Big toenail and hair samples as biomarkers for fluoride exposure – a pilot study

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Abstract

Background: Biomarkers can aid in detecting and preventing clinical disease through the recognition of change in biological samples. The objective of this case-control study was to further the knowledge on the use of big toenail and hair samples as biomarkers for fluoride exposure.

Methods: A total of 48 participants from an endemic (IC) and a non-endemic (SC) fluorosis region were included. Big toenail and hair samples were collected from each participant and the fluoride concentration was determined. The results of 42 participants were reported: 21 participants (11 males and 10 females, mean age 15.66 ± 2.61 years) from IC and 21 participants (11 males and 10 females, mean age 15.06 ± 0.79 years) from SC.

Results: The mean fluoride concentration of big toenail (2.34 ± 0.26 mg/kg) and hair (0.24 ± 0.04 mg/kg) in the endemic region was significantly higher than the mean fluoride concentration of big toenail (0.98 ± 0.08 mg/kg) and hair (0.14 ± 0.02 mg/kg) in the non-endemic region (p < 0.001 and p = 0.004, respectively). The Receiver Operating Characteristic (ROC) analysis showed that the Area Under the Curve (AUC) value was 0.889 for big toenail (p < 0.001) and 0.762 for hair (p = 0.004) samples. The fluoride assay for big toenails exhibits greater observed accuracy than does the fluoride assay for hair.

Conclusion: Nail and hair samples can serve as biomarkers to detect biological fluoride exposure according to the data of this pilot study. Nevertheless, hair is less sensitive and specific as a biomarker when AUC values of big toenail and hair samples were compared.

Keywords: Big toenail, Hair, Fluoride exposure, Biomarker

Background

Biomarkers, i.e. biological markers, can aid in detecting and preventing clinical disease through the recognition of change in biological systems or samples [1]. Specifically, they have been defined as “cellular, biochemical, or molecular alterations which are measurable in biological media such as human tissues, cells, or fluids and are indicative of exposure to environmental chemicals” [1], such as fluoride.

Bone [2, 3], dentin [3, 4], plasma, saliva and urine [5–7] as well as nails [3, 8–12] and hair [3, 13, 14] are biomarkers that were investigated to assess fluoride exposure. It has been pointed out that among the mineralized tissues, bone is the main site for fluoride accumulation, thereby making bone a good choice as a fluoride biomarker [3]. However, the difficulty and the invasiveness of bone sample collection has been underlined. Thus, the collection of dentin, from extracted third molars, has been suggested as more appropriate when compared to bone sample collection [3].

Less invasive methods, using body fluid samples, such as plasma, saliva and urine also have been considered in the literature to analyze body fluoride concentration [5–7]. Nevertheless, these body fluids are affected by a number of variables, such as fluoride intake within the last few hours. Consequently, these body fluids present short-term, i.e. ‘snapshot’, information only [3, 7].

Nail samples, on the other hand, can be obtained non-invasively. They can be easily transported and stored for long periods of time without degradation [3,
Table 1 Criteria for selection of big toenail and hair samples

Criteria for selection of big toenail samples
- No dermatological disease, trauma or injury affecting the nails.
- No nail polish or any other chemicals on nails for at least three months before the date of nail sample collection.
- The patients were notified about the study in advance and advised not to cut their nails for four weeks before sample collection. Patients who had nail polish or any other chemicals on their nails waited for three months and then one extra month for nails to grow before sample collection, i.e. four months.

Criteria for selection of hair samples
- No dermatological disease affecting the hair.
- No dyed or bleached hair. Hair was free of creams, oil and gels before sample collection.
- The patients were informed six weeks in advance about the study and instructed about the dates for hair sample collection and also advised that they should not get a hair-cut during this time. If a patient has had a hair-cut, perm or colouring during the last six weeks, sample collection was postponed for six weeks as hair grows at about 0.4 mm/day or an average of about 1 cm/month.

Criteria for selection of hair samples
- No dyed or bleached hair. Hair was free of creams, oil and gels before sample collection.
- The patients were informed six weeks in advance about the study and instructed about the dates for hair sample collection and also advised that they should not get a hair-cut during this time. If a patient has had a hair-cut, perm or colouring during the last six weeks, sample collection was postponed for six weeks as hair grows at about 0.4 mm/day or an average of about 1 cm/month.

Table 2 Fluoride concentration in big toenail and hair samples

| Biomarker                  | IC            | SC            | Difference                        |
|----------------------------|---------------|---------------|-----------------------------------|
| Nail Fluoride concentration| Mean ± SE     | 2.34 ± 0.26 (95% CI 1.80–2.88) | 0.98 ± 0.08 (95% CI 0.82–1.14) | 1.36 ± 0.25 (95% CI 0.85–1.88) |
|                           | Median        | 2.296 (SIQR 0.917) | 0.914 (SIQR 0.189) | 1.426 (SIQR 0.933) |
| Hair Fluoride concentration| Mean ± SE     | 0.24 ± 0.04 (95% CI 0.17–0.32) | 0.14 ± 0.02 (95% CI 0.10–0.17) | 0.11 ± 0.04 (95% CI 0.03–0.19) |
|                           | Median        | 0.194 (SIQR 0.059) | 0.126 (SIQR 0.045) | 0.073 (SIQR 0.085) |

SE Standard Error, CI Confidence Interval for Mean, SIQR Semi Interquartile Range for Median
sample collection for four weeks and six weeks, respectively.

On the scheduled day, the participants/parents visited their respective dental school to enable the investigator (SET) to collect big toenail and hair samples. Occipital hair is the only source recommended for the analysis for both male/female subjects. High-grade stainless steel scissors were utilized to cut the hair. The hair tuft sample was collected at a distance of 0.5 centimeters (cm) from the scalp and approximately 3 cm in length. The weight required for the hair specimen was 50–100 milligrams (mg). Hair samples were stored in labelled polyethylene bags, in a dry place, at room temperature. Again, the investigator (SET) clipped the big toenail's free end and cut from the right side to the left side using each participant's nail clipper. The clipped nails were stored separately in labelled plastic boxes per participant. The nail and hair samples were initially forwarded to the University of Sydney, Australia. Subsequently, the samples were sent to Bauru School of Dentistry, University of São Paulo, Brazil for analyses.

Fluoride concentration in nail and hair samples was determined after overnight HMDS-facilitated diffusion, applying the Taves method [21] as modified by Whitford [16] using a fluoride ion-specific electrode (Orion Research, Cambridge, Mass., USA, model 9409) and a miniature calomel reference electrode (Accumet, No. 13–620–79), both coupled to a potentiometer (Orion Research, model EA 940). All readings were made in duplicate.

Statistical analysis
Statistical analyses were performed with SPSS 23.0 for windows. The mean repeatability of the readings, based on duplicate samples, was 96%. Data were presented as mean ± standard error (SE) and median (Semi Interquartile Range – SIQR). The Shapiro–Wilk test was used to analyze the normal distribution assumption of the quantitative outcomes. Mann-Whitney test was used to compare the fluoride concentration in big toenail as well as hair samples between endemic and non-endemic regions. The receiver operating characteristic (ROC) curve was used to illustrate and evaluate the performance of big toenail and hair samples as biomarkers in case of fluoride exposure. The area under the ROC curve (AUC) was evaluated as the measure of a diagnostic test’s discriminatory power. Confidence intervals can be computed for AUC. In this article, sensitivity and specificity values were evaluated. A p value less than 0.05 was considered as statistically significant.

Results
The fluoride concentration in 6 participants could not be detected (3 from each city) due to technical difficulties, i.e. the fluoride concentration was not within the detection limit of the electrode since the amount of the sample collected was insufficient. Thus, 21 residents from IC (11 males and 10 females, mean age 15.66 + 2.61 years) and 21 residents from SC (11 males and 10 females, mean age 15.06 + 0.79 years) remained for the final analyses.

Overall, the mean fluoride concentration collected from nail clippings of the residents of IC was 2.34 + 0.26 mg/kg, while it was 0.98 + 0.08 mg/kg for those from SC (Table 2). Hair sample analysis of the participants from IC displayed a fluoride concentration of 0.24 + 0.04 mg/kg, while it was 0.14 + 0.04 mg/kg for those from SC (Table 2). There was a significant difference in the concentration of fluoride in nail clipping (p < 0.001; Table 3; Fig. 1) and hair (p = 0.004; Table 3; Fig. 2) samples between the two cities, higher in participants from IC than those from SC.

The results of ROC analysis are given in Table 4 and Fig. 3. The relative positions of the plots indicate the relative accuracies of the tests. A plot lying above and to the left of another plot indicates greater observed accuracy. In Fig. 3, the fluoride assay for big toenails exhibits greater observed accuracy than does the fluoride assay for hair.

The results of ROC analysis showed that the area under the curve (AUC) of big toenail samples was 0.889 (p < 0.001). The optimal sensitivity and specificity were 0.810 and 0.857, respectively (Table 4, Fig. 3). The AUC for hair samples was 0.762 (p = 0.004). The optimal sensitivity and specificity were 0.762 and 0.762 respectively (Table 4, Fig. 3).

Discussion
The relationship between fluoride concentrations in big toenail (hallux) clippings, hair and the level of fluoride in the water of an endemic (IC) and non-endemic (SC) fluorosis region was assessed in this study.

| Table 3 Comparison of fluoride concentration in big toenail and hair samples between IC and SC |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Biomarker       | City            | N   | Mean Rank | Sum of Ranks | U      | P      |
|-----------------|-----------------|-----|-----------|--------------|--------|--------|
| Nail Fluoride concentration (mg/Kg) | IC    | 21  | 29.67     | 623.00       | 49.00  | <0.001 |
|                 | SC              | 21  | 13.33     | 280.00       |        |        |
| Hair Fluoride concentration (mg/Kg) | IC    | 21  | 16.00     | 336.00       | 105.00 | =0.004 |
|                 | SC              | 21  | 16.00     | 336.00       |        |        |
The results of the present study, namely a higher and significant fluoride concentration in nail clippings and hair collected from IC participants when compared to SC participants, are similar to the findings of other studies [11–14]. These findings may be attributed to the fact that the regular level of fluoride in IC (≥2 ppm) drinking water was higher than that in SC (≤0.05 ppm) [18, 19]. Therefore, systemic fluoride circulation in the residents of IC is considerably higher, which in turn raised the fluoride uptake by nails and hair.

Absorbed fluoride deposits in the growing toenail by either continuous incorporation or secondary concentration [12]. One might argue that the detected level of fluoride in the nail clippings and hair collected from IC had a wide standard deviation indicating a significant individual discrepancy. This trend might be related to factors such as variation in the amount of consumed water [11–14], diet [14, 22], tea consumption [23] and external contamination among participants [24] as well as interpersonal variation in the amount of absorbed, circulated, metabolized and deposited fluoride [14].

The area under the ROC curve showed that nail and hair samples can serve as a biomarker to detect biological fluoride exposure according to the data of this pilot study. AUC is an effective way to summarize the overall diagnostic accuracy of a test [25]. In general, an AUC value of 0.7 to 0.8 is considered acceptable; whereas, an AUC value of 0.8 to 0.9 is considered excellent [26]. Thus, nail and hair samples have a reasonable discriminating ability to diagnose fluoride exposure. Nevertheless, hair is less sensitive and specific as a biomarker when AUC values of big toenail and hair samples are compared.

It is worth noting that the fluoride concentration for hair was lower than that for toenails. This outcome might be due to the fact that hair is characterized by a cyclic growth rate with different stages [27], whereas nails grow continuously and do not have a growth cycle analogous to that of hair [28]. In contrast, the fluoride concentration in toenails was higher than hair fluoride concentration and almost equal to the associated water. The growth rate of toenails [29] is substantially lower when compared to the growth rate of hair [30]. This lower growth rate of toenails might allow a more significant accumulation of fluoride. Furthermore, incorporation of fluoride through the nail

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**Table 4** Comparison of the area under the ROC curve (AUC) for fluoride concentration

| Biomarker  | AUC  | Std. Error | P     | 95% Confidence Interval Lower Bound | Upper Bound |
|------------|------|------------|-------|-----------------------------------|-------------|
| Nail samples | 0.889 | 0.050      | < 0.001 | 0.791                | 0.987       |
| Hair samples | 0.762 | 0.075      | =0.004 | 0.615                | 0.909       |

**ROC** Receiver Operating Characteristics, **AUC** Area Under Curve
bed, not only through the matrix (growth end), might contribute to the total fluoride concentration in toenails [3, 31].

Conclusion
The null hypothesis was rejected, i.e. nail and hair samples can serve as biomarkers to detect biological fluoride exposure according to the data of this pilot study. Nail and hair samples have a reasonable discriminating ability to diagnose fluoride exposure from the water supply from an endemic and non-endemic fluorosis region. Nevertheless, hair is less sensitive and specific as a biomarker when AUC values of big toenail and hair samples were compared. This area merits further research with a larger sample size.

Abbreviations
AUC: Area Under the Curve; cm: Centimeter; cm/month: Centimeter per month; HMDS: Hexamethyldisiloxane; IC: Isparta City; mg: Milligram; mg/kg: Milligram per kilogram; mm/day: Millimeter per day; Ppm: Parts per million; ROC: Receiver Operating Characteristics; SC: Samsun City; TFI: Thylstrup and Fejerskov Fluorosis Index

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Availability of data and materials
The data of this research is available from Selma Elekdag-Turk (corresponding author)/ Associate Professor/ Department of Orthodontics, Faculty of Dentistry/ Ondokuz Mayis University, Samsun, Turkey as well as from M. Ali Darendeliler / Professor and Chair/ Discipline of Orthodontics/ Faculty of Dentistry/ University of Sydney, Australia upon request.

Authors’ contributions
All authors assisted in the critical revision of this manuscript (Big Toenail and Hair Samples as Biomarkers for Fluoride Exposure – A Pilot Study) and gave final approval for submission to your journal. Furthermore, “each author participated sufficiently in the work to take responsibility for appropriate portions of the content; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated.” Additional details concerning the contributions of the authors are as follows: Responsible for: organization and appointment scheduling of the patients, acquisition of data from SC and IC, interpretation of data, drafting, editing and submission of the final manuscript: SET. Responsible for: contribution to the drafting of the manuscript, interpretation of data: MA. Responsible for: substantial contribution to the statistical analysis, interpretation of data: TT. Responsible for: laboratory analyses (HMDS-facilitated diffusion method): MARB. Responsible for: contribution to the statistical analysis of the data and the interpretation of this data: AA. Responsible for: contribution to conception of research, transfer of samples: OD. Responsible for: substantial contribution to conception of research, responsible for the funding support: MAD.

Ethics approval and consent to participate
Ethical approval was obtained from the Medical Faculty Ethics Committee of Ondokuz Mayis University, Samsun, Turkey (No. 2008/143). This research was undertaken with the understanding and written consent of each participant/guardian. Furthermore, the participants of this research were informed that the results will be published.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Hulka BS, Wilcosky T. Biological markers in epidemiologic research. Arch Environ Health. 1988;43:83–9.
2. Turner CH, Boivin G, Meunier PJ. A mathematical model for fluoride uptake by the skeleton. Calcif Tissue Int. 1993;52:130–8.
3. Pessan JP, Buzalaf MAR. Historical and recent biological markers of exposure to fluoride. Monogr Oral Sci. 2011;22:52–65.
4. Vieira AP, Hancock R, Limeback H, Maia R, Grynpas MD. Is fluoride concentration in dentin and enamel a good indicator of dental fluorosis? J Dent Res. 2004;83:76–80.
5. Ekstrand J. A micromethod for the determination of fluoride in blood plasma and saliva. Calcif Tissue Res. 1977;23:225–8.
6. Usuda K, Kono K, Dote T, Nishiura K, Miyata K, Nishiura H, et al. Urinary biomarkers monitoring for experimental fluoride nephrotoxicity. Arch Toxicol. 1998;72:104–9.
7. Rugg-Gunn AJ, Villa AE, Buzalaf MAR. Contemporary biological markers of exposure to fluoride. Monogr Oral Sci. 2011;22:57–51.
8. Whitford GM, Sampaio FC, Aneberg R; von der Fehr FR. Fingernail fluoride: a method for monitoring fluoride exposure. Caries Res. 1999;33:462–7.
9. Whitford GM. Monitoring fluoride exposure with fingernail clippings. Schweiz Monatsschr Zahnhemm. 2005;115:685–9.
10. Buzalaf MAR, Pessan JP, Alves KNRP. Influence of growth rate and length on fluoride detection in human nails. Caries Res. 2006;40:231–8.
11. 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.
12. Sankhala SS, Harshwal R, Palival P, Agarwal A. Toe nails as a biomarker of chronic fluoride exposure secondary to high water fluoride content in areas with endemic fluorosis. Fluoride. 2014;47:235–40.
13. Mandic Z, Curic M, Antonijevic B, Carevic M, Mandic J, Djukic-Cosic D, et al. Fluoride in drinking water and dental fluorosis. Sci Total Environ. 2010;408:3507–12.
14. Parimi N, Viswanath V, Kashyap B, Patel PU. Hair as biomarker of fluoride exposure in a fluoride endemic area and a low fluoridated area. Int J Trichology. 2013;5:148–50.
15. Hoppes HJ. The biologic bases for using hair and nails for analyses of trace elements. Sci Total Environ. 1977;77:71–89.
16. Whitford GM. In: Myers HM, editor. Absorption and plasma concentrations of fluoride. Basel: Karger: The metabolism and toxicity of fluoride; 1996. p. 26–7.
17. Pocock SJ. The size of a clinical trial. In: Pocock SJ, editor. Clinical trials: a practical approach. Chichester: John Wiley & Sons; 1983. p. 125–9.
18. Davraz A, Senir E, Sener S. Temporal variations of fluoride concentration in Isparta public water system and health impact assessment (SW-Turkey). Environ Geol. 2008;56:159–70.
19. Karadeniz EI, Gonzales C, Turk T, Isci D, Sahin-Saglam AM, Alis H, et al. Effect of fluoride on root resorption following heavy and light orthodontic force application for 4 weeks and 12 weeks of retention. Angle Orthod. 2013;83:418–24.
20. Thylstrup A, Fejerskov O. Clinical appearance of dental fluorosis in permanent teeth in relation to histological changes. Community Dent Oral Epidemiol. 1978;6:315–28.
21. Taves DR. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. Talanta. 1968;15:969–74.
22. Chowdhury C, Khijmatgar S, Kumari DP, Chowdhury A, Grootveld M, Hedge C, et al. Fluoride in fish flesh, fish bone and regular diet in south-coastal area of Karnataka state of India. Indian J Dent Res. 2018;29:414–7.
23. Das S, de Oliveira LM, da Silva E, Liu Y, Ma LQ. Fluoride concentrations in traditional and herbal teas: health risk assessment. Environ Pollut. 2017;231:779–84.
24. Czarnowski W, Krechniak J. Fluoride in the urine, hair, and nails of phosphate fertiliser workers. Br J Ind Med. 1990;47:349–51.
25. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5:1315–6.
26. Hosmer DW, Lemeshow S. Assessing the fit of the model. In: Cessie NAC, Fisher NI, Johnstone IM, Kadane JB, Scott DW, Silverman BW, et al., editors. Applied logistic regression. New York: John Wiley & Sons; 2000. p. 160–4.
27. Alonso I, Fuchs E. The hair cycle. J Cell Sci. 2006;119(Pt3):391–3.
28. Baumgartner MR. Nails: an adequate alternative matrix in forensic toxicology for drug analysis? Bioanalysis. 2014;6:2189–91.
29. Yaemsiri S, Hou N, Slining MM, He K. Growth rate of human fingernails and toenails in healthy American young adults. J Eur Acad Dermatol Venereol. 2010;24:420–3.
30. Myers RJ, Hamilton JB. Regeneration and rate of growth of hairs in man. Ann N Y Acad Sci. 1951;53:562–8.
31. Correa Rodrigues MH, de Magalhães Bastos JR, Rabelo Buzalaf MA. 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.