Original Article

Comparative Evaluation of Hair, Fingernails, and Toenails as Biomarkers of Fluoride Exposure: A Cross-Sectional Study

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ABSTRACT

Background: The increased prevalence of fluorosis has led to a search for biomarkers of fluoride exposure. Among the biomarkers of sub-chronic exposure to fluoride, hair, fingernails, and toenails have the advantage of being noninvasively collected, easily transported, and stored. **Objective:** The objective of this study was to comparatively evaluate coronal hair, fingernails, and toenails as biomarkers of fluoride exposure from drinking water; the study was designed as a population-based observational cross-sectional study. **Materials and Methods:** A population-based observational cross-sectional study was conducted in 60 children (20 subjects per group) of ages 12–17 years in three villages of Nilakottai block, Dindigul district, Tamil Nadu, India (Thomaspuram, Bangalapatti, and Singampatti). The fluoride concentration in the household drinking water was analyzed and compared with the fluoride content in the coronal hair, fingernail, and toenail clippings, which was estimated by potentiometric method (fluoride-ion-selective electrode) and expressed in ppm (parts per million). A two-tailed probability value of $P < 0.05$ was considered significant. **Results:** The mean fluoride concentration in drinking water was 0.63 ppm in Thomaspuram, 1.63 ppm in Bangalapatti, and 2.92 ppm in Singampatti. The mean fluoride content in hair samples was 2.84 ppm, 4.67 ppm, and 6.53 ppm; fingernail clippings was 2.99 ppm, 4.94 ppm, and 6.84 ppm; and toenail clippings was estimated as 3.13 ppm, 5.10 ppm, and 7.24 ppm in Thomaspuram, Bangalapatti, and Singampatti residents, respectively. The mean fluoride content in the hair, fingernails, and toenails was significantly higher as compared to the mean fluoride content in the drinking water (viz., toenail fluoride > fingernail fluoride > hair fluoride). **Conclusion:** Coronal hair, fingernails, and toenails are useful biomarkers for both sub-chronic and chronic fluoride exposure from drinking water. Due to ample sample availability and the highest fluoride content, toenails are the most suitable biomarkers of fluoride exposure from drinking water.

Keywords: *Biomarkers, coronal hair, fingernails, fluorides, fluorosis, toenails*

Introduction

Fluoride is often called a "double-edged sword" because deficient fluoride intake leads to dental caries whereas excess consumption leads to dental and skeletal fluorosis. Due to the increase in the prevalence of dental fluorosis, the search for biomarkers of fluoride exposure that are easy to collect and analyze has been intensified. A biomarker is an indicator of a disease or biological alteration, providing evidence for disease at a

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preventable stage.[1] Human exposure to fluoride can be monitored through the analysis of bone, enamel, hair, nails, plaque, plasma, saliva, and urine.[2-4] The most reliable indicator of exposure to fluorides is their level in urine and blood. Collection of urine and blood sample is difficult in epidemiological studies covering a large population. In a community setting, an ideal biomarker should be easily retrievable in a noninvasive manner, with measured fluoride values showing a temporal and dose–response relationship with fluoride intake.[5] Coronal hair, finger nails, and toe nails have been used as biomarkers of sub-chronic and chronic exposure to fluoride because they offer a simple and noninvasive bioassay method that is easily consented by all donors. The storage of these samples does not require any sophisticated methods and there is minimal risk of decay. The fluoride concentration in the hair and nails reflects the average fluoride intake and plasma concentration during which the particular portion of hair and nail is formed; that is, the concentration in the clipping is directly related to the average fluoride exposure that occurred during a 3-month period. There are many reports suggesting the use of nails and hair as biomarkers for fluoride exposure in humans.[6,7] The available literature is contradictory regarding the relationship between hair, fingernail, toenail, and water fluoride concentration. Moreover, none of the studies have comparatively evaluated the use of hair, fingernails, and toenails as biomarkers of sub-chronic fluoride exposure. The aim of this study was to evaluate and compare relationship between fluoride content in hair, fingernails, and toenails at different fluoride concentrations in drinking water in 12- to 17-year-old children in three villages of Nilakottai block (Thomaspuram, Bangalapatti, and Singampatti), Dindigul district, Tamil Nadu, India.

Materials and Methods

This study was designed as a population-based observational cross-sectional study after obtaining institutional ethical committee clearance (reference no. PMS/IEC/2013(b)/19). Prior to definition of the final sample, the purpose of the study and the nature of the clinical procedures were explained verbally and in writing to the prospective participants (12–17 years old) in three villages of Nilakottai block, Dindigul district, Tamil Nadu, whose drinking water fluoride concentrations were known [Table 1]. These villages were identified as geographically fluoride belt areas with high incidence of dental fluorosis.[8] This study was conducted to quantify the dental fluorosis burden among school-going adolescent age (12–17 year) group in all the three villages.[9]

Stratified random sampling was used to define the study sample. Three villages were selected from Nilakottai block, Dindigul district, and stratified into three groups based on low, intermediate, and high fluoride concentration in drinking water. All adolescent children who are lifelong residents of their respective communities were randomly selected and included in the study. A detailed questionnaire was devised comprising demographic details, known medical history or illnesses, and sources and duration of fluoride exposure (drinking water source, use of fluoridated toothpaste, frequency of intake

Table 1: Comparison of known and determined levels of fluoride in drinking water in three villages of Nilakottai block, Dindigul district, Tamil Nadu

Group	Village	Known levels of fluoride in drinking water (ppm)		Determined levels of fluoride in drinking water (ppm)	
		Mean ± SD	Range	Mean ± SD	Range
Group A	Thomaspuram	0.62 ± 0.12	0.48–0.76	0.63 ± 0.08	0.49–0.75
Group B	Bangalapatti	1.59 ± 0.28	1.23–1.88	1.63 ± 0.11	1.39–1.85
Group C	Singampatti	2.84 ± 0.35	2.48–3.24	2.92 ± 0.10	2.75–3.08

SD = standard deviation

Table 2: Demographic details of the study participants

Demographic details		Group A Thomaspuram (n)	Group B Bangalapatti (n)	Group C Singampatti (n)
Age	12–13 years	3	4	4
	13–14 years	4	6	5
	14–15 years	5	3	4
	15–16 years	4	4	3
	16–17 years	4	3	4
Gender	Male	11	8	10
	Female	9	12	10
Diet	Vegetarian	16	2	3
	Nonvegetarian	4	18	17

of fluoride-containing food and beverages, etc.) [Table 2]. Children with a history suggestive of acute or chronic involvement of the kidneys, on medication, or any illness, and those using fluoride supplements were excluded from the study. A urine test was conducted to rule out any kidney problem as patients with kidney problems are at increased susceptibility to fluoride toxicity.

Five drinking water samples were collected from different areas in the village from ground water source used for drinking purpose. The water samples were collected in clean 15 mL polypropylene centrifuge tubes without any air bubbles. The tubes were tightly sealed after collection and labeled in the field. The samples were kept in refrigerator maintained at 40°C. Fluoride analysis of drinking water samples was made by the direct method using an ion-selective electrode (Model 9609; Orion Research, Cambridge, MA), after buffering the samples with an equal volume of total ionic strength adjustment buffer (TISAB II; Thermo Electron Corp, Beverly, MA). Standards were prepared by serial dilution of a 100 parts per million (ppm) NaF stock solution (Orion).[10]

After having determined the drinking water fluoride content [Table 1] and confirming that the fluoride content in the household drinking water fells within the known range of fluoride content in drinking water of that particular area, the final sample for the study was defined[8] [Table 2].

The subjects and their parents willing to participate in the study signed an informed consent. The sample size was calculated using the formula:

$$\frac{N = 2\,S^2\,f\,(\alpha,\,\beta)}{d^2}$$

Figure 1: Collected samples

where N is the sample size, S is the standard deviation, $f(\alpha, \beta)$ the value calculated from α and β, α is the type I error/level of statistical significance, β is the type II error, and d is the clinically significant difference.

With an error of 5%, the total sample size comprised 60 (20 subjects per group; Group A [Thomaspuram], Group B [Bangalapatti], and Group C [Singampatti]) subjects in three villages of Nilakottai block willing to participate in the study with similar lifestyles and dietary patterns, representing lifelong exposure to one of three concentration ranges of fluoride in their household water. In all the three groups, the samples collected included 15–20 strands of coronal head hair (minimal length 1.5 cm) and fingernails (>1 mm length) and toenails (>1 mm length) from the 10 digits of each subject were transported in separate sealed plastic pouches [Figure 1]. The samples were brushed and rinsed on a fritted glass filter with acetone, detergent, 2N sulfuric acid, and deionized water. After drying, 100 mg aliquots were placed into centrifuge tubes, treated with concentrated sodium hydroxide solution and heated in a boiling water bath until complete solution (approximately 60 min). Cooled and neutralized with 1M hydrochloric acid, the samples were made up with deionized water to 4 mL [Figure 2A]. The samples were buffered with an equal volume of total ionic strength adjustment buffer (TISAB) [Figure 2B]. Fluoride concentration in each of the prepared solutions was estimated potentiometrically with the help of a fluoride-ion-specific electrode [Figure 2C]. Recovery of fluoride from analyzed material amounted to 100 ± 8%.[11,12] The fluoride concentration in each of the prepared solution, expressed in ppm, was statistically analyzed using one-way analysis of variance, post hoc tests (Turkey's post hoc and unpaired t test), Kruskal–Wallis tests of significance, and multivariate Pearson's correlation tests. Data were analyzed using computer software, Statistical Package for Social Sciences (SPSS), version 16.0 (IBM, Armonk, NY).

Results

The mean fluoride concentration in drinking water was 0.63 ppm in Thomaspuram, 1.63 ppm in Bangalapatti,

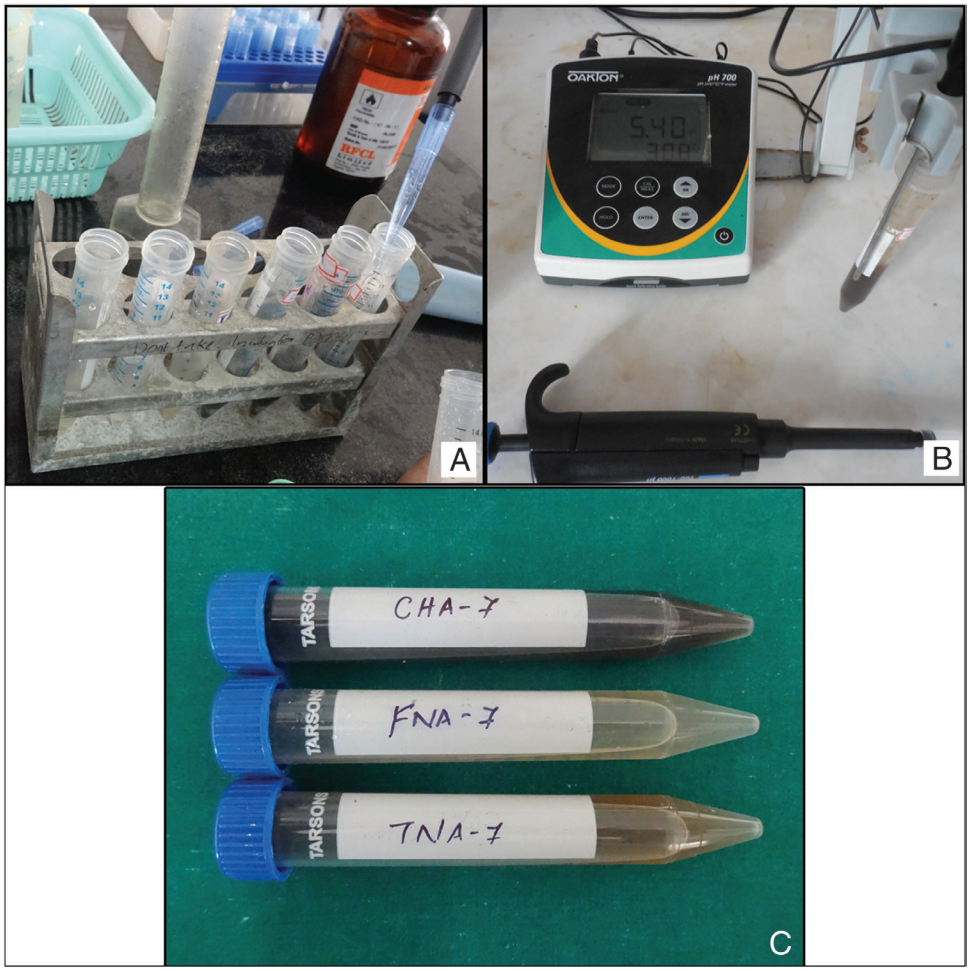

Figure 2: (A) Adding freshly prepared 1N Sodium hydroxide. (B) Adjusting the pH of prepared solution to 5.3–5.5. (C) Prepared solutions for fluoride estimation

Table 3: Descriptive statistics of fluoride content in drinking water, hair, fingernails, and toenails of inhabitants of Thomaspuram, Bangalapatti, and Singampatti

Observation	Water	Hair	Fingernails	Toenails
Fluoride content in the inhabitants of Thomaspuram				
Mean	0.63	2.84	2.99	3.13
Median	0.64	2.85	3.03	3.11
Range	0.49–0.75	2.73–2.90	2.01–3.47	2.10–4.12
SD	0.08	0.04	0.27	0.36
Fluoride content in the inhabitants of Bangalapatti				
Mean	1.63	4.67	4.94	5.10
Median	1.63	4.74	4.98	5.09
Range	1.39–1.85	3.70–5.30	3.95–5.92	4.08–6.12
SD	0.11	0.35	0.40	0.47
Fluoride content in the inhabitants of Singampatti				
Mean	2.92	6.53	6.84	7.24
Median	2.93	6.59	6.94	7.12
Range	2.75–3.08	5.56–7.61	5.92–7.96	6.01–9.53
SD	0.10	0.40	0.46	0.78

SD = standard deviation

Table 4: One-factor ANOVA with post hoc analysis of fluoride content in drinking water, hair, fingernails, and toenails of inhabitants of Thomaspuram

One-factor ANOVA				
	Mean	*n*	SD	
	0.623	20	0.0779	Water fluoride
	2.826	20	0.0859	Hair fluoride
	2.946	20	0.3369	Fingernail fluoride
	3.078	20	0.4114	Toenail fluoride
	2.368	80	1.0525	Total

ANOVA table					
Source	SS	*df*	*MS*	*F-statistic*	*P value*
Treatment	81.8802	3	27.29339	368.49	3.56E-45
Error	5.6291	76	0.07407		
Total	87.5093	79			
		P < 0.001			

Post hoc analysis					
P values for pair-wise *t* tests		Water *F-statistic*	Hair *F-statistic*	Fingernail *F-statistic*	Toenail *F-statistic*
		0.623	2.826	2.946	3.078
Water fluoride	0.623				
Hair fluoride	2.826	4.10E-39			
Fingernail fluoride	2.946	1.08E-40	0.1673		
Toenail fluoride	3.078	2.39E-42	0.0046	0.1307	
		P < 0.01		*P* < 0.05	
Tukey simultaneous comparison *t* values (*df* = 76)		Water *F-statistic*	Hair *F-statistic*	Fingernail *F-statistic*	Toenail *F-statistic*
		0.623	2.826	2.946	3.078
Water fluoride	0.623				
Hair fluoride	2.826	25.60			
Fingernail fluoride	2.946	27.00	1.39		
Toenail fluoride	3.078	28.53	2.92	1.53	
Critical values for experiment-wise error rate			*0.05*	*2.63*	
			0.01	*3.23*	

ANOVA = analysis of variance, SD = standard deviation, SS = sum of squares due to the source, MS = mean sum of squares due to the source

Table 5: One-factor ANOVA with post hoc analysis of fluoride content in drinking water, hair, fingernails and toenails of inhabitants of Bangalapatti

One-factor ANOVA				
	Mean	***n***	**SD**	
	1.631	20	0.1129	Water fluoride
	4.665	20	0.3524	Hair fluoride
	4.937	20	0.4029	Fingernail fluoride
	5.095	20	0.4682	Toenail fluoride
	4.082	80	1.4753	Total

ANOVA table					
Source	**SS**	***df***	***MS***	***F-statistic***	***P value***
Treatment	162.0897	3	54.02989	416.84	4.39E-47
Error	9.8510	76	0.12962		
Total	171.9407	79			
		P < 0.001			

Post hoc analysis					
***P* values for pair-wise *t* tests**		**Water *F-statistic***	**Hair *F-statistic***	**Fingernail *F-statistic***	**Toenail *F-statistic***
		1.631	4.665	4.937	5.095
Water fluoride	1.631				
Hair fluoride	4.665	2.65E-40			
Fingernail fluoride	4.937	6.91E-43	0.0194		
Toenail fluoride	5.095	2.62E-44	0.0003	0.1693	
		P < 0.01		*P < 0.05*	
Tukey simultaneous comparison *t* values (*df* = 76)		**Water *F-statistic***	**Hair *F-statistic***	**Fingernail *F-statistic***	**Toenail *F-statistic***
		1.631	4.665	4.937	5.095
Water fluoride	1.631				
Hair fluoride	4.665	26.65			
Fingernail fluoride	4.937	29.04	2.39		
Toenail fluoride	5.095	30.43	3.78	1.39	
Critical values for experiment-wise error rate			*0.05*	*2.63*	
			0.01	*3.23*	

ANOVA = analysis of variance, SD = standard deviation

and 2.92 ppm in Singampatti. The mean fluoride concentration in hair samples was 2.84 ppm, 4.67 ppm, and 6.53 ppm; fingernail clippings was 2.99 ppm, 4.94 ppm, and 6.84 ppm; and toenail clippings was estimated as 3.13 ppm, 5.10 ppm, and 7.24 ppm in Thomaspuram, Bangalapatti, and Singampatti residents, respectively. The mean fluoride content in the hair, fingernails, and toenails was significantly higher as compared to the mean fluoride content in the drinking water (viz., toenail fluoride > fingernail fluoride > hair fluoride) [Table 3]. The post hoc analysis revealed a statistically significant increase in the mean fluoride content in toenail clippings in Thomaspuram and both fingernail and toenail clippings in Bangalapatti and Singampatti as compared to mean fluoride content in hair [Tables 4–6]. The mean fluoride concentration (ppm) found in hair, fingernail, and toenail clippings tends to increase with increase in mean fluoride concentration in drinking water [Figure 3]. The Kruskal–Wallis test of significance for multiple independent samples showed a statistically significant difference in hair, fingernail, and toenail fluoride concentrations between the three groups [Table 7]. Multivariate Pearson's correlation tests showed a positive, strong correlation between fluoride concentration in drinking water and coronal hair, fingernails, and toenails [Table 8].

Discussion

In India, occurrence of skeletal and dental fluorosis in endemic geographical areas due to high fluoride content in drinking water is a public health problem. Therefore, it is important to monitor the effects of fluoride on these populations so that effective action can be taken to combat this problem.[13] A major obstacle in this regard is the absence of an accurate and practical method for measuring combined fluoride intake. This has led to the search for biomarkers of fluoride exposure in various body tissues.[14] Compared to other biomarkers, hair and nails have the advantage of being easily collected, stored, and transported.

Table 6: One-factor ANOVA with post hoc analysis of fluoride content in drinking water, hair, fingernails, and toenails of inhabitants of Singampatti

One-factor ANOVA					
	Mean	*n*	SD		
	2.920	20	0.0983	Water fluoride	
	6.534	20	0.4043	Hair fluoride	
	6.838	20	0.4562	Fingernail fluoride	
	7.241	20	0.7806	Toenail fluoride	
	5.883	80	1.8073	Total	
ANOVA table					
Source	**SS**	***df***	***MS***	***F-statistic***	***P value***
Treatment	239.2274	3	79.74245	321.98	4.20E-43
Error	18.8223	76	0.24766		
Total	258.0497	79			
			P < 0.001		
Post hoc analysis					
***P* values for pair-wise *t* tests**		**Water *F-statistic***	**Hair *F-statisticF***	**Fingernail *F-statistic***	**Toenail *F-statistic***
		2.920	6.534	6.838	7.241
Water fluoride	2.920				
Hair fluoride	6.534	6.21E-36			
Fingernail fluoride	6.838	2.76E-38	0.0575		
Toenail fluoride	7.241	3.35E-41	2.47E-05	0.0123	
		P < 0.01		*P* < 0.05	
Tukey simultaneous comparison *t* values (*df* = 76)		**Water *F-statistic***	**Hair *F-statistic***	**Fingernail *F-statistic***	**Toenail *F-statistic***
		2.920	6.534	6.838	7.241
Water fluoride	2.920				
Hair fluoride	6.534	22.97			
Fingernail fluoride	6.838	24.90	1.93		
Toenail fluoride	7.241	27.46	4.49	2.56	
Critical values for experiment-wise error rate			*0.05*	*2.63*	
			0.01	*3.23*	

ANOVA = analysis of variance, SD = standard deviation

Table 7: Mean fluoride content in hair, fingernails, and toenails with different levels of exposure to fluoride from drinking water

	Area			Kruskal–Wallis H value	Level of Significance*
	Thomaspuram (low)	Bangalapatti (intermediate)	Singampatti (high)		
Level of fluoride exposure from drinking water	0.63 ± 0.08 (0.49–0.75)	1.63 ± 0.11 (1.39–1.85)	2.92 ± 0.10 (2.75–3.08)		
Fluoride content in hair	2.84 ± 0.04 (2.73–2.90)	4.67 ± 0.35 (3.70–5.30)	6.53 ± 0.40 (5.56–7.61)	52.535	0.004
Fluoride content in fingernails	2.99 ± 0.27 (2.01–3.47)	4.94 ± 0.40 (3.95–5.92)	6.84 ± 0.46 (5.92–7.96)	52.416	0.002
Fluoride content in toenails	3.13 ± 0.36 (2.10–4.12)	5.10 ± 0.47 (4.08–6.12)	7.24 ± 0.78 (6.01–9.53)	51.933	0.001

Values are mean ± SD with the range in parentheses (in ppm)

*Kruskal–Wallis test of significance $P < 0.05$

The main source of fluoride intake by the subjects included in the study was household drinking water. Ground water was the main source of drinking water in all the three villages. The determined levels of fluoride in drinking water in all the areas were at par with the data published by Viswanathan *et al.*[8] in 2009. Obtained results indicated that fluoride levels in drinking water of Thomaspuram (0.63 ± 0.08 ppm) were lower than levels recommended by World Health Organization (0.7–1.2 ppm), whereas fluoride levels in drinking water of Bangalapatti (1.63 ± 0.11 ppm) and Singampatti (2.92 ± 0.10 ppm) were higher than levels recommended.

Table 8: Multivariate Pearson's correlation matrix of fluoride content in drinking water, hair, fingernails, and toenails

	Water F	Hair F	Fingernail F	Toenail F
Water F	1.000			
Hair F	0.983*	1.000		
Fingernail F	0.977*	0.992*	1.000	
Toenail F	0.963*	0.975*	0.985*	1.000

*Correlation is significant at the 0.01 level (two tailed)

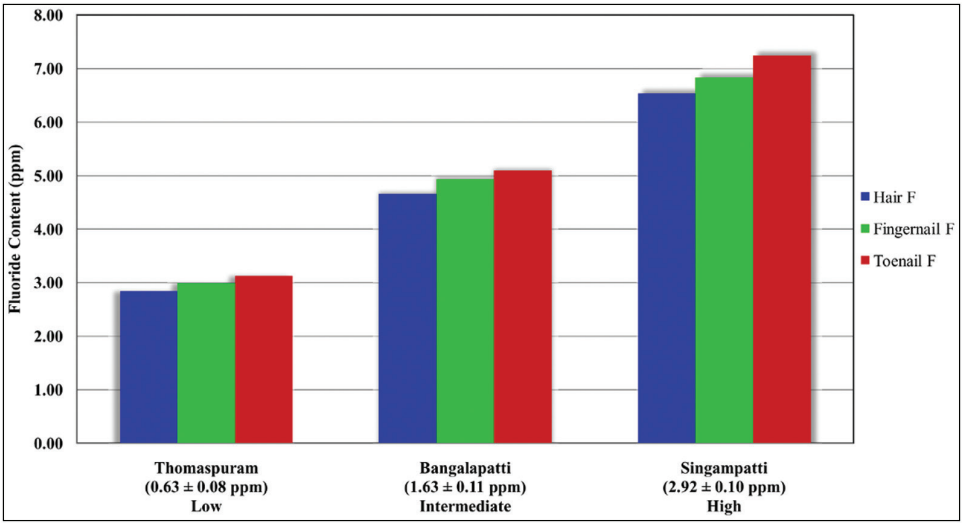

Figure 3: Mean fluoride content in hair, fingernails, and toenails at different levels of exposure to fluoride from drinking water

The method used for analyzing fluoride concentration in any biological sample should be valid, reliable, and easy to perform and incur minimum expenditure. Extensive literature available pertains to the analysis of fluoride in solution by means of ion-selective electrode/specific ion electrode. A total-ionic strength adjustment buffer (TISAB) is used to adjust the samples and standards to the same ionic strength and pH to eliminate interference in measuring the fluoride concentration.[15] In this study, alkali digestion was used for separation and concentration of fluoride in solid samples, a technique that is similar to that used by Schamschula *et al.*[11] in 1985, Czamowski and Krechniak[12] in 1990, and Parimi *et al.*[4] in 2013. Although, the recovery of fluoride from analyzed material amounted to 100 ± 8%, the usefulness of this technique is limited by its inherent inability to transfer organic fluorides into solution.

The possibility of using coronal hair and fingernails as indicators of exposure to fluoride was first described by Schamschula *et al.*[11] in 1985. The content of fluorine in hair depends on the daily intake from food, water, tea, fish (especially sea fish), and the use of fluoridated toothpastes.[16] In this study, the mean fluoride content in hair samples was 2.84 ppm, 4.67 ppm, and 6.53 ppm in Thomaspuram, Bangalapatti, and Singampatti residents, respectively. The children of Thomaspuram followed a predominantly vegetarian diet (green leafy vegetables that are rich in fluoride content); hence, high level of fluoride was obtained in the hair sample of Thomaspuram in spite of the low level in drinking water. This may be also due to seasonal variation in water intake. Mean fluoride content in the hair was significantly higher than the mean fluoride content in the drinking water in all the three populations, which may be due to seasonal variation in water intake or due to the fluoride intake from food. This is in accordance with the results published by various authors.[17,18] A contradictory report was published by Schamschula *et al.*[11] stating fluoride content in hair was lower than the fluoride content in the drinking water.

Buzalaf *et al.*[19] in 2012 reported the usefulness of fingernail fluoride concentration in public health research to identify the risk of developing dental fluorosis. In this study, the mean fluoride content in fingernail clippings was estimated as 2.99 ppm, 4.94 ppm, and 6.84 ppm in the inhabitants of Thomaspuram, Bangalapatti, and Singampatti, respectively. Mean fluoride content in the fingernail clippings was significantly higher than the mean fluoride content in the drinking water in all the three populations, which may be due to seasonal variation in water intake or due to the fluoride intake from food.

Correa Rodrigues *et al.*[21] in 2004 first reported toenails as indicators of fluoride exposure. In this study, the mean fluoride content in toenail clippings was estimated as 3.13 ppm, 5.10 ppm, and 7.24 ppm in the inhabitants of Thomaspuram, Bangalapatti, and Singampatti, respectively. Mean fluoride content in the toenail clippings was significantly higher than the mean fluoride content in the drinking water in all the three populations, which may be due to seasonal variation in water intake or due to the fluoride intake from food. The results obtained for fingernail and toenail fluoride concentration are in accordance with the results published by various authors.[20,21]

When comparing all the biomarkers of fluoride exposure, the mean fluoride concentrations in the hair samples were lower than that of fingernail clippings, in all the three villages, with statistical significance noted in only in Bangalapatti (intermediate exposure group). The mean fluoride concentrations in the hair samples were lower than that of toenail clippings, in all the three villages, with statistical significance noted in all the three villages. The fingernail fluoride concentrations were lower than those for toenails in all the three villages, with statistical significance noted in none of the three villages. Higher fluoride concentration in toenails may be linked to its faster growth rate.[21-23] The difference in the analytical techniques used in these studies help to explain the different results observed.

The most important finding of this study is the presence of a positive, strong correlation between fluoride concentration in drinking water and coronal hair, fingernails, and toenails. Thus, if fluoride intake is chronic, human hair and nails may be good predictors of plasma fluoride concentration over time. These findings are important because hair and nails from babies and young children with a risk for developing dental fluorosis can be clipped and assayed for plasma fluoride estimation. For instance, the maxillary central incisors have increased risk of developing fluorosis between ages 15 and 24 months for males and between 21 and 30 months for females. Thus, periodic hair and nail analysis starting by the age 1–2 years will have a practical prophylactic value to prevent dental fluorosis of the early erupting permanent incisors.[24] The salient advantages of using hair and nails as biomarkers of fluoride exposure include ample sample availability; ease of collection, storage, and transport; and user-friendly technique for the measurement of fluoride. The preliminary data of this study suggest that among the biomarkers studied, toenails may be regarded as the most suitable biomarker for measuring chronic/sub-chronic fluoride exposure from the drinking water. However, extrapolation of the study results is limited by the following shortcomings:

(1) Relevant confounding factors such as age, gender, and growth rate of hair/nails were not taken into account.
(2) Relatively small sample size.
(3) Sources of fluoride exposure other than drinking water were not taken into account.
(4) Inability of alkali digestion to quantitatively transfer organic fluorides into solution.

Conclusion

The mean fluoride concentration in coronal hair, fingernail, and toenail clippings is higher when compared to mean fluoride concentration in drinking water, in all the three groups studied and was the highest for toenail clippings, followed by fingernail clippings and coronal hair. Fluoride concentration tends to increase with increase in drinking water fluoride concentration. Considering the ample sample availability and the highest fluoride content, our data suggest toenails may be regarded as the most suitable biomarker for measuring chronic/sub-chronic fluoride exposure from the drinking water.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Author contributions

Dr. Mathew V. and Dr. Jyothi S. Issac were involved in the study conception and data collection. Dr. Angel M. Joseph and Dr. Ashwin Joseph were involved in data acquisition and analysis. Dr. Dhanya John and Dr. Vinutha K. Varadharaju were involved in data interpretation and manuscript writing. All the authors have approved the final version of this manuscript for publication.

Ethical policy and institutional review board statement

This study was conducted after obtaining Institutional Ethical Committee Clearance (Reference no. PMS/

IEC/2013(b)/19 dated 19/11/2013). All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki (1964).

Patient declaration of consent

The children and their parents willing to participate in the study signed a written informed consent. The patients were informed that the data collected will be used only for research and educational purposes.

Data availability statement

The data set used in the current study is available on request to the corresponding author only (Dr. Mathew V.; mathewjustincase@gmail.com).

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