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Determination of fluoride in drinking water and in urine of adolescents living in three counties in Northern Chihuahua Mexico using a fluoride ion selective electrode

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Abstract

This study was carried out to determine fluoride in drinking water and in urine of adolescents, ages 15–20 years, living in Northern Chihuahua Mexico. Participants are from a cross-sectional study on health effects of chronic fluoride exposure from drinking water. A total of 201 participants (106 female and 95 male) in the study were recruited from three counties. Samples of drinking water of each county were collected and analyzed using the U.S. EPA Fluoride Ion-Selective Method. Statistically significant difference of fluoride content in water was found among the three counties of recruitment (Cd. Juarez; 0.3 mg/L, Samalayuca, 1.0 mg/L, and Villa Ahumada, 5.3 mg/L). Fluoride content in wells and tap water samples of Villa Ahumada ranged from 5.0 to 5.7 mg/L. Fluoride content of these samples was above the level permitted by Mexican regulations. The fluoride content in bottled water obtained from local stores in Villa Ahumada ranged from 0.3 to 3.7 mg/l.

Fluoride in urine samples of each participant was also analyzed using the U.S. EPA Ion-Selective Method. The mean fluoride urine concentration (reported in mg/g creatinine) in adolescents living in these counties was 0.792 ± 0.39, 1.33 ± 0.67 and 2.22 ± 1.16 (Cd. Juarez, Samalayuca and Villa Ahumada), respectively. The high fluoride urinary levels found in participants from Villa Ahumada may be associated to the high fluoride level (5.3 mg/L) in dinking water.

The accuracy of measurements was assessed with reference materials in water and in urine. Mean fluoride recovery was 99.0% and 99.6% in water and in urine, respectively. The levels obtained were within the assayed 5% confidence levels.

Keywords: Fluoride; Urinary fluoride; Fluoride drinking water; Ion selective methodf

1. Introduction

Fluoride (F) is a ubiquitous element present in soil and water in low concentrations. Fluoride may represent a concern to environmental and occupational health when its presence in the environment increases due to natural or anthropogenic sources [1]. Fluoride exposure via drinking water is an endemic problem in several parts of the world such as China, India, Africa and Mexico [2–6].

The effects of fluoride on health are multiple. Some are characterized by mineralization changes in the calcified tissues which results in mottling of the teeth or dental fluorosis, and skeletal deformities [7,20]. Metabolic changes have been reported on soft tissues such as the thyroid, reproductive organs, brain, liver and kidneys. In the thyroid gland, fluoride causes an increase in the concentration of thyroid stimulating hormone (TSH) and a decrease in the concentration of T3 and T4 hormones, rising hypothyroidism in some populations [8–10]. Reproductive effects have been reported in humans [11]. In addition, in the rat brain fluoride causes alterations of nicotinic acetylcholine receptors (nAChRs). These receptors are located in the cellular

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membrane of neurons and play an important role in cognitive processes such as learning and memory. Additionally, studies in China revealed that the levels of mental work capacity and the Intelligence Quotient of children who were born and raised in an area with endemic fluorosis were found to be lower than normal [12].

In other animal studies, cell alterations were found at the biochemical level in kidneys, liver, adrenal glands and reproductive organs of rats [13,14].

The toxic effects of fluoride in humans are most evident in regions where levels of fluoride are high. The purpose of this investigation was to measure and compare urinary fluoride levels in adolescents participating in a cross-sectional study of the health effects of chronic fluoride exposure from drinking water. We used an electrochemical ion-selective method to determine fluoride in drinking water and urine samples. We are reporting fluoride levels in water from three different counties in Northern Mexico as well as urinary fluoride from participants from each county. Our results are reported herein.

2. Materials and methods

2.1. Subjects

A cross-sectional study was conducted in three counties in Northern Mexico (Cd. Juarez, Samalayuca and Villa Ahumada) located in the State of Chihuahua. Chihuahua State is located south of the US State of Texas. El Paso, Texas and Cd. Juarez, Chihuahua form the largest border community on the US–Mexico border. The study received approval from the Institutional Review Board from the University of Texas at El Paso and from the Health Department of each participating county. All participants and their parents signed an informed consent form. A total of 201 middle and high school students, ages 15–20 years, participated in the study. Seventy participants (32 boys and 38 girls) were recruited from Villa Ahumada, Chihuahua. Villa Ahumada is a county known by its high fluoride content in drinking water (5.35 mg/L). A second group of participants was recruited from Samalayuca (34 boys and 31 girls) where the level of fluoride in drinking water has been reported to be around 1.0 mg/L. A third group consisted of 66 participants (29 boys and 37 girls) was recruited from Cd. Juarez where the levels of fluoride in drinking water were around 0.3 mg/L. Each participant in the study donated the first voided urine sample.

2.2. Chemicals

Commercial fluoride standard was purchased from Orion (Cat. No. 040908). The Standard reference material for freeze-dried urine (SRM2671a), certified for fluoride, was supplied by the National Institute of Standards and Technology (NIST). Both sets of standards were diluted using TISAB (Total Ionic Strength Adjustment Buffer Orion 940911). Stock solutions of fluoride, containing 100 mg/L were used for preparation of the standards. Standard Reference Material (2671a) Fluoride in freeze-dried urine, trace elements in water, SRM 1643d were obtained from the U.S. National Institute of Standards and Technology (Gaithersburg, MD) and were used to validate the method.

2.3. Sampling

2.3.1. Water

Tap water samples of 500 mL each were collected (in triplicate) in clean fluoride polyethylene plastic bottles and directly from home faucets. Additionally, water samples were taken from wells or storage tanks that were used to collect the water before distribution to homes. Samples were obtained directly from the water pump after water was allowed to run for at least 15 min and were stored at 4 °C until they were analyzed. Water samples from other sources such as wells and surface reservoirs, were collected in a similar manner as the tap water samples. Trace grade nitric acid was added in order to preserve the samples for future analysis. In order to determine if there were seasonal variations in fluoride concentrations, water samples were obtained three times during the year at intervals of four months.

2.3.2. Urine

The first voided urine samples were collected in polyethylene bottles containing 0.2 g Ethylen Diamine Tetra Acetic Acid (EDTA), transported and stored in dry ice for no more than 12 h. The samples were then stored at −20 °C until the analysis were performed. Fluoride and creatinine concentrations were determined in these samples. Creatinine was determined according to Artiss et al. [16].

2.4. Electrochemical analyses

2.4.1. Fluoride in water

Fluoride concentration was determined electrochemically, using the U.S. EPA ion selective method (Method 340.2). This method is applicable to the measurement of fluoride in drinking water, surface and saline waters and domestic and industrial wastes in a range of concentration from 0.1 up to 1000 mg/L [15]. A detailed explanation of this analytical method has been reported elsewhere [17]. The electrode used was an Orion 96-09 fluoride electrode, which was coupled to an Orion 420A Electrometer. Standards (0.1–10 mg/L) were prepared from a stock solution (100 mg/L) of sodium fluoride. Three replicates of each water sample were analyzed. For quality control purposes, the standard reference water (SRM 1643d) was analyzed together with the collected water samples.

Bottled water in Villa Ahumada is processed in local companies. Samples of bottled water obtained at local stores were also analyzed for fluoride content.

Table 1
Fluoride levels in wells in Villa Ahumada

Sample location	Fluoride concentration (mg/L)
Well No. 2	5.0±0.26
Well No. 3	5.7±0.17

2.4.2. Fluoride in urine

In order to analyze fluoride in urine, the National Institute for Occupational Safety and Health (NIOSH) method for fluoride in urine was used [18]. The fluoride concentration in the urine samples was determined directly after dilution with equal volumes of TISAB solution (pH=5.2). Then, fluoride was quantified using a fluoride ion-specific electrode Orion 420A Electrode, as previously described. Quality control for fluoride analysis included the analysis of freeze-dried urine standard references material (SRM 2671a) concurrently with urine samples from individuals.

2.5. Quality control

As previously stated, the accuracy of measurements was assessed with reference materials in water (SRM2671a) and in urine (SRM1643d NIST Gaithersburg, MD). Mean fluoride recovery was 99.0% from water and 99.6% from urine. These levels were within the assayed 5% confidence levels.

2.6. Statistical analysis

Statistical analysis was performed using ANOVA and Student's t-test. Significance level was fixed at 0.05 and calculated with Minitab software. The significance of differences between urinary fluoride concentrations was analyzed using Duncan's test.

3. Results and discussion

Table 1 shows the number of wells and their fluoride concentration. Villa Ahumada County has only 2 wells and fluoride concentration for each one was 5.0±0.26 and 5.7±0.17 mg/L, respectively. Bottled water is used as an alternative source of drinking water and its use may be also a significant source of fluoride. Table 2 displays the fluoride content of two different brands of bottled water locally processed in Villa Ahumada. Bottled water San Juan had a fluoride concentration of 0.2±0.29 mg/L and the fluoride concentration in the Superior brand was 3.7±0.21 mg/L. This brand has a fluoride concentration above the accepted level in the Mexican regulation for bottled water (0.7 mg/L) [20].

Table 2
Fluoride levels in bottled water from Villa Ahumada

Bottled water	Fluoride concentration (mg/L)
San Juan	0.2±0.29
Superior	3.7±0.21

Table 3
Fluoride concentration in drinking water and urine of adolescents in Villa Ahumada, Samalayuca and Cd. Juarez

County	*n*	Water, mg/L (mean±SD)	*n*	Urine, mg/g creatinine (mean±SD)
Cd. Juarez	2	0.250±0.707	66	0.792±0.39
Samalayuca	1	0.950±0.212	65	1.333±0.67[a]
Villa Ahumada	2	5.350±0.495	70	2.220±1.16[a,b]

Urinary fluoride was adjusted to creatinine.
[a] $p<0.05$ versus Cd. Juarez.
[b] $p<0.05$ versus Samalayuca. Duncan's test.

In the summer the temperature ranges in Villa Ahumada from 32 to 37 °C, thus a higher ingestion of water can be expected. According to recommendations from the World Health Organization, the optimal concentration in drinking water should be 0.5 to 0.7 mg/L in regions with extreme climates [21]. Conversely, the Mexican national standard for drinking water is 1.5 mg/L [19]. Another problem for the people living in Villa Ahumada is the availability of only one source of water and as observed before this source contains high levels of fluoride. Only a small portion of the population has access to bottled water. As a result, the population in Villa Ahumada is chronically exposed to high levels of fluoride from drinking water. Several studies have indicated a possible correlation between arsenic and fluoride levels in drinking water. Del Razo found a correlation between fluoride and arsenic concentration in an area of northern Mexico [22]. In the present study we also analyzed the concentration of arsenic drinking water of Villa Ahumada and found no arsenic in the drinking water.

Urinary fluoride concentrations in participants from Cd. Juarez, Samalayuca and Villa Ahumada are displayed in Table 3. Participants in the study were between 15 and 20 years old, with similar age distribution between Cd. Juarez and Villa Ahumada. However individuals from Samalayuca were slightly younger than those from Cd. Juarez and Villa Ahumada. When compared to participants from Cd. Juarez, the fluoride concentration in urine showed a significant difference of 2- and 3-fold in individuals from Samalayuca

Table 4
Urinary fluoride concentrations of adolescents in Villa Ahumada, Samalayuca and Cd. Juarez

County	Sex	*n*	Mean mg/g creatinine	SD	Min	Max
Villa Ahumada	M	32	2.002[a]	0.728	0.867	3.804
Villa Ahumada	F	38	2.409[a]	1.411	0.976	7.061
Samalayuca	M	34	1.309[a]	0.552	0.502	3.223
Samalayuca	F	31	1.337[a]	0.7802	0.574	4.235
Cd. Juarez	M	37	0.872	0.446	0.242	1.300
Cd. Juarez	F	29	0.691	0.274	0.182	2.167

Urinary fluoride was adjusted to creatinine.
[a] Significant ($p<0.001$).

and Villa Ahumada. The mean urine fluoride concentration in participants from Cd. Juarez was 0.792±0.39 mg /g creatinine. This fluoride level has usually been reported in people not exposed to fluoride [23]. Participants living in Samalayuca reported a mean urinary fluoride of 1.388±0.75 mg/g creatinine. This level has usually been found in people not exposed to excessive amounts of fluoride [23]. The mean urine fluoride concentration of participants from Villa Ahumada was significantly higher (2.22±1.16 mg/g creatinine; $p<0.05$) when compared to the urine fluoride levels reported from Cd. Juarez. This high fluoride urine concentration may be associated to the high fluoride content in drinking water in Villa Ahumada. As indicated in Table 4, urinary fluoride concentrations in girls did not exhibit a difference when compared to the urinary fluoride concentration in boys. No age dependent differences were found in adolescents in any of the cities.

4. Conclusions

Chronic exposure to high levels of fluoride has been associated to multiple health problems. It is generally accepted that fluoride in urine is the best indicator of environmental or occupational exposure to fluoride. Urine samples are easily obtained and more than 90% of ingested or inhaled fluoride is excreted in urine. The high levels of fluoride in urine found in participants from Villa Ahumada may be linked to a chronic exposure of fluoride from drinking water. Analysis of drinking water from the different counties in this study showed that fluoride concentration in drinking water from Villa Ahumada was 3.5 times above the Official Mexican Norm. The city of Villa Ahumada has only one source of water and the entire population is exposed to fluoride. Chronic exposure to high concentrations of fluoride in this population represents a concern due to possible health effects from their long-term exposure. It is assumed that this population is stable (resulting in long-term exposure) and alternative water sources (e.g., bottled water) are not widely utilized. Thus, this population offers the opportunity to examine the fluoride exposure and its association with long-term health effects.

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