Original Article

Dental fluorosis, fluoride in urine, and nutritional status in adolescent students living in the rural areas of Guanajuato, Mexico

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Abstract

Objective: The aim of this study was to assess urine fluoride concentration, nutritional status, and dental fluorosis in adolescent students living in the rural areas of Guanajuato, Mexico. **Materials and Methods:** A cross-sectional study was conducted including participants aged 11–20 years. The presence and severity of dental fluorosis was registered according to the Thylstrup and Fejerskov index (TFI) criteria. Anthropometric measures were also recorded. Urine sample of the first morning spot was recollected to assess urine fluoride concentration by using the potentiometric method with an ion-selective electrode. Water samples were also recollected and analyzed. Bivariate tests were performed to compare urine fluoride concentration according to different variables such as sex, body mass index, and TFI. Nonparametric tests were used. A logistic regression model was performed (SPSS® 21.0). **Results:** This study included 307 participants with a mean age of 15.6 ± 1.6; 62.5% of the participants showed normal weight. A total of 91.9% of the participants had dental fluorosis, and 61.6% had TFI > 4. Mean fluoride content in urine ranged between 0.5 and 6.65 mg/L, with a mean of 1.27 ± 1.2 mg/L. Underweight children showed greater urine fluoride concentration. The increment of urine fluoride was a related (OR = 1.40) to having severe dental fluorosis. **Conclusions:** Most of the studied population had moderate or severe dental fluorosis. Urine fluoride concentration was related to fluorosis severity and nutritional status. Underweight children showed greater urine fluoride concentration as well as severe dental fluorosis.

Key words: *Dental fluorosis, nutritional status, urine fluoride*

INTRODUCTION

Fluoride intake in appropriate concentrations prevents the formation of dental caries. However, exposure to high concentrations of fluoride can generate several alterations. Chronic fluoride poisoning is a global health problem that occurs endemically in areas where the fluoride content in water is above the optimal level. World Health Organization's (WHO) international standards of 1958 and 1963 referred to fluoride for drinking water, claiming that consumption of water with fluoride concentrations above 1.0–1.5 mg/L can result in pathological changes in teeth causing dental fluorosis (DF), which is characterized by light yellow to brown–black horizontal lines on the teeth surface

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and chipped off edges. High concentrations of fluoride can also produce long-term bone damage in children and adults such as skeletal fluorosis.[1] Even intelligence quotient (IQ) has been associated with fluoride exposure.[2]

Traditionally, dental fluorosis has been connected with a higher intake of fluoride coming from drinking water, which may contain high fluoride concentrations, especially in groundwater of areas of volcanic rocks. These high-risk areas are mostly located in arid and semi-arid regions that are characterized by a rapid rate of chemical weathering of geological materials.[3] According to the WHO, permissible fluoride concentration limit in drinking water is 1.0 mg/L,[4] and Mexican normativity stipulates a limit of 1.5 mg/L.[5] Nonetheless, in Mexico, there are some areas that have high amounts of fluoride in water, mainly in the states of the north and center of the country, most notably in Chihuahua,[6] Durango,[7] Aguascalientes, and Guanajuato.[8]

Fluoride ingested remains for a long time in the human body, however, approximately 80% of fluoride entering the body is excreted mainly through urine; the rest of it is absorbed into body tissues from where it is released very slowly.[9] Excreted fluoride can be monitored by biomarkers of fluoride, which are values that serve to identify deficient or excessive consumption and bioavailability of fluoride in the body. WHO defines different fluoride biomarkers; current (urine, plasma, and saliva), recent (nails and hair), and historical biomarkers (bones and teeth).[10] Urine fluoride concentration among the biomarkers of fluoride exposure is generally accepted as the best indicator of fluoride exposure[11] because it can be recollected noninvasively and systematically reflects the burden of fluoride exposure from drinking water. Hence, special attention has been given to it as a biomarker, and is used as an indirect indicator of fluoride intake.

Official Mexican Norms (NOM-013-SSA2-1994) stipulates,[12] periodically monitoring urine fluoride concentration has been stipulated; nonetheless, there is scarce data regarding DF prevalence and urinary fluoride excretion in adolescents living in rural communities with no central water supplies and where concentrations of fluoride may be above optimal. This descriptive study aimed to assess urinary fluoride concentration in a community Where water supplies contain higher amounts of fluoride than recommended.

MATERIALS AND METHODS

This cross-sectional study was conducted in an endemic fluorosis area in Guanajuato State where natural high concentrations of fluoride in groundwater and endemic dental fluorosis have been reported.[13] Fluoride concentration in this area ranges from 0 to 16 mg/L, averaging 1.2 mg/L. Areas with the highest concentration of fluoride are located toward the northwest between San Felipe, San Luis de la Paz, and Dolores Hidalgo.[8]

Participants were individuals aged 11–20 years who were born and had resided in the area since their birth. Those with orthodontic appliances were excluded. A questionnaire was administered to all the participants at the time of admission to collect demographic data. Diagnosis of dental fluorosis was performed by clinical examination using the Thylstrup–Fejerskov Index (TFI),[14] which was selected because of its accuracy to identify DF severity. One trained examiner performed clinical evaluations; previously intraexaminer reliability was assessed using the Kappa test (Kappa value = 0.82). Anthropometric measures such as weight and height were registered, and using these data body mass index (BMI) was calculated. Percentage of body fat was also assessed by the bioimpedance method obtained by using a Tanita scale SC240.

Early morning spot urine sample were recollected in polyethylene containers and stored at −20°C until analysis. Urine fluoride (UF) concentrations were determined using an electronic meter (Orion 720A) and a fluoride-specific ion electrode, which was calibrated with fresh, serially diluted standard solutions. During the measurement, ionic strength buffer solution was added to each sample for analysis. Water samples were also recollected and analyzed.

Written informed consent was obtained from all the participants or by their legal guardians in case they were minors. All data were managed to ensure the protection of individual rights and maintaining confidentiality. This study was approved by the Committee of Ethics the National Autonomous University of Mexico, ENES León.

Descriptive analysis of the data (arithmetic mean, standard deviation and percentages) were obtained, bivariate analyses were performed to compare variables, and then a logistic regression model was created. Population was divided into two groups according to the presence or absence of severe fluorosis (TFI < 6 vs TFI > 6). Data was processed using SPSS version 21 for Windows (Statistical Package for the Social Sciences, SPSS Inc. Chicago, Illinois, USA).

RESULTS

A total of 307 participants were included; 59.9% (n = 184) were females and 40.1% (n = 123) were males. Mean age was 15.6 ± 1.6 years. Fluoride content in urine ranged between 0.5 and 6.65 mg/L, with a mean of 1.27 ± 1.2 mg/L [Table 1]. Fluoride concentration in water was 4.42 ppm.

Most of the population (62.5%) showed normal weight; 21.5% were underweight, 11.1% were overweight, and 4.9% were obese. DF was present in 91.9% of the participants, of which 61.6% were (TFI > 4) moderate or severe cases, as observed in Table 2. Teeth more frequently affected were premolars and those less affected were central inferior incisors.

Bivariate tests were performed to compare UF concentration according to different variables such as sex, BMI, and TFI. According to the Kolmogorov–Smirnov test, the distribution of data was not normal and hence nonparametric tests were used. No differences in UF concentrations among girls and boys were observed (Mann–Whitney U test = 10589.50, P = 0.335).

Regarding BMI and UF, significant differences were found when using the Kruskal–Wallis test (Chi square test = 16.200; P = 0.003). Underweight children showed greater UF concentration, as show in Table 3. No difference in the prevalence of DF according to nutritional status was observed (Chi square test = 29.746; P = 0.326). Nonetheless, significant differences (Chi square test = 11.22; P = 0.011) were observed when comparing the prevalence of severe DF (TF ≥ 6) and nutritional status; 42% of the children having TFI ≥ 6 were underweight while of those having TFI < 6 18.6% were underweight, as shown in Table 3.

Table 1: Descriptive data about age and urine fluoride concentration by sex

	n	Min	Max	Mean	SD
Age					
Female	184	11.0	19.0	15.5	1.68
Male	123	11.0	20.0	15.8	1.64
Total	307	11.0	20.0	15.6	1.6
Urine fluoride (ppm)					
Female	184	0.05	6.65	1.23	1.15
Male	123	0.05	5.87	1.41	1.34
Total	307	0.05	6.65	1.26	1.20

Table 2: Nutritional status and dental fluorosis severity by sex

Nutritional status				Dental Fluorosis severity			
BMI *	Females *n* (%)	Males *n* (%)	Total *n* (%)	TFI	Females *n* (%)	Males *n* (%)	Total *n* (%)
Underweight	41 (22.3)	25 (20.3)	66 (21.5)	0	13 (7.1)	12 (9.8)	25 (8.1)
Normal	111 (60.3)	81 (65.9)	192 (62.5)	1	15 (8.2)	11 (8.9)	26 (8.5)
Overweight	21 (11.4)	13 (10.6)	34 (11.1)	2	18 (9.8)	10 (8.1)	28 (9.1)
Obesity	11 (8.0)	4 (3.3)	15 (4.9)	3	25 (13.6)	14 (11.4)	39 (12.7)
Total	184 (100)	123 (100)	307 (100)	4	19 (10.3)	8 (6.5)	27 (8.8)
				5	36 (19.6)	29 (23.6)	65 (21.2)
				6	30 (16.3)	29 (23.6)	59 (19.2)
				7	24 (13.0)	5 (4.1)	29 (9.4)
				8	3 (1.6)	4 (3.3)	7 (2.3)
				9	1 (0.5)	1 (0.8)	2 (0.7)

BMI=Body mass index, TFI=Thylstrup–Fejerskov index

Table 3: Urine fluoride concentration and dental severe fluorosis according to nutritional status

	n	Urine fluoride concentration				Severe dental fluorosis	
		Min	Max	Mean	SD	TFI <6 *n* (%)	TFI ≥6 *n* (%)
Underweight	67	0.09	6.01	1.76	1.57	50 (18.6)	16 (42.1)
Normal	190	0.05	6.65	1.18	1.11	175 (65.1)	17 (44.7)
Overweight	35	0.09	3.72	1.08	0.88	30 (11.2)	4 (10.5)
Obesity	15	0.30	4.28	1.29	1.22	14 (5.2)	1 (2.6)
Kruskal-Wallis test: Chi=7.98; *P*=0.046						Chi square test=11.22 *P*=0.011	

TFI=Thylstrup–Fejerskov index

Positive correlation was observed among urine fluoride concentration and fluorosis severity (Rho Spearman = 0.224; $P < 0.001$). To perform bivariate analysis to identify the association between UF concentration and dental fluorosis severity status, this last variable was recoded in order to reduce it into 5 categories; significant differences were noted (Kruskal–Wallis test = 16.200; $P = 0.003$). Children having dental fluorosis TFI = 7–9 had greater concentration of fluoride in urine [Table 4].

Table 4: Urine fluoride concentration according to dental fluorosis severity

TFI	*n*	Min	Max	Mean	SD
0	25	0.09	4.91	1.02	1.04
1-2	54	0.07	4.86	1.03	0.93
3-4	66	0.09	3.80	1.04	0.87
5-6	124	0.05	6.01	1.39	1.21
7-9	38	0.05	6.65	2.02	1.88

TFI=Thylstrup-Fejerskov index; Kruskal-Wallis test=16.200; P=0.003

A logistic model was constructed to explore the association between severe dental fluorosis (TFI < 6 vs TFI ≥ 6) and fluoride concentration in urine, controlled by age, sex, nutritional status, (BMI) and body fat percentage. It was observed that sex, age, body fat, and UF concentration were variables that were statistically significant in this model. Male gender (OR = 0.127), increment of percentage in body fat (OR = 0.875) and age (OR = 0.640) were protective factors. The increment of UF (OR = 1.40) concentration was a related risk to have severe DF [Table 5].

Table 5: Logistic model using as dependent variable the presence or absence of severe dental TFI ≥6

	Wald	*P*	OR	95% (CI)	
				Lower	Upper
Sex (male)	11.421	0.001	0.127	0.038	0.420
Age	9.032	0.003	0.640	0.479	0.856
Underweight	1.680	0.195	0.126	0.005	2.892
Normal weight	1.681	0.195	0.174	0.012	2.445
Overweight	0.000	1.000	1.001	0.088	11.318
Obesity	5.040	0.169	-	-	-
Body fat	8.125	0.004	0.875	0.798	0.959
Urine fluoride (ppm)	7.088	0.008	1.407	1.094	1.808

DISCUSSION

DF was present in 91.9% of the participants in our study. In San Luis Potosí State, in an area where fluoride level was similar (4.54 ppm), DF was present in all participants, of which 95% had severe cases.[15] In a Mexican community where fluoride concentration was lower (1.9 ppm) than that found in our study area, DF prevalence was 98%, being severe in 47%.[16] Rodriguez *et al*.[17] in Chihuahua, Mexico found DF in 85.5% of the population when fluoride in water was 3.00–5.99 ppm. Ambient temperature, meters above mean sea level,[18] risk practices as direct consumption of boiled water,[19] and preparing food with tap water may explain the differences in DF prevalence, even when concentration of fluoride in water are similar.

Range of fluoride content in urine was similar to that reported in other Mexican children population, aged 6-12, authors reported a range of UF concentration of 11.1 to 5.9 mg/L; with a mean of UF content of (3.14 ± 1.09mg/L.[15,17] In an Indian group aged 6 to 18, the highest UF concentration recorded was 17 mg/L when fluoride water concentration was of 2.11 mg/l.[2] In other study in Indian population, in individuals aged 11–16 years, fluoride concentration found in urine samples ranged from 0.90 to 3.25 mg/L with an average of 2.35 mg/L.[20] These variations might derived not only from variation on water fluoride content but from different use and consumption practices of water and other sources of fluoride among populations.

We observed a positive correlation among UF concentration and fluorosis severity; these results are congruent with those reported by Jarquín-Yañez *et al*.[15] who found that urine fluoride concentration was more elevated in those showing greater fluorosis severity. They reported levels of 2.66 (0.89) in children with TFI of 4–5, 3.11 (1.06) in the TFI of 6–7, and 3.75 (1.10) ppm in TFI of 8–9. Nonetheless, Heintze *et al*.[21] reported no correlation between UF levels and DF, however, that study was conducted in low-fluoride areas, which may be one of the causes of these different results.

Age was significantly associated with DF, suggesting that age is a protective factor; nonetheless, this does not imply that DF decreases as age. Increases, but it is not possible because DF is irreversible. Rather, these results might suggest that the problem is exacerbating, probably reflecting an increase in the consumption of fluoride in new generations that could come from higher concentration of fluoride in the water of the zone, as suggested by some authors who reported that as the depth of water extraction increases the concentration of this element also increases, raising the risk of developing DF. Pontigo-Loyola *et al*.[22] also reported similar results showing that children aged 12 years had greater chance to have fluorosis compared to those aged 15 years. The epidemiology system for oral diseases (SIVEPAB)[23] in 2010 also proposed an increase in DF prevalence, especially in the younger age groups (under 25 years). Similarly, this has been observed in other countries, for instance, in a study performed in rural areas of Brazil was observed that children between 10 and 12 years and those between 13 and 15 years of age had greater chance of having severe dental fluorosis in comparison with the younger children and individuals aged between 16 and 22 years.[24] These results contrast to the study by Rwenyonyi *et al*.[25] who found significant increase in the severity of fluorosis with increasing age in a community with high concentration of fluoride in water. On the other hand, it was found that underweight children showed greater urine fluoride concentration. Not many studies exist reporting this association; one study by Das and Mandal.[2] reported that overall fluoride exposure dose has negative correlation with BMI ($r = -0.083$), which would be similar to that found in our population.

We found no difference in the prevalence of DF according to nutritional status, nonetheless significant differences were observed when comparing the prevalence of severe DF, with 42% of the children having TFI ≥ 6 being underweight. Some epidemiological studies have indicated that manifestations of fluorosis are more marked among communities exposed to chronic malnutrition.[26] Choubisa *et al*.[27] showed that, among participants with poor nutrition, the prevalence of DF increased to 61.6% And skeletal fluorosis increased to 23.9%. Furthermore in a study by Irigoyen *et al*.[28] in Mexico, association between malnutrition and defects in the enamel were observed in an area where the water contained 2.7 mg/L of fluoride. Similarly Pérez-Pérez *et al*. observed statistically significant differences in height for age, and reported a OR = 2.66 for children with short stature to present fluorosis TF ≥ 4.[29] In addition, it was observed that males have less risk to present severe DF than girls, these results are congruent to those reported by Ramezani *et al*.[30] who observed greater prevalence of severe fluorosis in girls (65%) than in boys (34.2%). Nonetheless, in Pakistan, boys were more affected.[31]

Fluoride concentration in the water of this population exceeds the permissible limits for human consumption (0.7–1.5mg/L); owing to the potential of adverse health effects of this situation, immediate actions are needed to reduce the exposure, thus diminishing adverse health effects in this population as DF, which is an irreversible alteration. Hence, actions Taken would not only reduce dental fluorosis in future generations but also would prevent the prevalence or severity of other alterations that excessive consumption of fluoride can cause.

CONCLUSIONS

Most of the studied population had DF and most of the cases were moderate or severe. Positive correlation between fluorosis severity and UF concentration was observed, also nutritional. Nutritional status was associated with severe DF. Water fluoride concentration of this population exceeds the limits stipulated by national and international norms. Given the potential of adverse health effects that this may produce, immediate actions are needed to reduce the exposure to this element.

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Conflicts of interest

There are no conflicts of interest.

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