Validation of Fingernail Fluoride Concentration as a Predictor of Risk for Dental Fluorosis

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Fluoride is widely recognized as the main factor responsible for the dramatic decline in caries incidence and prevalence worldwide [Bratthall et al., 1996]. However, concomitantly with the caries decline, an increase in the prevalence of dental fluorosis, the only proven side effect of fluoride for caries control, has also been documented [Clark, 1994; McDonagh et al., 2000; Whelton et al., 2004; Khan et al., 2005]. Dental fluorosis is caused by excessive fluoride intake during tooth formation [Bronckers et al., 2009]. Despite the fact that a beneficial pre-eruptive effect of water fluoridation on caries control has been identified, particularly on fissure caries, the preventive effect of fluoride is mainly post-eruptive, not requiring ingestion [Buzalaf et al., 2011]. Thus, it is possible to take maximum advantage of this element, with minimum risk of dental fluorosis. Moreover, while dental fluorosis is not regarded as an adverse health effect, the prudent use of fluoride fluoride concentrations should be useful in public health research, since it has the potential to identify around 80% of children at risk of developing dental fluorosis.

Key Words
Biomarker · Dental fluorosis · Fingernail · Fluoride

Abstract
The aim of this study was to validate the use of fingernail fluoride concentrations at ages 2–7 years as predictors of the risk for developing dental fluorosis in the permanent dentition. Fifty-six children of both genders (10–15 years of age) had their incisors and premolars examined for dental fluorosis using the Thylstrup-Fejerskov index. Fingernail fluoride concentrations were obtained from previous studies when children were 2–7 years of age. Data were analyzed by unpaired t test, ANOVA, and Fisher’s exact test when the fingernail fluoride concentrations were dichotomized (≤ 2 or > 2 μg/g). Children with dental fluorosis had significantly higher fingernail fluoride concentrations than those without the condition, and the concentrations tended to increase with the severity of fluorosis ($r^2 = 0.47$, $p < 0.0001$). Using a fingernail fluoride concentration of 2 μg/g at ages 2–7 years as a threshold, this biomarker had high sensitivity (0.84) and moderate specificity (0.53) as a predictor for dental fluorosis. The high positive predictive value indicates that fingernail

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requires surveillance not only over the amount that is ingested but also over the amount that is bioavailable during the critical period of tooth development.

Monitoring total fluoride intake is not an easy task due to the multiplicity of sources [Mascarenhas, 2000]. Attempts have been made by using questionnaires to estimate fluoride intake from diet, by collecting duplicate diets and by using the subtraction method to estimate intake from dentifrice [for review, see Clarkson et al., 2010]. One limitation of these methods is that they estimate fluoride intake only on the specific day that is under evaluation. If a more precise assessment of fluoride intake over time is desired, it is necessary to repeat the estimations periodically. In a longitudinal cohort study (Iowa Fluoride Study) it was possible to verify a significant positive association between fluorosis prevalence and level of early fluoride intake assessed by periodic questionnaires [Hong et al., 2006b]. However, considerable overlap among groups presenting both caries and fluorosis was observed in terms of mean fluoride intake. In addition, a wide variability was seen in individual fluoride intakes for volunteers without fluorosis or caries history. This led to the conclusion that precisely recommending an ‘optimal’ fluoride intake is quite difficult [Warren et al., 2009].

The most important limitation of the above-mentioned methods to estimate fluoride intake is that they are not able to assess bioavailable fluoride, which is the fraction of ingested fluoride that is absorbed and potentially responsible for disturbing amelogenesis. In this sense, the use of biomarkers that are related to circulating fluoride levels emerges as an important tool. Among them, plasma and ductal saliva have been used, but they are difficult to collect and reflect only present, or very recent, exposure to fluoride [Rugg-Gunn et al., 2011]. On the other hand, nail fluoride concentrations reflect plasma fluoride levels over a protracted period of time [McDonnell et al., 2004; Pessan and Buzalaf, 2011]. Another advantage crucial for their use as biomarkers is that nails can be collected in a non-invasive manner and stored for long periods of time without degradation.

Although many studies have shown that nails can be used to assess acute, subchronic and chronic exposure to fluoride [for review, see Pessan and Buzalaf, 2011], none of them had a longitudinal design that allowed the evaluation of the association between dental fluorosis prevalence/severity and nail fluoride concentrations. The aim of the present study was to test the hypothesis that fingernail fluoride concentrations in children with developing teeth can serve as an effective, predictive biomarker for the subsequent development of dental fluorosis.

Subjects and Methods

Participants

The participants of this study were recruited from among volunteers of previous studies conducted by our research group who had had their fingernail fluoride concentrations evaluated when they were 2–7 years [Whitford et al., 1999; Correa Rodrigues et al., 2004; Pessan et al., 2005; Fukushima et al., 2009]. This was done because we wanted to relate the fingernail fluoride concentrations that were determined when the teeth were forming to the prevalence and severity of dental fluorosis after the teeth erupted. The children were lifelong residents of Bauru, state of São Paulo, Brazil (artificially fluoridated water, 0.6–0.8 mg/l) [Ramires et al., 2006] or of communities in the state of Paraíba, Brazil (naturally fluoridated water, 0.1, 1.6 or 2.3 mg/l) [Whitford et al., 1999]. Among the 70 volunteers included in the previous studies whose fingernail fluoride concentrations were available, 52 agreed to participate in the present study. None of the volunteers living in the naturally fluoridated community (1.6 mg/l) could be found. An additional 4 volunteers residing in Paraiba (in the area containing 2.3 mg/l fluoride) who were not included in the study by Whitford et al. [1999] but had their fingernail fluoride concentrations determined were also included in the present study, giving a total of 56 participants (33 from Bauru and 23 from Paraiba) (table 1). All participants had their premolars completely erupted upon clinical examination and assented to the clinical examination. The protocol of the study was approved by the Institutional Review Board (IRB) of Bauru Dental School; parents signed an IRB-approved consent document.

Although the fingernail fluoride concentrations were obtained from previous studies and analyzed in two different laboratories (Bauru Dental School and Georgia Health Sciences University), the analytical method employed was essentially the same, as described in the original publications [Whitford et al., 1999; Correa Rodrigues et al., 2004; Pessan et al., 2005; Fukushima et al., 2009].

Dental Examinations

Fluorosis assessments of the labial surfaces of permanent upper and lower incisors and premolars were made by two trained and calibrated examiners (C.S.M. and F.C.S., kappa inter- and intra-examiner >0.75) using the Thylstrup-Fejerskov (TF) index [Thylstrup and Fejerskov, 1978]. The degree of fluorosis was determined using the highest score verified for each volunteer. The examiners were blinded to the fingernail fluoride concentrations.

For all the volunteers living in Paraiba and 3 volunteers living in Bauru, dental examinations were conducted at home, while the others were examined in a clinic at Bauru Dental School. In all cases the volunteers brushed their teeth under supervision of the examiner to remove dental plaque. The teeth were then dried using gauze and the examinations were done under natural light by visual inspection using an exploratory probe as recommended by the World Health Organization (WHO), a plane mirror and a tongue depressor. During the examinations, the volunteers were seated on chairs.

Statistical Analysis

The software GraphPad InStat version 3.0 for Windows (GraphPad Software Inc., La Jolla, Calif., USA) was employed. Data were tested for normal distribution and homoscedasticity by Kolmogorov and Smirnov and Bartlett’s tests, respectively.
Comparison of fingernail fluoride concentrations in volunteers residing in communities with different drinking water fluoride concentrations was done by ANOVA after logarithmic transformation of the data and Tukey’s test for individual comparisons. Unpaired t test after logarithmic transformation of the data was employed to compare fingernail fluoride concentrations in volunteers with and without dental fluorosis. The relationship between severity of dental fluorosis and fingernail fluoride concentrations was evaluated using Pearson’s correlation coefficient. Dichotomized data (fingernail fluoride concentration ≤ 2 or > 2 µg/g) were assessed by bivariate analysis using Fisher’s exact test. In all cases the significance level was set at 5%. The data are expressed as mean and standard deviation.

Results

The volunteers’ (30 male and 26 female) ages upon dental examination ranged from 10 to 15 years with a mean (SD) of 11.8 (1.2) years.

A dose-response relationship was observed for fingernail fluoride concentrations in relation to the drinking water fluoride concentrations. The mean (SD) fingernail fluoride concentrations in volunteers residing in the areas containing 0.1, 0.6–0.8 and 2.3 mg/l fluoride in the drinking water were 1.75 (0.46), 3.01 (1.36) and 6.28 (2.69) µg/g, respectively, and they were significantly different from each other (fig. 1a).

The overall prevalence of dental fluorosis was 66.1%. None of the volunteers living in the low-fluoride area (0.1 mg/l) had dental fluorosis. This might have been due to the fact that these volunteers lived in a rural community and reported no use of dentifrices for toothbrushing (data not shown), in addition to the low fluoride intake from the water. However, all volunteers living in the high-fluoride area (2.3 mg/l) had dental fluorosis and the severity was high (mainly TF 3, 4 and 5). For those living in the area with 0.6–0.8 mg/l fluoride in the drinking water, 72.7% had dental fluorosis, most of whom were classified in the mildest forms (TF 1 and 2) (fig. 1b).

Individuals who did not have dental fluorosis had a significantly lower mean (SD) fingernail fluoride concentration [2.24 (1.09) µg/g] than those with dental fluorosis [4.22 (2.45) µg/g] (t = 3.68, p < 0.001), and no overlap of the 95% confidence intervals (CIs) was observed. Moreover, a tendency for increases in the severity of dental fluorosis with increases in fingernail fluoride concentrations was found (fig. 2, table 2). This was confirmed by the significant positive correlation between these variables (r² = 0.47, p < 0.0001).

Based on the mean fingernail fluoride concentration observed in individuals who did not have dental fluorosis (2.24 µg/g), we decided to dichotomize the fingernail data into two categories: ≤ 2 or > 2 µg/g fluoride. Using 2 µg/g as a threshold, it was observed that fingernail fluoride concentrations at ages 2–7 years had high sensitivity (0.84, 95% CI 0.68–0.94) and moderate specificity (0.53, 95% CI 0.29–0.76) as predictors for dental fluorosis in the permanent dentition. The positive predictive value was high (0.78, 95% CI 0.62–0.89), while the negative predictive value was moderate (0.63, 95% CI 0.35–0.85) (table 3).

Table 1. Characteristics of children in previous studies from whom fingernail fluoride concentrations were available and who were volunteers in the present study

<table>
<thead>
<tr>
<th>Community (water fluoridation status)</th>
<th>Age (years) when nails were analyzed</th>
<th>Total number of volunteers</th>
<th>Number of volunteers included</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraíba* (0.1 mg/l)</td>
<td>6–7</td>
<td>10</td>
<td>10</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Paraíba* (0.6–0.8 mg/l)</td>
<td>2–3</td>
<td>10</td>
<td>8</td>
<td>Correa Rodrigues et al. [2004]</td>
</tr>
<tr>
<td>Paraíba* (2.3 mg/l)</td>
<td>6–7</td>
<td>9</td>
<td>13</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Bauru** (0.6–0.8 mg/l)</td>
<td>4–7</td>
<td>20</td>
<td>15</td>
<td>Pessan et al. [2005]</td>
</tr>
<tr>
<td>Bauru** (0.6–0.8 mg/l)</td>
<td>3–7</td>
<td>15</td>
<td>10</td>
<td>Fukushima et al. [2009]</td>
</tr>
</tbody>
</table>

* Naturally fluoridated water. ** Artificially fluoridated water. *** Four children residing in Paraíba (area containing 2.3 mg/l fluoride) that were not included in the study by Whitford et al. [1999] but had their fingernail fluoride concentrations available were also included.

Table 2. Analysis of variance (ANOVA) and correlation of severity of dental fluorosis with fingernail fluoride concentrations

<table>
<thead>
<tr>
<th>Community (water fluoridation status)</th>
<th>Mean (SD) fingernail fluoride concentration (µg/g)</th>
<th>SE</th>
<th>t-test</th>
<th>p-value</th>
<th>r²</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraíba* (0.1 mg/l)</td>
<td>1.75 (0.46)</td>
<td>0.07</td>
<td>10.76</td>
<td>&lt; 0.001</td>
<td>0.64</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Paraíba* (0.6–0.8 mg/l)</td>
<td>3.01 (1.36)</td>
<td>0.12</td>
<td>18.03</td>
<td>&lt; 0.001</td>
<td>0.71</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Paraíba* (2.3 mg/l)</td>
<td>6.28 (2.69)</td>
<td>0.50</td>
<td>34.52</td>
<td>&lt; 0.001</td>
<td>0.81</td>
<td>Whitford et al. [1999]</td>
</tr>
</tbody>
</table>

Table 3. Bivariate analysis of the relationship between dental fluorosis and fingernail fluoride concentrations

<table>
<thead>
<tr>
<th>Community (water fluoridation status)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraíba (0.1 mg/l)</td>
<td>0.84</td>
<td>0.53</td>
<td>0.78</td>
<td>0.63</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Paraíba (0.6–0.8 mg/l)</td>
<td>0.84</td>
<td>0.53</td>
<td>0.78</td>
<td>0.63</td>
<td>Whitford et al. [1999]</td>
</tr>
<tr>
<td>Paraíba (2.3 mg/l)</td>
<td>0.84</td>
<td>0.53</td>
<td>0.78</td>
<td>0.63</td>
<td>Whitford et al. [1999]</td>
</tr>
</tbody>
</table>
Dental fluorosis results from excessive exposure to fluoride during the period of tooth formation. The period of susceptibility for dental fluorosis in the whole permanent dentition (excluding the third molars) has been considered to be the first 6–8 years of life [Pendrys, 1990, 1999]. For the permanent maxillary central incisors, which are of greatest cosmetic importance, the first 3 years of life appear to be more important [Hong et al., 2006a]. This implies that dental fluorosis can be clinically detected only some years after excessive exposure to fluoride has occurred. Thus, preventing dental fluorosis by controlling fluoride intake and circulating fluoride levels during the critical period of tooth formation assumes great importance.

The volunteers included in the present study had their fingernail fluoride concentrations measured at the age of risk for dental fluorosis, i.e. when they were 2–7 years of age. After eruption of their premolars, they were recalled and examined for dental fluorosis. They had different levels of exposure to fluoride from the drinking water, since they were lifelong residents of areas with negligible, optimum or high fluoride concentrations in the drinking water. This allowed us to obtain a wide range of fingernail fluoride concentrations (means of 1.75–6.28 μg/g for the different areas) that were reflected in a wide range of TF scores (0–5) (fig. 1, table 2). This was essential to assess the validity of the biomarker. It is important to mention that there was a small variation in the age range of the volunteers included in the present investigation (2–7 years) (table 1), since they were recruited among participants of previous studies with different designs. It has been shown that nail fluoride concentration is expected to increase with age [Fukushima et al., 2009] as a conse-

![Fig. 1.](image1.png) **Fig. 1.** a Fingernail fluoride concentrations in children living in areas with different fluoride concentrations in the drinking water. Distinct letters indicate significant differences (ANOVA after log transformation and Tukey’s test, p < 0.05). Bars indicate SD. b Distribution of children according to the severity of dental fluorosis (n = 10–33 for the different areas).

![Fig. 2.](image2.png) **Fig. 2.** Relationship between severity of dental fluorosis at ages 10–15 years and fingernail fluoride concentrations at ages 2–7 years (linear regression, r² = 0.47, p < 0.0001, n = 56).
While there were many cases of TF 1–2 (22), the number of volunteers included in the TF categories shown in Table 2 were considerable differences in the number of volunteers among the categories of TF index. In fact, 95% CIs of the different categories of TF, suggesting that fingernail might not be a good biomarker for checking the severity of dental fluorosis. It should be emphasized that this positive correlation is mostly due to the inclusion of the two ‘extreme’ communities, i.e. areas with 0.1 and 2.3 ppm mg/l fluoride in the drinking water. The correlation obtained for the optimally fluoridated water separately is not significant. While the overlap in 95% CIs between TF 0 and TF 1–2 is negligible, it is greater between TF 0 and TF 3–4, which may be attributed in part to the small number of cases in these categories. This can be considered as the main limitation of the present study, since TF 1–2 usually does not pose any aesthetic concern [Chankanka et al., 2009]. Furthermore, from the clinical point of view, it is more important to have a good predictor of dental fluorosis in areas where exposure to fluoride is not so high, since in endemic areas the value of an additional biomarker is small. In order to refine the use of fingernail fluoride concentrations as biomarkers for the risk of dental fluorosis, further studies should be conducted, including a more even distribution of volunteers among the categories of TF index.

In addition, 4 individuals with TF 3 scores lived in Bauru, the artificially fluoridated community. All of them had fingernail fluoride concentrations <2.8 μg/g (data not shown), which reduced the mean fingernail fluoride concentration and increased the 95% CI in this category. In the communities of Paraíba, fluoride is naturally present in the drinking water, which ensures more constant levels, but in Bauru the water is artificially fluoridated. Fluctuations in the drinking water fluoride concentrations in Bauru have been reported in the past [Buzalaf et al., 2002]. After implementation of external monitoring they seem to occur less often [Ramires et al., 2006]. However, daily variations in water fluoride concentration that could alter the exposure to fluoride might have occurred. Having this in mind, it seems that the use of fingernail fluoride concentrations may be less appropriate to predict the risk of dental fluorosis for children who have moved from one town to another or for children whose exposure to fluoride is expected to vary considerably along time. Nail samples collected at different time points could give a more precise estimation of the child’s fluoride intake. Additionally, fluoride intake from fluoridated dentifrices, which is a common source of

<table>
<thead>
<tr>
<th>TF</th>
<th>n</th>
<th>Mean fingernail fluoride concentration, μg/g&lt;sup&gt;1&lt;/sup&gt;</th>
<th>95% CI, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>2.24 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71–2.76</td>
</tr>
<tr>
<td>1–2</td>
<td>22</td>
<td>3.35 ± 1.40</td>
<td>2.73–3.97</td>
</tr>
<tr>
<td>3–4</td>
<td>8</td>
<td>3.66 ± 2.11</td>
<td>1.89–5.42</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>7.58 ± 2.72</td>
<td>5.07–10.1</td>
</tr>
<tr>
<td>1–5</td>
<td>37</td>
<td>4.22 ± 2.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.40–5.03</td>
</tr>
</tbody>
</table>

<sup>1</sup> Figures are means ± SD. Values followed by distinct letters indicate significant differences (t test, p < 0.001).

### Table 3. Sensitivity, specificity as well as positive and negative predictive values for the use of fingernail fluoride concentrations >2 μg/g at ages 2–7 years as predictors of dental fluorosis in the permanent dentition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.84</td>
<td>0.68–0.94</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.53</td>
<td>0.29–0.76</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.78</td>
<td>0.62–0.89</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.63</td>
<td>0.35–0.85</td>
</tr>
</tbody>
</table>

Fisher’s exact test (p < 0.05).

The mean fingernail fluoride concentration in individuals without dental fluorosis was 2.24 μg/g compared with 4.22 μg/g for those with dental fluorosis, and there was no overlap of the 95% CIs (Table 2). However, there were considerable differences in the number of volunteers included in the TF categories shown in Table 2. While there were many cases of TF 1–2 (22), the number of cases in TF 3–4 (8) and TF 5 (7) were lower (Table 2, fig. 2). Despite the fact that there was an increase in mean fingernail fluoride concentrations with the increase in the severity of dental fluorosis, which led to a significant positive correlation between these variables, there is some overlap in the 95% CIs of the different categories of TF, suggesting that fingernail might not be a good biomarker for checking the severity of dental fluorosis.
fluoride intake for preschool children [Clarkson et al., 2010], might have diminished the impact of fluoridated water on fingernail fluoride concentrations, thus making interpretation of the data more difficult.

For the validation of diagnostic tests, the primary measures used are sensitivity, specificity and predictive values. Using the concentration of 2 μg/g fluoride in fingernail measured at ages 2–7 years as threshold, the sensitivity to predict dental fluorosis in the permanent dentition is high (0.84), while the specificity is moderate (0.53). This indicates moderate ability of fingernail fluoride concentrations to predict absence of dental fluorosis, i.e. false positive cases can be expected.

Considering that the recommendation when fingernail fluoride concentrations >2 μg/g are found is to reduce fluoride intake in order to have lower circulating fluoride levels, the moderate specificity of the test cannot be regarded as a drawback, since fluoride controls carries mainly through its topical effect [Buzalaf et al., 2011]. However, in an attempt to increase the specificity of the test, we decided to test the use of 2.8 μg/g fluoride in fingernails as a cut-off point. This was done because the 95% CI of fingernail fluoride concentrations in children without dental fluorosis ranged between 1.71 and 2.76 μg/g. In this case, sensitivity and negative predictive values were reduced (0.70 and 0.58, respectively), but specificity and positive predictive values increased (0.79 and 0.87, respectively) when compared to the cut-off point of 2 μg/g for fingernail fluoride concentrations. This could be a strategy if higher specificity of the diagnostic test is desired.

Furthermore, the high positive predictive value found indicates that this biomarker might be useful in public health, since it has the potential to identify around 80% of 2- to 7-year-old children at risk of developing dental fluorosis in the permanent dentition. This might allow more judicious and targeted counseling of the child’s parents or caregivers regarding fluoride intake in order to diminish circulating fluoride levels during tooth formation in an attempt to reduce the prevalence and severity of dental fluorosis. From the available evidence it seems that if the main concern is to avoid dental fluorosis in the permanent maxillary central incisors, fingernail fluoride concentrations should be assessed periodically during the first 3 or 4 years of life, when it is possible to control fluoride intake.

Acknowledgements

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Disclosure Statement

There is no conflict of interest that might introduce bias or affect the paper’s judgment.

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