Nails as Biomarkers of Fluoride in Children of Fluoridated Communities

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ABSTRACT

Purpose: The objective of this study was to verify the use of nails as biomarkers of chronic fluoride (F) exposure from the diets of children living in communities with negligibly or optimally fluoridated water.

Methods: Fifteen 2- to 6-year-old children living in Bauru-São Paulo (fluoridated) and 15 lifelong residents of Itápolis-São Paulo (nonfluoridated) participated in the study. Fluoride concentrations in nails and duplicate diet were analyzed with the electrode, following hexamethyldisiloxane (HMDS)-facilitated diffusion. Data were analyzed by student's *t*-test and linear regression ($P<.05$).

Results: Mean fluoride concentrations (μg/g) in fingernails and toenails of Bauru children were 3.56±1.3 and 2.81±1.29, respectively, and for Itápolis children 2.29±1.25 and 1.58±0.59, respectively. The differences between Bauru and Itápolis children, as well as between fluoride concentrations in fingernails and toenails, were statistically significant. The estimated fluoride intake from the diet was significantly higher for Bauru children (0.55±0.61 mg) when compared to Itápolis children (0.09±0.06 mg). A significant positive correlation was found between the mean of fingernail and toenail fluoride concentrations and estimated fluoride intake from the diet (r=0.57).

Conclusions: This study's preliminary data suggests that fingernails and toenails may be used as biomarkers of chronic fluoride exposure from the diet. Additional studies are necessary to determine the predictive values, sensitivity, and specificity of this biomarker so that nails are used to differentiate children at the age of dental fluorosis risk and who live in communities with negligibly or optimally fluoridated water. (*J Dent Child*. 2004;71:121-125)

KEYWORDS: FLUORIDE, FINGERNAILS, TOENAILS, DIET, CHILDREN, BIOMARKERS

Due to the increase in dental fluorosis prevalence, the search for biomarkers of fluoride exposure that are easy to collect and analyze has been intensified.[1]

Nail sampling is simple, noninvasive and there are many reports suggesting the use of nails as biomarkers for fluoride exposure in animals[2] and humans.[3-10] Both fingernails and toenails have been used for this purpose, but the literature is contradictory regarding the relationship between fingernail and toenail fluoride concentrations.[4,9,10] In addition, some reports show a direct relationship between the fluoride concentrations in the drinking water and nail clippings.[3,4,9] However, none of the aforementioned studies related the fluoride intake from the diet to the fluoride concentration found in nail clippings. This comparison is important, because nail fluoride concentrations could be used as a predictor of fluoride intake levels, and consequently, the risk of dental fluorosis.

Based on these assumptions, the aim of this study was to verify that nails can be used as biomarkers of chronic fluoride exposure from the diets of children living in communities with negligibly or optimally fluoridated water. Additionally, the fluoride concentrations of fingernail and toenail clippings were also compared.

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METHODS

VOLUNTEERS

Study participants included:

1. 15, 2-to 6-year-old children, lifelong residents of the optimally fluoridated (0.6-0.8 ppm) city of Bauru-São Paulo;
2. 15, 2-to 6-year-old children, lifelong residents of the negligibly fluoridated (0.09-0.20 ppm) city of Itápolis-São Paulo.

This study was approved by the Ethical Committee of Bauru Dental School–University of São Paulo, Bauru-São Paulo, Brazil. Parental informed consent was obtained. The children were born in the respective cities and consumed water only from the public supply. All children appeared to had good oral health, were not using medicines, no gastrointestinal, bone, or renal problems, and medical indication for blood collection.

NAIL SAMPLING

At the beginning of the study, parents were instructed to let their children's nails grow for 15 days before clipping. On the scheduled day, researchers went to the volunteers' houses and clipped the nails. The researcher was trained to clip all the nails and store fingernails and toenails separately in identified vials. After another 15-day period, the researcher returned to the volunteers' houses and clipped their nails again. Thus, 2 samples of fingernails and 2 samples of toenails were obtained for each child.

PLASMA SAMPLING

Fasting blood (3-4 mL) was collected in certified laboratories with disposable plastic syringes containing 25 μL heparin (0.184 μg fluoride per mL). The blood samples were centrifuged at 3,000 rpm for 5 minutes and plasma was obtained.

DIETARY FLUORIDE INTAKE ESTIMATE

To estimate intake of fluoride from the children's diets, "duplicate-plate" samples of all foods and beverages, including water, ingested during 1 weekend day were collected, as described by Guha-Chowdhurry et al.[11] Parents were instructed to maintain their children's usual dietary habits and duplicate the diet as precisely as possible by observing the amounts that the children had really eaten and drunk. Parents were asked to remove parts of foods not normally eaten, such as seeds, cores, skin, and bones, before including the food in a container.

Parents were asked to use household measures, such as teaspoons, tablespoons, or cups, to approximate quantities of food ingested. In the case of cooked meals, parents were asked to:

1. serve 2 similar portions on 2 separate plates;
2. wait until the children had finished their portion;
3. add or remove comparable portions on the separate plate.

In addition to collecting duplicate diets, parents were also requested to maintain a 24-hour diet record of all foods and drinks their children ingested.

Appointments were scheduled for the day after the diet collection. At this time the:

1. duplicate diet and diet records were collected;
2. diet was homogenized using deionized water;
3. total volume was measured;
4. 50 mL aliquot sample was taken and frozen (–20°C) until analysis.

ANALYTICAL PROCEDURE

The nail clippings obtained for each date were pooled, resulting in 4 analytical results (2 from fingernails, 2 from toenails) for each child. Each nail clipping was cleaned briefly with deionized water using an interdental brush and then sonicated in deionized water for 10 minutes, dried at 60±5°C, and weighed (±0.01 mg). The weights of the pooled samples ranged from 2.53 to 19.98 mg. Thus, it was not possible to perform duplicate analysis.

The nail clipping fluoride concentrations were determined after overnight, hexamethyldisiloxane (HMDS)-facilitated diffusion[12] as modified by Whitford[13] using a fluoride ion-specific electrode (model no. 9409, Orion Research, Cambridge, Mass) and a miniature calomel reference electrode (model no. 13-620-79, Accumet), both coupled to a potentiometer (model no. EA 940, Orion Research, Cambridge, Mass). For diffusion, deionized water was placed in the bottom of a nonwettable diffusion dish (model no. 1007, Falcon) along with the samples. The base trap, 50 μL of 0.05 M NaOH, was placed in 3 drops on the inside of the dish's top portion.

The inside periphery of the lid was ringed with petroleum jelly and sealed to the bottom carefully to avoid leaving any air bubbles in the petroleum jelly. Two milliliters 3 M H_2SO_4 saturated with HMDS, were added to the bottom through a small hole previously burned into the top portion of the diffusion dish. The hole was immediately sealed with petroleum jelly. During the diffusion process, which was conducted overnight at room temperature, the solutions in the nonwettable Petri dishes (model no. 1007, Falcon) were gently swirled on a rotatory shaker.

On the next day, the dishes were opened, the lid was inverted, and the trap was buffered with 25 μL of 0.2 M acetic acid. The final volume was then adjusted to 75 μL by the addition of fluoride-free water using a pipettor. The solution was then returned to the lid and fluoride and miniature calomel reference electrodes were immersed for analysis. Fluoride standards were prepared in triplicate and diffused in the same manner as the nail clippings. In addition, nondiffused fluoride standards were prepared with the same solutions (0.05 M NaOH, 0.20 M acetic acid, plus NaF) that were used to prepare the diffused standards and samples. The nondiffused standards were created to have exactly the same fluoride concentrations as the diffused standards.

Comparison of the millivolt readings demonstrated that the fluoride in the diffused standards had been completely trapped and analyzed. The fluoride concentrations in the diet and plasma were analyzed in the same way, except the

Table 1. Mean Fluoride Concentration (μg/g) in Fingernail (FN) and Toenail (TN) Clippings From 2- to 6-year-old Children Residing in Bauru (Fluoridated) and Itápolis (Nonfluoridated) on 2 Different Dates*

	City											
	Bauru						Itápolis					
	1st		2nd		Both		1st		2nd		Both	
	FN	TN	FN	TN	FN	TN	FN	TN	FN	TN	FN	TN
Mean (±SD)	3.56±1.37	2.85±1.41	3.55±1.52	2.77±1.59	3.55±1.29	2.81±1.28	2.10	1.56±0.66	2.48±1.34	1.59±0.78	2,29±1.25	1.58±0.58
Minimum	0.87	1.21	2.00	0.81	0.87	0.81	0.61	0.08	0.42	0.91	0.42	0.08
Maximum	5.83	6.13	7.06	6.38	7.06	6.38	6.11	2.59	4.83	3.89	6.11	3.89
95% CI	2.8-4.31	2.07-3.64	2.69-4.42	1.93-3.62	2.84-4.28	2.1-3.53	1.38-2.83	1.19-1.93	1.73-3.22	1.16-2.02	1.6-2.98	1.25-1.91

*Fingernail and toenail collection data are shown separately and in combination. The mean fluoride fingernail concentrations found among Bauru children were significantly higher than those for Itápolis children in the first collection, and for the combination of both dates. In the 2 collections, as well as for the combination of both, the toenail values for Bauru children were significantly higher than those for Itápolis children. No significant differences were detected between the 2 dates of collection, both for fingernail and toenail clippings (P<.01).

analyses were made in duplicates. The mean repeatability of the readings, based on duplicate samples, was 94%. For plasma, the samples were prepared with previously heated HMDS-H_2SO_4 to remove CO_2 before the diffusion was conducted.

The same researcher, who was unaware of the volunteers from whom samples were taken, made all the analysis.

STATISTICAL ANALYSIS

The fluoride concentrations of plasma, nails, and duplicate diets from the Bauru children were compared to Itápolis children using unpaired student's *t* tests. A significance level of 5% was selected a priori.

Paired student's *t* tests were used to compare the fluoride concentration difference between fingernails and toenails and detect differences in fluoride concentrations found in the 2 different dates, both for fingernails and toenails.

Pearson's coefficient was used to detect correlations between plasma fluoride concentrations and fingernail and toenail fluoride levels, as well as the amount of ingested fluoride. Also evaluated was the correlation between: (1) fingernail and toenail fluoride concentrations; and (2) the first and second dates of sampling.

RESULTS

Table 1 shows the mean fluoride concentrations in fingernail and toenail clippings of the Bauru and Itápolis children. The data are displayed separately by each collection and combined. Mean fluoride concentrations found in fingernail clipping at the first collection were higher among Bauru children than Itápolis childen. This difference was statistically significant (t=2.97; P=.01). In the second sampling, similar values were obtained for Bauru children, but a slight increase was observed among Itápolis children when compared to the first sampling. In this case, the difference between the cities was not statistically significant (t=2.02; P=.05). When the data from both collections were combined, statistically significant differences were observed between the 2 cities for fingernails fluoride concentration (t=2.72; P=.01).

As for toenails, fluoride concentrations found in samples from Bauru were statistically higher than those from Itápolis, for the first (t=3.20; P<0.01) and second collections (t=2.67; P<0.01), and also for the combination of both (t=3.37; P<0.01). In addition, based on 95% confidence intervals, some overlap of the concentrations found in both communities was seen for fingernails, but no overlap was observed for toenails (Table 1).

The paired *t* test did not detect statistically significant differences between fingernail clippings collected at the different dates (t=0.87; P=.39). In addition, a strong, significantly positive correlation was observed between the values obtained in the 2 different dates of collection (r=0.71, P<.0001). The same was observed for toenail clippings. No significant differences were detected between the 2 dates of collection (t=0.11; P=.91), and a positive correlation was observed between them (r=0.62, P<.0001).

Mean fluoride concentration of toenail clippings was lower than that of fingernail clippings collected at the same dates. Among Bauru children, the mean difference was 20%, compared to 30% for Itápolis children. The paired student's *t* test revealed that this difference was significant (t=2.82; P<0.01).

Table 2 shows the mean fluoride concentration found in plasma and duplicate diets of the Bauru and Itápolis children.

Table 2. Mean (±SD [CI 95%], N=15 Per City) Fluoride Concentration Found in Plasma and Duplicate Diets of Bauru and Itápolis Children*

	City	
Variable	Bauru	Itápolis
Plasma (μg/mL)	0.019±0.011 (0.013-0.025)[a]	0.024±0.020 (0.013-0.035)[a]
Diet (mg)	0.551±0.610 (0.213-0.888)[a]	0.088±0.056 (0.057-0.120)[b]
Diet (mg/kg body weight)	0.029±0.029 (0.013-0.045)[a]	0.004±0.003 (0.002-0.006)[b]

*Values in the same rows followed by distinct letters are significantly different (P<.01).

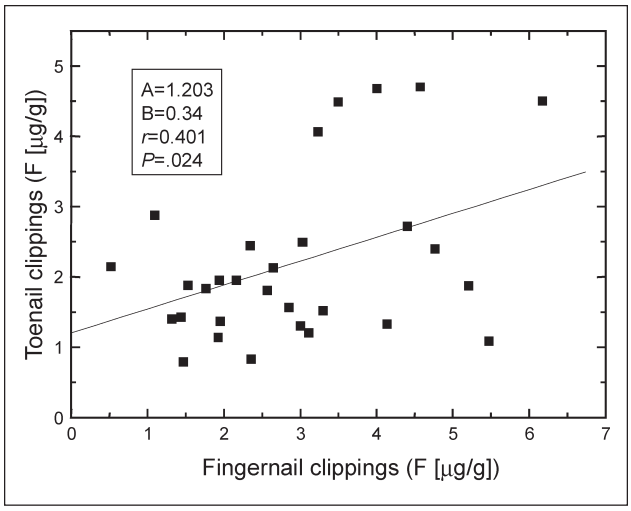

Figure 1. Correlation between fingernail and toenail fluoride concentrations. A=y intercept; B=slope; N=15 per city. Data from the 2 dates were combined.

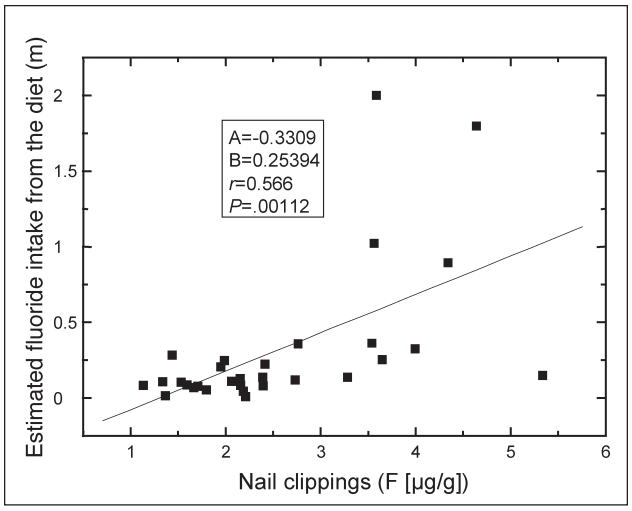

Figure 2. Correlation between nail clipping fluoride concentration and estimated dietary fluoride intake. A=y intercept; B=slope; N=15 per city. The nail clipping values are the mean of toenail and fingernail fluoride concentrations.

Regarding plasma fluoride concentrations, despite the fact that values found among Itápolis children were slightly higher than for Bauru children, this difference was not statistically significant (t=–0.89; P=.38). As for the diet, significantly higher values were found among Bauru children when compared to Itápolis children (t=2.92; P<0.01).

No significant correlations were found between:

1. plasma and fingernail fluoride concentrations (r=0.14; P=.47);
2. plasma and toenail fluoride concentrations (r=–0.27; P=.15);
3. plasma fluoride levels and estimated fluoride intake from the diet (r=–0.21; P=.26).

However, a positive statistically significant correlation was found between fingernail and toenail fluoride concentrations (r=0.41; P=.02), as shown in Figure 1. A similar correlation was found between the estimated amount of ingested fluoride and the nail fluoride concentrations (r=0.57; P<0.01), as shown in Figure 2.

DISCUSSION

In a previous study, the authors' research group[2] found a significant positive correlation between nail and plasma fluoride concentrations in rats given different concentrations of drinking water fluoride. In this study, however, no significant plasma fluoride level differences were seen when the volunteers from the fluoridated area were compared to those from the nonfluoridated area. Three hypotheses may help to explain this unexpected finding:

1. The children were supposed to be fasting when the blood samples were taken. However, the plasma fluoride levels found in some of them were higher than expected for fasting subjects. Values up to 0.09 µg/mL were detected.
2. It is very likely the children swallowed fluoride from the toothpaste during morning brushing. This would tend to narrow plasma level differences found in the 2 cities, since both groups of children use fluoride dentifrice (approximately 1,100-1,500 µg fluoride per gram).
3. Plasma is a short half-life biomarker of fluoride exposure.[14] Thus, it is necessary to repeat sampling over several hours if reliable data are to be obtained that describe plasma levels.[15] This was a limitation of the present study that could be overcome if parotid ductal saliva was collected instead of blood, since ductal saliva reflects plasma fluoride levels.[16]

This work demonstrated statistically significant higher fluoride concentrations both in fingernail and toenail clippings for the children living in the optimally fluoridated community when compared to those living in the community with negligible water fluoride levels. The relationship between the fluoride concentrations in the drinking water and fingernail clippings had already been described.[9] However, this is the first known report on the relationship between estimated dietary fluoride intake and the fluoride concentration in nail clippings.

Despite the fact that duplicate diets were collected only once, a significant correlation was observed, which contributes to the validation of nails as biomarkers of chronic fluoride exposure. Some reports did not find significant differences in the fluoride content of the duplicate diet collected on different dates,[10,17] but others did.[18] Thus, the possibility exists that, had the authors collected duplicate diets on different dates, a stronger correlation between the diet's fluoride content and the nail clippings' fluoride content could be obtained. Furthermore, the estimate of dentrifice fluoride intake, if adequately obtained, would contribute to the improvement of this relationship.

The sample size (N=15) of the analyzed population is too low to draw definitive conclusions on the suitability of nails as biomarkers of fluoride exposure to differentiate optimally and negligibly fluoridated communities. Studies involving larger sample sizes would contribute to this biomarker's validation on

a community basis and help determine predictive values, sensitivity, and specificity. However, the absence of significant differences between fluoride concentrations of nail clippings collected on different dates—as well as the strong correlation found between the values collected on the 2 dates for each child—reinforce the suitability of nails as biomarkers of chronic fluoride exposure.

This study found fingernail fluoride concentrations to be approximately 25% higher than those for toenails. This difference was significant. Another study conducted by the authors' research group found fingernail fluoride concentrations to be slightly higher than those for toenails, but this difference was not statistically significant.[10] Whitford et al[9] also reported fingernail fluoride concentrations to be higher than toenail fluoride concentrations, but in this case the difference was approximately 50%. The authors attributed this to lower blood-flow rate to the toes and/or the slower growth rate of toenails.

However, Machoy[4] found similar values for fingernail and toenail fluoride levels. The studies of Whitford[9] and Rodrigues et al[10] used the same analytical technique used in this study, but the study of Machoy[4] used a different technique. This may help explain the different results observed. Another important consideration is that a small overlap of the concentrations found in both communities was observed for fingernails, while no overlap was observed for toenails (Table 1). The authors suspect that the small overlap for fingernails was due to differences in fluoride intake and balance (total intake minus total excretion). Studies in which these variables are measured will be required to evaluate this hypothesis.

Due to the small overlap for fingernails and absence of overlap for toenails, both may be appropriate biomarkers of chronic dietary fluoride exposure for children living in communities with negligibly or optimally fluoridated water. Additional studies, with increased sample sizes, are needed to confirm these findings and refine the biomarkers in terms of predictive values, sensitivity, and specificity.

CONCLUSIONS

This study's preliminary data suggest that fingernails and toenails may be used as biomarkers of chronic dietary fluoride exposure. Additional studies are needed to determine the predictive values, sensitivity, and specificity of this biomarker so that nails are used to differentiate children at the age of dental fluorosis risk, who live in communities with negligibly or optimally fluoridated water.

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