patients in Thailand. This study aimed to assess severity of dental fluorosis, fluoride content in urine and drinking-water of schoolchildren in fluoride endemic areas.

Methods: Schoolchildren in fluoride endemic areas which located in Lamphun and Ratchaburi provinces, Thailand were screen for fluorosis severity (n=50). The 24-hours urine and drinking-water were collected. The fluoride concentrations in urine and drinking-water were evaluated by ion-selective fluoride electrode.

Results: Among 50 schoolchildren aged between 6-16 years; 30 were non-dental fluorosis and 20 were moderate to severe (M-S) dental fluorosis. Urine fluoride concentrations in M-S group were significantly higher than those in non-fluorosis group while water fluoride concentrations in M-S group were not significantly different from non-fluorosis group. A positive correlation (r=0.49) between urine and water fluoride concentrations (p<0.001) was observed. The living duration of the parents in fluoride endemic areas was significantly correlated with urinary fluoride concentration of children.

Conclusion: The study shows that the children who have moderate-severe fluorosis have increased urinary fluoride concentration. Apart from drinking-water, other sources such as foods and dental products can contribute to urinary fluoride concentration and development of dental fluorosis.

Keywords: Dental fluorosis, Fluoride endemic areas, Urinary fluoride excretion, Water fluoride concentration

Introduction

Fluoride is found in water, food and especially dental products. It is considered as a double-edged sword. Fluoride provides a protection against dental caries when consuming in adequate quantity, but chronic and excessive fluoride intake can cause dental and skeletal fluorosis.[1]

Dental fluorosis is a developmental disturbance of dental enamel, caused by chronic and excessive ingestion of fluoride during tooth development.[2] In Thailand, the national standard of fluoride concentration in drinking water is ≤ 0.7 mg/liter and not exceed 1.0 mg/liter during high water consumption in summer. Dental fluorosis is endemic among certain areas depending on geological background such as the northern and western parts of Thailand. A high incidence of dental and skeletal fluorosis has been reported in Chiangmai, Lamphun and Phayao provinces due to high fluoride concentration in natural water sources.[3] It has been estimated that fluoride concentration in underground water at endemic area can reach 10 mg/L(ppm).[4]

The common source of fluoride exposure is the drinking/cooking water derived from underground water. Fluoride in water cannot be removed by boiling or distillation. Reverse osmosis is the successful defluoridation process in community scale, but it comes with high investment and maintenance cost.[5] After ingestion within minutes, fluoride is rapidly absorbed from the stomach to plasma. The peak plasma fluoride level usually occurs within the next 30-60 minutes, and then declines due to bone uptake and urinary excretion. Urinary fluoride is a widely accepted biomarker of current fluoride exposure and has frequently been used as an indicator of fluoride exposure from drinking-water recommended by WHO.[6]

Dean's fluorosis index (DFI) is often used by the World Health Organization and remains as the standard gold index in public health armamentarium. It consists of 6 categories based on the second most severely affected clinical appearance. The normal teeth are given a score of 0. The score gradually increases by the severity to score of 4 which stands for the most severe type of fluorosis.[7]

This aims of this study were to assess severity of dental fluorosis, and fluoride content in urine and drinking-water in schoolchildren in fluoride endemic areas of Thailand.

Materials and Methods

The protocol for this study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (Study Code HREC-DCU 2021-061, approved on 1 October 2021)

Sample Size Calculation

The sample size was calculated by using the n4Studies program version 1.4.1 for 80% power and 95% confidence interval level according to the previous study.[8] The final estimated total sample size was 50.

Screening and Selection of Subjects

Participant screenings were held in fluoride endemic area of Thailand located in Lamphun and Ratchaburi provinces according to their high prevalence of dental fluorosis and low population mobility.

The inclusion criteria of case group were Thai population with moderate to severe dental fluorosis (Dean fluorosis score 3-4) and those of the control group were Thai population without dental fluorosis (Dean fluorosis score 0). DFI and diagnostic criteria are shown in Table 1. A consensus score for DFI was given for each subject by four examiners (P.G., T.P., P.A and a pediatric dentist). The final score for DFI was recorded directly by the interface excel file.

All subject recruited must not have the following conditions: clinical dental caries, systemic condition, history of antibiotic, antifungal, antiviral or steroid intake or use of mouthwash in 3 months prior to sample, presence of periodontitis and mucosal lesions, smoking, drinking alcohol, and using narcotic drugs.

Informed consents were obtained from parents and/or child participants (ages 6 to 16 years) included in this study.

History Taking and Questionnaire

The questionnaire including information of age, gender, grade, period of staying in the area and medical history of the participants and their parents were collected.

Sample Collection and Fluoride Measurement

All subjects were asked to collect their 24-hr urine in 2.4 L container and the most consumption drinking-water in household in 60 ml. bottle. The samples were transported to the laboratory at Intercountry Centre for Oral Health, Chiangmai for fluoride measurement. The amount of fluoride was evaluated by TISAB III, containing CDTA (cyclohexylenedinitrilotetraacetate), sodium hydroxide, sodium chloride and acetic acid (ethanoic acid) dissolved in deionized water. Fluoride electrode (4-star benchtop, Orion, USA) was used as the measuring equipment.

Statistical Analysis

Statistical analysis was performed using SPSS software version 22 (SPSS Inc. Chicago, IL, USA). Demographic and clinical conditions were reported with descriptive statistics. The normal distribution for each variable was determined by the Kolmogorov-Smirnov test and follow by nonparametric Mann-Whitney U test. Statistical significance level of less than 0.05 was considered significant.

Results

Seventy-one subjects from 3 schools in endemic area were approached to participated in this study. Twenty-one subjects did not provide consent to participate or fail to collect samples. Fifty subjects were included. Oral examination and sample collection took place between December 2021 to January 2022.

Subjects were male 44% and female 56%, mean age 11.26 ± 2.76 years. The children were divided into 2 groups; control group (n=30) who were non-dental fluorosis (Dean fluorosis score 0); male 46.7% and female 53.3% and case group (n=20) who were moderate to severe dental fluorosis (Dean fluorosis score 3-4); male 40% and female 60%.

Mean fluoride value in total water samples were measured to be 1.52 (range from 0.10 to 16 ppm) and mean fluoride value in total urine sample of children were found to be 3.20 (range from 0.10 to

Table 1. Criteria for Dean's fluorosis index for dental fluorosis.

Classification	Score	Criteria
Normal	0	The enamel represents the usual translucent semivitriform (glass-like) type of structure. The surface is smooth, glossy, and usually of a pale, creamy white color.
Questionable	0.5	The enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flakes to occasional white spots. This classification is used in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and classification of "normal" not justified.
Very Mild	1	Small, opaque, paper white areas scattered irregularly over the tooth, but not involving as much as approximately 25 percent of the tooth surface. Frequently included in this classification are teeth showing no more than about 1-2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
Mild	2	The white opaque areas in the enamel of the teeth are more extensive, but do not involve as much as 50 percent of the tooth.
Moderate	3	All enamel surfaces of the teeth are affected, and surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature.
Severe	4	All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread, and teeth often present a corroded-like appearance.

11.40 ppm). In control group, mean fluoride value of urine sample was 2.43 ppm (range from 0.10 to 6.78 ppm) and mean fluoride value of water sample was 1.40 ppm (range from 0.10 to 16 ppm) while in case group, mean fluoride value of urine sample was 4.35 (range from 0.70 to 11.40) and was found to be 1.71 (range from 0.10 to 7.50 ppm) in water sample. There was not significantly different between case and control group in parent's duration of living characteristic (*p*=0.13). The result showed that in case group, the parent of participant also experienced tooth discoloration (50%) and others (50%) had no report of any fluoride related diseases while in control group none of those were experienced tooth discoloration but 20% experienced joint problem and other 80% had no report of any fluoride related diseases. General characteristics of the study population are shown in Table 2.

Analyzing the urinary and water fluoride concentrations between M-S group and non-fluorosis group showed that urine fluoride concentrations in fluorosis group were significantly higher than those in non-fluorosis group ($p<0.05$) while the water fluoride concentrations in fluorosis group was not significantly different from non-fluorosis group ($p=0.30$) (Fig. 1).

A significantly positive correlation was observed between urinary fluoride and water fluoride ($r=0.49$, $p<0.05$) as shown in Fig. 2.

In relation to the presence of fluoride in urine and water samples of children and parent's duration of living in endemic area,

Table 2. General characteristics of the study population.

Characteristics	Total (N=50)	Control (N=30)	Case (N=20)	*p*-value
Age (years)				0.005
○ Mean ± SD	11.26 ± 2.76	12.13 ± 2.80	9.95 ± 2.00	
○ Median	11	12.50	11	
○ Range	6 to 16	6-16	6-12	
Sex				0.645
○ Male	22 (44%)	14 (46.7%)	8 (40%)	
○ Female	28 (56%)	16 (53.3%)	12 (60%)	
Dean Index				
○ Score 0	34 (60%)	30 (100%)	0	
○ Score 3	8 (16%)	0	8 (40%)	
○ Score 4	12 (24%)	0	12 (60%)	
Urine Fluoride (ppm)				0.005
○ Mean ± SD	3.20 ± 2.52	2.43 ±1.99	4.35 ± 2.83	
○ Median	2.78	1.81	3.41	
○ Minimum	<0.10	<0.10	0.70	
○ Maximum	11.40	6.78	11.40	
Water Fluoride (ppm)				0.30
○ Mean ± SD	1.52 ± 2.97	1.40 ±3.26	1.71 ± 2.54	
○ Median	0.20	0.20	0.22	
○ Minimum	<0.10	<0.10	<0.10	
○ Maximum	16.00	16.00	7.50	
Location				0.05
○ Lamphun	26 (52%)	19 (63.3%)	7 (35%)	
○ Ratchaburi	24 (48%)	11 (36.7%)	13 (65%)	
Parent's Duration of Living				0.13
○ 1-5 years	0	0	0	
○ 5-10 years	24 (48%)	18 (60%)	6 (30%)	
○ 10-20 years	4 (8%)	2 (6.7%)	2 (10%)	
○ More than 20 years	22 (44%)	10 (33.3%)	12 (60%)	
Parent History				0.004
○ None	34 (68%)	24 (80%)	10 (50%)	
○ Bone Fracture	1 (1.4%)	0	0	
○ Joint Pain	6 (12%)	6 (20%)	0	
○ Tooth Discolor	10 (20%)	0	10 (50%)	

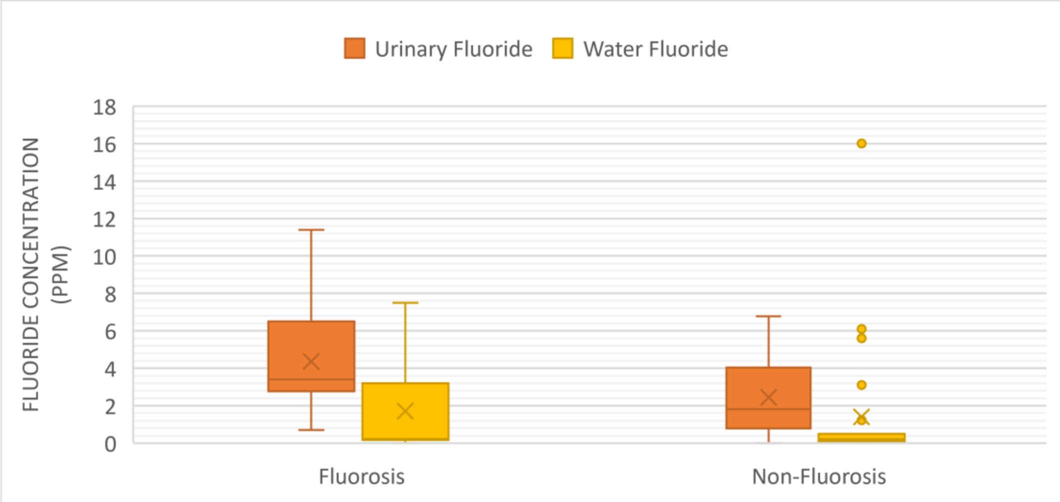

Figure 1. The fluoride concentration (ppm) of urine and water samples between dental fluorosis group and non-dental fluorosis group.

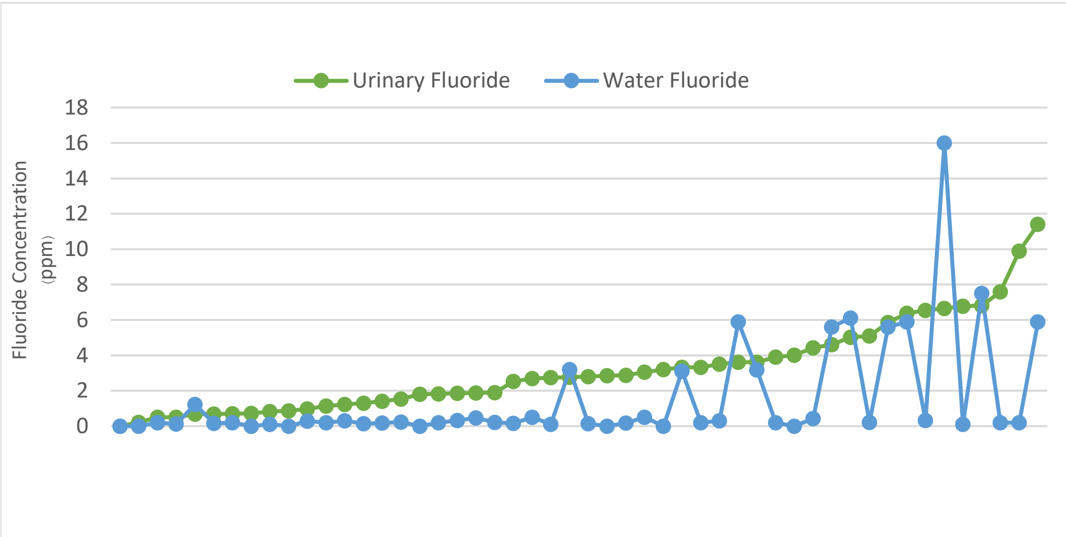

Figure 2. The association between fluoride concentration in water sample and the urinary fluoride concentration for each participant, the data is ordered according to urinary concentration.

Table 3 and Fig. 3 show the mean fluoride urinary value of 2.39 ±1.91 ppm in group 5-10 years of parents' living duration in endemic area (n=24), 3.63 ± 2.3 ppm in group 10-20 years of living duration (n=4), and 4.01 ± 2.93 ppm in group more than 20 years of living duration (n=22). A significant positive moderate strength correlation (r=0.23, $p<0.05$) between the parent's duration of living and urine fluoride concentration was observed while water fluoride concentration did not show a significant correlation with parent's duration of living (r=0.03, $p=0.79$).

Discussion

The maximum concentration of fluoride in natural water at 1.5 ppm is set as the guideline value to prevent the incidence of dental fluorosis according to "Guidelines for Drinking-Water Quality" by WHO 2006, while the recommendation for artificial fluoridation of water supply is at 0.5-1.0 ppm.[9]

Drinking water consumption depends on the atmospheric temperature. This study was carried out in hot climates region where the temperature ranged 38-40 degree Celsius. The optimal concentration of fluoride in drinking water for region with hot climates should be 0.7-1.0 ppm recommended by World Health Organization.[10] In Thailand, the national standard of fluoride concentration in drinking water was limited at 0.7 ppm. which laid down by the Bureau of Dental Health.[11] In our study, we found that the mean values of water fluoride in the endemic areas (1.52 ± 2.97 ppm) exceeded the national concentration and had a wide range of fluoride values from <0.1 to 16 ppm.

The fluoride concentrations in biomaterials such as urine and blood have been proposed as the most reliable indicators of exposure to fluoride.[12] The acceptable concentration of urine fluoride is 1 ppm.[13] In our study, the fluoride concentration in urine samples from the endemic areas was higher than the accepted level. The mean urinary fluoride was at 3.20 (range from 0.1 to 11.40 ppm). Other studies of urinary fluoride in other endemic areas showed 0.17-47.50, 0.15-1.99, 0.9-3.25, 1.07-4.0 and 0.05-2.8 ppm in Youssoufia city of Morocco,[14] Barcelona,[15] Gurgaon,[16] Nellore[13] and Jhajjar,[17] respectively.

Table 3. The relationship between parent's duration of living in fluoride endemic areas and fluoride concentrations in urine and water samples of children.

Parent's Duration of Living	N	Urine Fluoride (ppm)	Water Fluoride (ppm)
Less than 5 years	0		
○ Mean ± SD		N/A	N/A
○ Range		N/A	N/A
5-10 years	24		
○ Mean ± SD		2.39 ±1.91	1.67 ± 3.63
○ Range		<0.1 – 6.81	< 0.1 – 16.0
10-20 years	4		
○ Mean ± SD		3.63 ± 2.3	1.50±2.73
○ Range		0.7 - 5.86	<0.1 - 5.6
More than 20 years	22		
○ Mean ± SD		4.01 ± 2.93	1.36 ± 2.24
○ Range		0.68 – 11.40	<0.1 – 5.9

Epidemiological studies have showed that people who live in fluoride endemic or artificial-fluoridated area are more susceptible to dental fluorosis than those living in non-endemic/non-fluoridated area. However, many studies showed that susceptibility and severity of fluorosis is influenced by many factors, including total fluoride intake, type of fluoride intake (i.e., ingested vs. inhaled), renal function, rate of bone metabolism, metabolic activity, high altitude of residence associated with altered acid-base balance in body, nutritional status, composition of diet, and genetics.[18] These factors may contribute to the incidence and severity of dental fluorosis in individuals although they have been living in the same fluoride endemic area.

The effects of fluoride are cumulative and depend mostly, but not entirely, on the amount and duration of exposure.[19] This study supported the recent evidence that higher urinary fluoride was found in M-S dental fluorosis patients and can be useful for determining the current availability of fluoride.[20] Nevertheless, the recent biomarkers of fluoride such as hairs and nails should be determined in further study.

Our study shows a positive correlation between urinary fluoride and water fluoride concentrations although it is not linear which could be due to other sources of fluoride exposure. Apart from drinking-water which is the main source of fluoride exposure,[21] there are other fluoride sources including foods/vegetables planted in endemic areas, brick tea consumption, cooking methods and dental products.

Previous studies reported that fluoride can pass through the placenta.[22] After removal of fluoride from community waters, the half-life of fluoride loss in adults is 120 weeks and in children is 70 weeks proximately.[23] Here, we observed that the duration that the

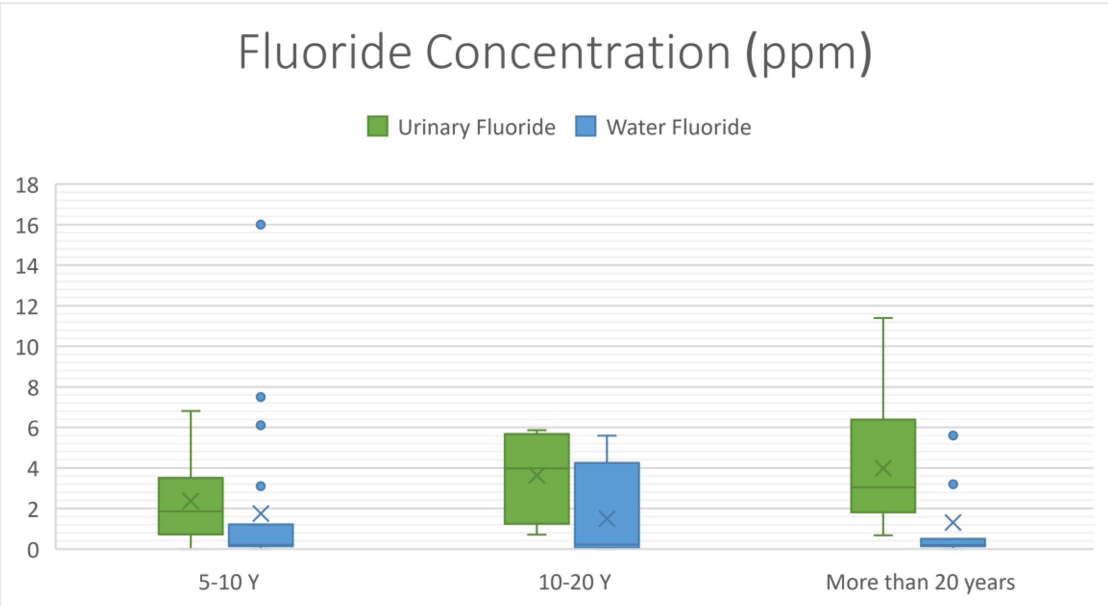

Figure 3. The fluoride concentration (ppm) in urine and water samples of children according to the durations of parents' living in fluoride endemic areas.

parents were living in endemic area was associated with the increase in children' urinary fluoride concentrations, suggesting a cumulative fluoride deposition in these children. Future studies recruiting the children who migrate from the endemic areas would clarify the above correlation.

Conclusion

This study reports the exceeding standard value of mean urinary and water fluoride concentrations in both fluorosis and non-fluorosis group in the endemic area reflecting that the schoolchildren in the areas are likely to be overly exposed to fluoride. The urinary fluoride can be used as the reliable biomarker for recent fluoride exposure other than drinking water although there is a positive correlation between urinary fluoride and water fluoride concentration. The longer duration of living in endemic area can raise the urinary fluoride concentration over the accepted level. The intervene of defluoridation and mitigation in community level should be revised and further research of fluoride toxication should be funded.

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