TOENAIL FLUORIDE ION CONCENTRATIONS, DENTAL FLUOROSIS IN THE PRIMARY DENTITION, AND VARIOUS SOCIO-BEHAVIORAL FACTORS IN CHILDREN LIVING IN A FLUORIDATED CITY IN BRAZIL

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ABSTRACT: ***Objectives:*** **To examine, in children living in a fluoridated city of an age when there is a risk of dental fluorosis developing, the prevalence of dental fluorosis in the primary dentition, and the relationship between the toenail fluoride ion (F) concentrations and the presence of (i) dental fluorosis in the primary dentition and (ii) various socio-behavioral factors.** ***Methods:*** **A calibrated dentist examined 242 children aged 4–6-yr-old in Bauru, Brazil, using the Dean Index (kappa=0.78). The guardians of the children answered a questionnaire with objective-descriptive questions about the child's profile (socio-behavioral factors). The children's toenails were clipped and collected by the guardians. The F analysis was made with an ion-specific electrode, after HMDS facilitated-diffusion. The data were analyzed by the Kruskal-Wallis and Mann Whitney tests ($p<0.05$).** ***Results:*** **The prevalence of very mild or mild dental fluorosis was 7.44% and range of the toenail F concentrations was 0.02–6.64 µg/g. The children without dental fluorosis had higher toenail F concentrations (1.64±1.06 µg/g) than those with dental fluorosis (1.42±1.00 µg/g) but the difference was not significant ($p>0.05$). Similarly, no significant relationships were present between the toenail F levels and age, gender, the type of preschool attended, the consumption of soy food, lactose intolerance, dentifrice ingestion, and the supervision of toothbrushing.** ***Conclusions:*** **We found that the primary dentition can be affected by dental fluorosis but no significant relationship was present between this and the toenail F levels. A reassessment of these children at the stage of the permanent dentition may contribute to a better understanding of the relationship between toenail F levels and the occurrence of dental fluorosis.**

Key Words: Biomarkers; Dental fluorosis; Fluoride; Nails; Primary teeth; Toenails.

INTRODUCTION

Children may be exposed to high doses of the fluoride ion (F) from various sources, such as fluoridated water, infant formulas, supplements, fluoridated dentifrices, and infant foods and beverages. This can lead to a high daily intake of F with an increased risk of developing dental fluorosis.[1] The critical period for occurrence of dental fluorosis in the maxillary central incisors after exposure to excessive doses of F extends from birth to three yr.[2]

As F comes from different sources, the determination of its ingestion is becoming increasingly more difficult. Furthermore, according to McDonnell et al.,[3] the monitoring of fluoride absorption, instead of fluoride intake, seems to be

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more accurate. Thus, the search for biomarkers of exposure to fluoride has been intensified. Nails seem to have some advantages, since they can be easily accessed, collected and analyzed, as well as having the possibility of being stored for a long time without degradation.[4] Nail clippings might then play a relevant role in monitoring F exposure and in estimating the risk of dental fluorosis developing.

Soy-based formulas have been widely used as a substitute for breast milk or cow's milk, for children who have lactose intolerance or cow's milk allergy[5-7]. Some studies have reported high levels of F in soy-based products.[8-10]

A study observed an association between dental fluorosis and lactose intolerance/cow's milk allergy, together with a tendency for soy-based products to contribute to this occurrence.[11]

The aims of this study were to examine, in children living in a fluoridated city of an age when there is a risk of dental fluorosis developing, the prevalence of dental fluorosis in the primary dentition, and the relationship between the toenail fluoride ion (F) concentrations and the presence of (i) dental fluorosis in the primary dentition and (ii) various socio-behavioral factors.

MATERIALS AND METHODS

Volunteers: The protocols of the present study were fully approved by the Institutional Review Board (IRB) of Bauru Dental School, University of São Paulo (Proc. 110/2006 and 073/2008). This research was conducted in full accordance with the World Medical Association Declaration of Helsinki.

The nature and purposes of the study were explained verbally and in writing to the guardians, who signed an IRB-approved informed consent document. The sample comprised 242 4–6-yr-old children, from four public preschools and two private preschools. These preschools were randomly selected to represent five different social-demographic regions in Bauru (State of São Paulo, Brazil), a city in which the public water supply is fluoridated at an optimal level (0.6–0.8 ppm F).[12] The inclusion criteria to participate in this study were to have an age of 4–6 yr and the guardians signing an informed consent document.

Questionnaire: The guardians received a questionnaire with objective-descriptive questions. It contained some questions about the children related to age, gender, the type of preschool, the consumption of soy food, the presence of lactose intolerance or cow's milk allergy, dentifrice ingestion, and the supervision of toothbrushing.

Survey: The examinations were made after supervised toothbrushing, with all the examinations being performed by one dentist who was helped by an assistant who took notes. Both of them were appropriately trained and calibrated.

The children were examined in the school yards, under natural light, by visual inspection, using a plane mirror and a tongue depressor. The examinations were repeated in 10% of the assessed sample and the kappa coefficient (κ) was used to assess the inter-examiner reproducibility. The κ was found to be good (κ=0.78).[13]

The Dean index was used to evaluate dental fluorosis, according to World Health Organization (WHO) criteria.[14] The criteria were used to assess the presence of very mild, mild, moderate, or severe degrees of dental fluorosis. Only the two most affected teeth were considered and when these teeth were not affected similarly, the value of the least affected among the two was recorded. In case of doubt, the tooth was considered normal.

Nail sampling/F concentrations in nails: The guardians were instructed to let the children's nails grow for 15 days before clipping. Samples were collected from all the toenails of each child and stored in labeled vials provided by the researcher. The nail clippings were cleaned with deionized water using an interdental brush, sonicated in deionized water for 10 min, dried at 60±5ºC, and weighed. The F concentrations of the nails clippings were determined after overnight hexamethyldisiloxane-facilitated diffusion,[15] as modified by Whitford,[16] using an ion-specific electrode (Orion Research, Cambridge, Mass., USA, model 9409) and a miniature calomel reference electrode (Fisher Scientific Accumer, No. 13-620-79), both coupled to a potentiometer (Orion, model EA 940).

Statistical analysis: The software Statistica for Windows version 7.0 (StatSoft Inc., Tulsa, Okla., USA) was used. The Kruskal-Wallis and Mann Whitney tests were used to detect significant relationships between the toenail F concentrations and the presence of dental fluorosis and the various socio-behavioral factors. A significance level of 0.05 was selected *a priori* as the indicator of statistical significance.

RESULTS

Table 1 shows the classification of the children regarding the prevalence of dental fluorosis. Dental fluorosis was detected in 7.44% of the children and it was mainly with a fluorosis score of 2 (very mild, 5.79%). Scores of 4 and 5 (moderate and severe) were not found. The molars were the most affected teeth (47.7%).

Table 1. Prevalence of dental fluorosis in 4–6-year-old children in Bauru, São Paulo

Fluorosis scores (Dean index)	n	%
0 (normal)	214	88.43
1 (questionable)	10	4.13
2 (very mild)	14	5.79
3 (mild)	4	1.65
4 (moderate)	0	0.00
5 (severe)	0	0.00

The mean±SD of the toenail F concentration for the children was 1.61±1.06 μg/g, and with a range of 0.02–6.64 μg/g.

Table 2 shows the mean±SD of the toenail F concentration for the children according to age, gender, the type of preschool, and the presence of dental fluorosis and various socio-behavioral factors.

Table 2. Toenail fluoride (F) concentrations in children from Bauru, Brazil, according to age, gender, the type of preschool, and the presence of dental fluorosis and various socio-behavioral factors (Values are mean±SD)

Parameter		n	Toenail F concentration (µg/g)	p
Age (yr)	4	63	1.71±1.32	0.83
	5	117	1.57±0.99	
	6	62	1.60±0.87	
Gender	Male	107	1.61±1.16	0.35
	Female	135	1.61±0.97	
Type of preschool	Private	55	1.75±1.39	0.14
	Public	187	1.57±0.94	
Dental fluorosis	Present	28	1.42±1.00	0.19
	Absent	214	1.64±1.06	
Consumption of soy food	Present	128	1.65±1.08	0.63
	Absent	114	1.58±1.06	
Lactose intolerance/cow's milk allergy	Present	23	1.90±1.30	0.34
	Absent	219	1.59±1.04	
Reported dentifrice ingestion	Present	50	1.87±1.37	0.36
	Absent	192	1.55±0.96	
Supervised toothbrushing	Present	49	1.55±1.17	0.53
	Absent	113	1.63±0.96	
	Mixed	80	1.63±1.14	

No significant association was found between the toenail F concentration and age, gender, the type of preschool, the presence of dental fluorosis, the consumption of soy food, and the presence of lactose intolerance or cow's milk allergy, dentifrice ingestion, and supervised toothbrushing ($p>0.05$).

DISCUSSION

The multiple sources of F intake, such as fluoridated water, fluoridated dentifrices, F supplements, infant formulas, and foods, can lead to a daily intake of F which is above the recommended levels, especially in children of the ages at which they are at risk for development of dental fluorosis.[1,17-19]

Some studies have suggested that fluorosis in the primary teeth can be associated with the development of fluorosis in the permanent teeth,[20,21] However, there is still a lack of information regarding the prevalence of dental fluorosis in the primary dentition.

The prevalence of dental fluorosis in the permanent dentition was previously assessed in 12–15-yr-old adolescents in the same city evaluated in the present study and shown to be 36%.[22] However, only a few reports have evaluated the prevalence of dental fluorosis in the primary dentition among Brazilian children. In the present study, the prevalence of very mild or mild dental fluorosis in the primary dentition was 7.44%, which is close to the value reported for children of a similar age range living in a fluoridated Brazilian city[23] and higher than the value reported in another.[24]

The identification of dental fluorosis in the primary teeth suggests the chronic and excessive ingestion of F by younger children. Additionally, it can represent a predictor of fluorosis developing in the permanent teeth and gives an opportunity to modify the F intake and reduce the chance of disturbances in the permanent teeth. The detection of primary tooth fluorosis in preschool children should alert clinicians and parents to the high likelihood of subsequent fluorosis in the permanent dentition.[21]

In order to know the risk for development of fluorosis before its appearance, research on biomarkers of F exposure that are easy to collect and analyze has been intensified. Whitford et al.[25] described the fingernail F concentrations as being useful indicators of F intake. Thereafter, nails have been suggested as suitable biomarkers of exposure to F in animals[26,27] and humans.[4, 28-30] The use of toenails as biomarkers of exposure to F instead of fingernails has been suggested, since toenails have a higher mass, lower variability, and are less prone to external contamination.[4, 30].

Despite dental fluorosis in the primary dentition being associated with an increased risk of developing dental fluorosis at the permanent dentition,[21] in the present study no significant differences were observed between the toenail F concentrations in the children and the presence of dental fluorosis in the primary dentition. Variations in the prevalence and severity of dental fluorosis within the same community and between communities with a similar drinking water F content, strongly indicate that dental fluorosis is a multifactorial problem.

Variables that can modify the general features of F distribution in the organism include chronic and acute acid-base disturbances, hematocrit, high altitude, physical activity, circadian rhythm, and hormones.[16] The effect of the genetic background has recently been reported, since some mice strains are susceptible to dental fluorosis while others are resistant, probably because these strains metabolize F in a different manner.[31] This might help to explain the lack of an association between toenail F levels and the presence of dental fluorosis in the primary dentition in the present study.

Additionally, this lack of association could be explained by the fact that the toenail F concentrations reflect subchronic exposure to F (around 3.5 months before)[25,29] and it is possible that the pattern of F intake of the children is not constant over time. Studies designed to verify the daily feeding and the analysis of the dentifrice intake may help to more accurately assess the daily F intake of children. A reassessment of these children at the stage of the permanent dentition may contribute to a better understanding of the relationship between toenail F levels and the occurrence of dental fluorosis

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest related to this study.

REFERENCES

1 Mascarenhas AK. Risk factors for dental fluorosis: a review of the recent literature. Pediatr Dent 2000;22:269-77.
2 Hong L, Levy SM, Warren JJ, Broffitt B, Cavanaugh J. Fluoride intake levels in relation to fluorosis development in permanent maxillary central incisors and first molars. Caries Res 2006;40:494-500.
3 McDonnell ST, O'Mullane D, Cronin M, Mac-Cormac C, Kirk J. Relevant factors when considering fingernail clippings as a fluoride biomarker. Community Dent Health 2004;21:19-24.
4 Fukushima R, Rigolizzo DS, Maia LP, Sampaio FC, Lauris JRP, Buzalaf MAR. Environmental and individual factors associated with nail fluoride concentration. Caries Res 2009;43:147-54.
5 Arato A, Horvath J. Soy formula in the feeding of infants with milk allergy. Orv Hetil 1995;136(27):1433-7.
6 Botey J, Eseverri JL, Dordal MT, Andreu J, Marin A. Alternative milk formulas in allergies to proteins in cow's milk. J Investig Allergol Clin Immunol 1993;3(2):100-2.
7 Host A, Koletzko B, Dreborg S, Muraro A, Wahn U, Aggett P, et al. Dietary products used in infants for treatment and prevention of food allergy. Joint statement of the European society for paediatric allergology and clinical immunology (ESPACI) committee on hypoallergenic formulas and the European society for paediatric gastroenterology, hepatology and nutrition (ESPGHAN) committee on nutrition. Arch Dis Child 1999;81:80-4.
8 Latifah R, Razak IA. Fluoride levels in infant formulas. J Pedod 1989;13:323-7.
9 Pagliari AV, Moimaz SAS, Saliba O, Delbem ACB, Sassaki KT. Analysis of fluoride concentration in mother's milk substitutes. Braz Oral Res 2006;20(3):269-74.
10 Van Winkle S, Levy SM, Kiritsy MC, Heilman JR, Wefel JS, Marshall T. Water and formula fluoride concentrations: significance for infants fed formula. Pediatr Dent 1995;17(4):305-10.

11 Carvalho CAP, Nicodemo CAZ, Mercadante DCF, Carvalho FS, Buzalaf MAR, Sales-Peres SHC. Dental fluorosis in the primary dentition and intake of manufactured soy-based foods with fluoride. Clinical Nutrition 2013;32:432-7.
12 Ramires I, Maia LP, Rigolizzo D dos S, Lauris JR, Buzalaf MA. External control over the fluoridation on the public water supply in Bauru, SP, Brazil. Rev Saude Publica 2006;40:883-9.
13 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.
14 World Health Organization. Oral health surveys: basic methods. 4th ed. Geneva: WHO; 1997.
15 Taves DR. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. Talanta 1968;15:969-74.
16 Whitford GM. The metabolism and toxicity of fluoride. 2nd ed. Basel: Karger; 1996.
17 Buzalaf MAR, Cury JA, Whitford GM. Fluoride exposures and dental fluorosis: a literature review. Rev Fac Odontol Bauru 2001;9:1-10.
18 Buzalaf MAR, Damante CA, Trevizani LMM, Granjeiro JM. Risk of fluorosis associated with infant formulas prepared with bottled water. J Dent Child 2004;71:110-3.
19 Almeida BS, Cardoso VES, Buzalaf MAR. Fluoride ingestion from toothpaste and diet in 1-3-year-old Brazilian children. Community Dent Oral Epidemiol 2007;35:53-63.
20 Milsom KM, Woodward M, Haran D, Lennon MA. Enamel defects in the deciduous dentition as a potential predictor of defects in the permanent dentition of 8 - and 9-year-old children in fluoridated Cheshire, England. J Dent Res 1996;75:1015-8.
21 Levy SM, Warren JJ, Broffitt B, Kanellis MJ. Associations between dental fluorosis of the permanent and primary dentitions. J Public Health Dent 2006;66:180-5.
22 Ramires I, Pessan JP, Levy FM, Rodrigues MH, Almeida BS, Kato MT, et al. Prevalence of dental fluorosis in Bauru, São Paulo, Brazil. J Appl Oral Sci 2007;15:140-3.
23 Hoffman RHS, de Sousa MLR, Cypriano S. Prevalence of enamel defects and the relationship to dental caries in deciduous and permanent dentition in Indaiatuba, São Paulo, Brazil. Cad Saude Publica 2007;23:435-44. [in Portuguese].
24 Cypriano S, de Sousa MLR, Rihs LB, Wada RS. Oral health among preschool children in Brazil, 1999. Rev Saude Publica 2003;37:247-53. [in Portuguese].
25 Whitford GM, Sampaio FC, Arneberg P, von der Fehr FR. Fingernail fluoride: a method for monitoring fluoride exposure. Caries Res 1999;33:462-7.
26 Buzalaf MAR, Fukushima R, Granjeiro JM, Cury JA. Correlation between plasma and nail fluoride concentrations in rats given different levels of fluoride in water. Fluoride 2002;35(3):185-92.
27 Buzalaf MAR, Caroselli EE, Oliveira RC, Granjeiro JM, Whitford GM. Nail and bone surface as biomarkers for acute fluoride exposure in rats. J Anal Toxicol 2004;28:249-52.
28 Levy FM, Bastos JRM, Buzalaf MAR. Nail as biomarkers of fluoride in children of fluoridated communities. J Dent Child 2004;71:121-5.
29 Correia Rodrigues MH, Bastos JRM, Buzalaf MAR. Fingernails and toenails as biomarkers of subchronic exposure to fluoride from dentifrice in 2- to 3-year-old children. Caries Res 2004;38:109-14.
30 Buzalaf MAR, Pessan JP, Alves KM. Influence of growth rate and length on fluoride detection in human nails. Caries Res 2006;40:231-8.
31 Carvalho JG, Leite AL, Yan D, Everett ET, Whitford GM, Buzalaf MA. Influence of genetic background on fluoride metabolism in mice. J Dent Res 2009;88:1054-8.