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Full Length Research Paper

Fluorides and dental fluorosis in students from Tula de Allende Hidalgo, Mexico

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This study correlates and presents the findings of the concentrations of fluorides – an important contaminant of ground water – present in two specific locations: The Ex-Hacienda well, which supplies the community of San Miguel Vindhó; and the Manzanitas-I well, which supplies La Malinche. The study concentrates on the prevalence and severity of dental fluorosis in students between 12 to 15 years old, who were born and live in these communities. The study was designed to be cross sectional and comparative. During the period of April 2008 to April 2009, the study determines the measurement of fluorides (F-) by using the Spectrophotometric method. A clinical exam was undertaken to observe the prevalence of dental fluorosis according to the Modified Dean Index. To determine the severity of fluorosis, the Community Fluorosis Index (CFI) was calculated. The concentrations of fluorides in the Ex-Hacienda well had a mean of 1.41 mg/L (CI 95%, 1.19, 1.64) with a fluorosis prevalence of 85% and a CFI of 1.6. Meanwhile, in the Manzanitas I well, the mean value was 0.62 mg/L (CI 95%, 0.53, 0.70) with a prevalence of 4% and CFI of 0.2. There is a significant difference between the two populations with a paired t test result of p < 0.005. There is a positive correlation between the concentration of fluorides of the Ex Hacienda well and the development of fluorosis in San Miguel Vindhó, and the study concludes that these results constitute a hazard for students' health.

Key words: Fluorides, dental fluorosis, modified dean index, community fluorosis index, Tula.

INTRODUCTION

Fluorides are universally present in the earth's surface and in water, from trace concentration to ppm (WHO, 2002). The erosion of alkaline in sedimentary rocks, sand, minerals and sediments with fluorospar, criolite, fluorapatite and hidroxiapatite, liberates fluorides to the ground and underground water (Misra and Mishra, 2006). Furthermore, volcanic activity and thermal waters can contribute to the increase in the levels of F- (Msonda et al., 2007).

There is a global epidemic problem of underground water that is used for public consumption being contaminated with F- (Amini et al., 2008). Hydrofluorosis has been reported in India (Misra and Mishra, 2006; Choubisa, 2001), China (Ando et al., 2001), Pakistan (Farooqi et al., 2007), Tanzania (Kaseva, 2006), Brazil (Casagranda et al., 2007), Kenya (Gaciri and Davies, 1993), Malawi (Msonda et al., 2007), Taiwan (Lung et al., 2008) and other countries like México (Secretariat of Health et al., 2003).

Mexico owns a territorial surface of 1, 972, 550 km^2. Its location is latitude North 32°43´06´´ and South 14°32´27´´, with a longitude of East 86°42´36´´ and West 118°27´24´´. The bordering countries are the United States of America to the North, and Belize and Guatemala to the South (www.inegi, 2006).

The Mexican territory is located around the Tropic of Cancer. The north of the country is made up of semi desert zones with a barren climate, while the south and south-east have humid and sub humid warm climates with tropical and marshy forests. Mexican Altiplano, located in the centre of the country, has cold or temperate climates, depending on the elevation of the regions which contain wooded zones, lakes and mountains with great volcanic activity. The presence of vast mountainous chains near the coasts means that the rivers of Mexico are generally short, hard to navigate and

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contain little volume (www.inegi, 2006). The water for the human consumption of 106 million Mexican inhabitants (www.vivirmexico, 2008) is mainly extracted from wells.

In Mexico, there exists chronic and endemic hydro-fluorosis in the underground waters of Durango (Alarcón-Herrera et al., 2001), San Luis Potosí (Díaz-Barriga et al., 1997). Sonora (Grijalva-Haro et al., 2001), Jalisco (Hurtado and Gardea-Torresday, 2005), Aguascalientes (Trejo-Vázquez and Bonilla-Petriciolet, 2001), Guanajuato (Ortega, 2009), North of Puebla, North Baja California and Zacatecas (Secretariat of Health et al., 2003), and Tula de Allende (Irigoyen et al., 1995; Pontigo-Loyola et al., 2008).

Tula, due to its volcanic origin and its geological characteristics of non metallic minerals and hydrotermalims, is suitable to the presence of naturally high concentrations of F- in water. The water is extracted from wells directly and used for human consumption with no other treatment than chlorination.

When ingested, 86 - 97% of F- can be absorbed in the gastro-intestinal tract and over 90% of it can be fixed in calcified tissues (Whitford, 1990). The toxin excretion is mainly renal; however, some factors can reduce the clearance of F-. Factors such as the presence of fixing agents like Ca_2+, Mg $_2$+ and Al $_3$+ in daily diet, some drugs, metabolic alterations such as diabetes mellitus, renal tubular acidosis, asthmatic states and chronic obstructive pulmonary disorders impede the clearance of F-. In addition to this, chronic respiratory alkalosis caused by high altitude can induce an F- re-absorption into renal tubes creating fluoride acid (HF) and decreasing the rate of excretion (Whitford, 1990; Ripa and Clark 2001; Birkeland et al., 2005).

Fluoride is a trace element which, for animals and humans, is essential (in low concentration levels) for the formation of dental and skeletal tissue (Ling and Jian, 2006; Griffin et al., 2007; Kaseva, 2006; Birkeland et al., 2005; Davies, 2004). According to Kaseva (2006), between 0.7 and 1.2 mg/L can prevent dental decay, but higher levels of F- and chronic exposure can provoke dental and skeletal fluorosis, and a bigger susceptibility to renal diseases and cancer (WHO, 2004). There is also a higher risk of brain damage, IQ reduction in children (Wang et al., 2007), and hepatic and parotid gland damage (Shantakumari et al., 2004; Wang et al., 2004). WHO (2002) indicates a maximum level in tap water of 1.5 ppm/day of F- for humans, and this same limit was established by Mexican Regulations (NMX-AA-077-SCFI-2001), but WHO has considered reducing this limit due to health risks (WHO, 2004).

Dental fluorosis is a hypomineralization of the sub-surface of enamel due to a chronic ingestion of F- at higher levels during its formation (Pendrys, 2001). The distribution is generally symmetrical and bilateral in the surface of the enamel. The teeth that develop and mineralize later are premolars, and they present the greater prevalence of fluorosis. The second molars and upper incisors are also severely affected (Secretariat of Health, 2003).

Dental enamel is constituted by a prismatic structure unit. The absence of F- is known as hidroxyapatite. With the presence of fluoride ions in the fluid phase, a significant amount of mineral is converted into fluorapatite, releasing OH groups. This could cause a pH drop which normally accompanies rapid crystal growth. This change in pH can cause amelogenin aggregation and prevent the diffusion of the protein out of the maturing enamel (enamel proteins are more soluble in acidic conditions, but form insoluble aggregates in neutral solutions). Therefore, local changes in pH can change the binding of amelogenesis to the surface of hidroxyapatite crystals. This may promote the growth of crystals more in thickness than in length, and in turn, this could trap matrix proteins and prevent their efficient removal during maturation (Limeback, 1994).

Different investigations have indicated specific periods of susceptibility for the development of fluorosis: From 11 months to seven years of age (Ishii and Suckling, 1991), between 15 and 30 months of age (Baden, 1991; Evans and Stamm, 1991) and during the first year. These are the most crucial periods for the load of fluoride in the teeth (Ismail and Messer, 1996). Dental fluorosis is evident when a child has consumed an excessive amount of fluoride during the enamel´s development (Ripa and Clark, 2001).

MATERIALS AND METHODS

Characteristics of location and population

Tula is located North 20° 10´, South 19° 57´ latitude, longitude East 99°15´ and West 99° 30´, at an altitude of 2477m above sea level. The climate is classified as semi-dry and tempered. During summer periods, the climate can be sub humid, tempered with rains; with an average humidity of 0.64 and 50.88%, depending on the exact location. It has an annual precipitation of 618.7 mm. (Government of the State of Hidalgo, 2008). Geologically, Tula possesses beds of clay, gypsum, sand, silicon, kaolin, quartz and dolomite which are associated with fluorides (Geological Monograph, 1992). The economy of the area depends on agriculture, and on the petrochemical, thermo electrical, cement and wax match industries. It is irrigated by several rivers: Salado, Rosas, Tlautla, Salto and Tula, which receive wastewaters from Mexico City with no treatment, and are used for agricultural production and cattle ranch. The municipality of Tula has 93,296 inhabitants (www.cuentame.inegi, 2005) and 15 communities (Government of the State of Hidalgo, 2008). The water for human consumption which comes from wells, as surface water is polluted.

The selection of the two communities for this work adheres to the Analysis Units (pupils of 12 to 15 years old) and to the purpose of the study. The criteria for inclusion were:

1. Both communities must be inside the municipality of Tula.
2. The water for human consumption that supplies communities must come from wells.
3. The levels of F- concentration in the well for the non-exposed community must be under the limit established by Mexican Regulations (NMX-AA-077-SCFI-2001) and for the exposed community must be on the limit.
4. Each community may have at least one elementary school, one

junior high school and one high school.
5. Dental fluorosis must be present in the exposed community and absent in the non-exposed.

Before selecting the two communities, permission was obtained from Comisión de Agua Potable y Alcantarillado de Tula (CAPyAT) to monitor water wells used for human consumption. During the study, only nine wells were permitted for sampling (from 14) in order to determine levels of F-. A pilot study was undertaken from January to March 2008, to determine which wells fitted the criteria for the exposed and non exposed communities. The Wells tested during the pilot study were, La Amistad (F- concentration mean, 2.07 mg/L), El Carmen (2.28 mg/L), Ex-hacienda (2.46 mg/L), Manzanitas I (0.61 mg/L), Manzanitas III (1 mg/L), Manzanitas IV (1.51 mg/L), San Marcos (1.38 mg/L), San Lorenzo (1.30 mg/L) and El Llano (3.07 mg/L).

From these wells, the Ex-Hacienda well and the Manzanitas I well were selected. The Ex-Hacienda well has a depth of 50 m and provides tap water to the community of San Miguel Vindhó (SMV) – the exposed community. The Manzanitas I well has a depth of 240 m and provides tap water to the community of La Malinche (LaM) – the non-exposed community. It should be mentioned that the communities SMV and LaM were the unique ones that fulfilled all the criteria of inclusion.

San Miguel Vindhó's urban location is South of Tula, at an altitude of 2100 m above sea level. It has 10, 488 inhabitants (www.cuentame.inegi, 2005). La Malinche's urban location is North of Tula, 2030 m above sea level. It has 2,000 inhabitants. Both communities reside at altitude.

Water sample

The water sampling was performed according to Mexican Official Law (NMX-AA-051-SCFI-2001) by personnel of Water Quality Research and Development Laboratory (IDECA S.A de CV, Mexico, DF). Monthly samples were taken for a year, from April 2008 to April 2009. For each sample, one litre of water was taken in polyethylene bottles previously washed with HCl 10% and rinsed with distilled water. The bottles were labeled with a sample number, date, time, point of sample, air temperature, water temperature, name and signature of the person that performed the sample. The dates were registered in a diary. The temperature was measured with a Checktemp pocket thermometer (HANNA instruments) in situ (calibration number H198501), and pH (*in situ*) with a digital measurer OAKTON (1234251). Samples of water were transported in an icebox to maintain a temperature of 4°C or 39.2°F. The samples were taken 28 days apart.

Water analysis

Once in the laboratory, water samples were distillated according to Standard Methods (APHA-AWWA-WEF, 1998) before fluoride levels were determined, in order to eliminate any interference due to color, turbidity or presence of other substances. Measurement was performed using the Spectrophotometric SPANDS method in acid media, based on Mexican regulations (NMX-AA-077-SCFI-2001), which determined the concentration of F- in the water by comparing it on a standard calibration curve with correlation coefficient > 0.997. Additional quantification of carbonates (NMX-AA-036-SCFI-2001) and aluminum (NMX-AA-051-SCFI-2001) were realized to avoid possible interferences in F- determination.

Statistics analysis

To estimate prevalence of dental fluorosis

Mean; Chi^2 with 95% CI, $p < 0.05$ (Statistic program STATA 8.0).

To compare data from both communities

Student *t* test, with $p < 0.05$, 95% CI, (Statistic program Graph Pad 3.0)

To estimate relative risk

Odds ratio (OR) 95% CI, $p < 0.05$ used Multivariable analysis multinomial regression used with dichotomous outcomes (Statistic program STATA 8.0).

Epidemiologic design

According to the Bucodental Health Survey, (WHO, 1999) students between 12 and 15 years old were selected because at this age all permanent teeth (except third molars) have eruption, and dental fluorosis is evident. In addition to this, they are able to answer a structured questionnaire and they are aware of the mottled affect on teeth.

The criteria of inclusion for the pupils were:

1. Both sexes.
2. Between 12 to 15 years old.
3. Were born and live in the community being sampled.
4. Have informed and signed parental consent.
5. Active students and voluntary participation in the study.
6. Students of SMV with dental fluorosis and without dental fluorosis in LaM.
7. The clinical crown must be at least 50% erupted in the permanent teeth present.
8. Have not undergone orthodontic treatment.

For this study, ethical aspects were considered that guaranteed the dignity and the well-being of the subjects involved, ensuring respect, confidentiality and the protection of human rights. The ethical considerations were based on the General Law of Health of Mexico (2007) which deals with investigations related to the inspection of students in communities.

An interview with the health and school authorities of SMV and LaM was undertaken in order to obtain a letter of approval for each community. In the same manner, during interviews with the participants' families, the study was explained, and their participation was outlined – that they would undergo a clinical examination, photographs of the teeth would be taken, and they would be required to answer a social-demographic questionnaire. A signed letter of consent was obtained from the family of each participant.

The schools were not randomly selected from each community because there were only 3 schools available (elementary school, junior high school and high school). The sample size was determined by Statistic program EPIDAT 8.0 and supported by using the following formula (Munch, 1998; Tamayo, 1998):

$$n = \frac{NZ^2\alpha - P(1-P)}{(N-1)e^2 + Z^2\alpha P(1-P)}$$

n= minimum size of sample required
N= population number (708 students)
P= expected prevalence of dental fluorosis proportion
Z^2= Stadigraphic estimator
α= significant level
1-P= prevalence complement=Q
e^2= muestral error.

As a reference for sample size in children with dental fluorosis, the

Table 1. 12 - 15 year old students with dental fluorosis.

Prevalence of fluorosis. (25)	Sample size	Level α	Precision	N	Adjustment factor	Measure of no response. 15	n real
0.57	708	99	1.8	100	88	13	101

Table 2. Distribution of sample by proportional affixing.

	San Miguel Vindhó			La Malinche		
Schoolastic grade	**Pupils**	**(%)**	**Proportional affixing**	**Pupils**	**(%)**	**Proportional affixing**
Elementary school	13	2	2	6	5	5
Junior high school	513	88	89	92	76	77
High school	61	10	10	23	19	19
Total	587	100	101	121	100	101

report by Irigoyen et al. (1995) was selected. In the report, 93 school children between the ages of 10 and 12 years old were studied in the community of Tula. The community stood at an altitude of 2066 m. The study found that the concentration of F- in water was 2.8 ppm with a 57% prevalence of moderate dental fluorosis.

The study of the 12 - 15 year old students was designed as a probabilistic two-stage process, stratified by sex and age, with a selection of the final unit of analysis by simple random sampling (MAS) and by EPIDAT 8.0. Final sample design used is as shown in Table 1.

The distribution sample was undertaken by proportional affixing according to locality, school level, number and percentage of pupils, this is shown in Table 2.

Assessment prevalence of dental fluorosis using modificated Dean index

An examiner was used; who was previously trained and standardize in dental fluorosis diagnosis according the criteria of WHO (1999) with a value kappa intraexaminer of 0.93.

The study was divided into two stages: First a questionnaire was undertaken with each student to collect social-demographic information: 1) Full name, 2) Age, 3) Sex, 4) Address, 5) Place of birth, 6) Place of residence, 7) Time of residence. Secondly, a dental fluorosis diagnosis was carried out according to Modified Dean Index (MDI) with the following criteria: Score 0- Normal, enamel surface is smooth; shiny with a white to cream light color. Score 1- Questionable; enamel with little aberrations with respect to normal enamel translucently with few white spots. Score 2- Very mild; few opalescent areas with paper white color with irregular distribution, but in less than 25% of the surface. Score 3- Mild; white opalescent areas are more extensive covering less than 50% of the surface. Score 4- Moderate; dental surface shows erosion, with maroon brown spots that become disfigured. Score 5- Severe; enamel surface very affected with remarkable hypoplastic areas, with alterations in the shape of the tooth, discontinue fosse, maroon brown spots, has the look of corrosion (WHO, 1999).

For each student, dental brushing was undertaken, and the teeth were dried with sterile gauze. Then all the permanent superior teeth were evaluated by visual inspection in natural morning light, using a sterile dental mirror and explorer, and other material necessary to guarantee the bio-security measures. The registry of dental fluorosis was based on the two most affected teeth.

Community fluorosis index (CFI)

In order to establish the CFI, statistic consideration (p) has the following values: Criteria of Normal has score 0 and p = 0, Questionable score 1 and p= 0.5, Very mild score 2 and p = 1, Mild score 3 and p = 2, Moderate score 4 p=3 and Severe score 5 and p = 4.

CFI was estimated with the summa of the number of affected students multiplied by the degree of affection (statistics considera-tion) and divided between the total numbers of examined pupils:

$$\text{CFI} = \frac{\sum \text{(Number of students with fluorosis} \times \text{consideration)}}{\text{Total number of students examined}}$$

Interpretation of the CFI: Dean indicates that if the CFI is above 0.6, it results to a public health problem and it justifies an increased attention to the population (Dean, 1942).

RESULTS

Water quality

Wells water quality

From April 2008 to April 2009, monthly samples were taken from the same sampling points at the same hour, to determine F- concentrations, with a mean value of 1. 41 mg/L (± 0.36, 95% CI, 1.19 -1.64) for the Ex–Hacienda well, and 0.62 mg/L (± 0.13, 95% CI, 0.53 - 0.70) for the Manzanitas I well. Data for every month, with air temperature and the temperature of sample water, were estimated (Table 3).

The measurement of pH at the Ex-Hacienda well had a mean value of 7.65 ± 0.39 and the same measurement at the Manzanitas I well was 7.56 ± 0.47. Hardness due to the presence of carbonates was 336.14 ± 37.34 mg/L and 216.71 ± 2.13 mg/L, respectively with a significant difference ($p < 0.0001$). However, both wells are under the levels indicated by the guidelines of WHO (2004) for

Table 3. Wells water quality.

Ex Hacienda well (San Miguel Vindhó)					Manzanitas I well (La Malinche)				
2008 - 2009 monthly	Fluoride (mg/L)	pH	Temp. Air (°C)	Temp. Sample (°C)	2008 - 2009 monthly	Fluoride (mg/L)	pH	Temp. air (°C)	Temp. sample (°C)
April	1.58	7.4	16	19.8	April	0.78	6.83	19.0	24.2
May	1.73	8.11	21	21	May	0.61	8.12	31.7	24.1
June	0.69	8.34	21.8	21.5	June	0.69	8.27	29.0	24.0
July	1.91	8.16	30.5	21.2	July	0.66	8.35	30.8	24.1
August	1.20	7.2	23	19.7	August	0.60	7.05	26.1	24.0
September	1.09	7.1	16.5	19.2	September	0.33	7.1	22.5	23.8
October	1.15	7.46	16.4	19.3	October	0.39	7.3	21.8	24.0
November	1.38	7.22	22.4	19.5	November	0.73	7.49	22.4	24.0
December	1.18	7.50	24.9	19.4	December	0.57	7.79	24.9	23.7
January	1.28	7.88	18.7	19	January	0.57	7.8	18.7	23.6
February	1.58	7.88	20	19	February	0.67	7.3	20	24.2
March	1.67	7.69	29.2	20	March	0.69	7.4	29.2	24.1
April	1.99	7.56	30	20	April	0.78	7.51	30	24.2

The mean of fluoride mg/L in San Miguel Vindhó (SMV) was 1.41 ± 0.36; for La Malinche (LM) 0.62 ± 0.13; with an extremely significant difference: P <0.0001. The mean of pH for SMV was 7.65 ± 0.39, for LM 7.56 ± 0.47, P value 0.51 considered not significant. Temperature air mean for SMV was 22.33 ± 5.08 for LM was 24.59 ± 4.58, P value 0.08. The temperature's mean for SMV's sample was 19.89 ± 0.83 while for LM A 24 ± 0.19 with a P value < 0.0001, very significative.

Table 4. Sample distribution by student's gender and age, between 12 and 15 years old in both communities.

San Miguel Vindhó	Gender				La Malinche	Gender			
	Feminine		Masculine			Feminine		Masculine	
Years old	n	(%)	n	(%)	Years old	n	(%)	n	(%)
12	13	26	10	20	12	18	33	17	36
13	17	33	16	32	13	12	22	15	32
14	15	29	16	32	14	14	26	6	13
15	6	12	8	16	15	10	19	9	19
Total	51	100	50	100	Total	54	100	47	100

The mean age for SMV was 13 ± 0.98, variance 0.97; for LM the mean value for age was 13 ± 1.12 and the variance 1.25.

quality drinking water. Aluminum concentrations for both wells were under 0.2 mg/L, warranting no interference of this element in F- determination.

Sample distribution by sex and age of the students from 12 - 15 years old in both communities

101 students from the three schools in SMV and 101 from LaM were taken for clinical analysis. In SMV, the age of greater prevalence was 13 years old for females n = 17 (33%). For males, the prevalent age was 13 and 14 years old n = 16 (32%). In LaM, the age of greater prevalence for females was 12 years old n = 18 (33%), and for males it was also 12 years old n = 17 (36%) (Table 4).

Dental fluorosis

The MDI reported in this work refers to the mean value of the most affected teeth by dental fluorosis. The overall dental fluorosis prevalence in the exposed sample population of San Miguel Vindhó was 85%, where 42% were very mild, 22% mild, 9% moderate and 12% severe. Meanwhile, in the sample population of La Malinche, prevalence was 4%, where 3% were very mild and 1% moderate. In the exposed community, all the grades of fluorosis were present in the students from 12 - 15 years old, whereas in non-exposed community there were no cases of mild or severe dental fluorosis. There were significant statistical differences inside the dental fluorosis degrees associated with the different concentrations of F- in the Ex-Hacienda well (SMV) and the Manzanitas I well

Table 5. Dean´s index to determine prevalence of dental fluorosis and community fluorosis index (CFI) in students of San Miguel Vindhó and La Malinche.

	San Miguel Vindhó				La Malinche			
Dean´S index	**n**	**95% CI**	**Score**	**Σ score**	**n**	**95% CI**	**Score**	**Σ score**
Normal	1	0.025 - 5.3	0	0	68	81.3 - 94.9	0	0
Questionable	14	6.6 - 21.0	0.5	7	29	19.3 - 38.0	0.5	14.5
Very mild	43	32.4 - 52.7	1	43	3	0.61 - 8.4	1	3
Mild	22	13.2 - 30.3	2	44	0	-	2	0
Moderate	9	2.8 - 14.9	3	27	1	0.025 - 5.3	3	3
Severe	12	5.0 - 18.6	4	48	0	-	4	0
Total	101			169	101			20.5

The attainment of CFI is based on Dean´s (1942) index score (0 to 4.0) symptoms of dental fluorosis: no public problems (0), questionable (0.5), very mild (1), mild (2), moderate (3) and severe (4); in the sample of both communities studied.

Table 6. Odds ratio estimation for prevalence of fluorosis and risk factors in students of San Miguel Vindhó and La Malinche.

Risk factors	Dental fluorosis	Odds ratio*	Confidence intervals		P value
			Inferior	Superior	
Well water consumption	Severe	12.111	1.359	100.039	0.0006
Use of fluoride salt for food preparation	Moderate	10.018	1.293	446.337	0.0097
Swallowing dentifrice when teeth brushing	Severe	37	2.489	1957.793	0.0001

*Odds ratio for prevalence. Students that consumed well's water had 12 times more risk to suffer severe dental fluorosis than those who did not consumed it. Students that ate foods with fluoride salt had 10 times more risk to have moderate dental fluorosis. Swallowing dentifrice had 37 times more risk to have severe dental fluorosis. All P values are considered very significant.

(LaM), with a paired *t* test p = 0.0001 (Table 5).

Community fluorosis index in students of 12 - 15-years-old in San Miguel Vindhó and La Malinche

A Community Fluorosis Index was calculated for both communities, determining 1.67 for San Miguel Vindhó and 0.20 for La Malinche. According to Dean (1942) values, if CFI is above 0.6 it results to a public health problem. The results obtained in this work indicate that for the exposed community, it is very possible that there is a public health problem (Table 5).

The dental fluorosis results had associations with sex and age. All the scores of dental fluorosis for males and females in the bivariate analysis showed that there was no significant correlation between dental fluorosis and sex Chi^2 = 5.54, p = 0.353; similarly, all the degrees of dental fluorosis were stratified in the rank of 12 - 15 years old and there was no statistically significant difference regarding age Chi^2 = 22.47, p = 0.96.

An odds ratio (OR) (Table 6) was used as an estimator of relative risk and dichotomizing variables (dummy) were used. The OR was estimated for dental fluorosis (dependent variable, outcome) with water for human consumption from the well (independent variable, risk factor). The measured variable was adjusted.

The value of the OR for severe fluorosis was 12.11 (1.35 – 100.039) p = 0.0006. This indicates that the risk was twelve times greater for the exposed community that consumes well water from the Ex Hacienda well, than for the non-exposed community that consumes water from the Manzanitas I well. The risk factor was associated positively. Moderate fluorosis OR = 4.03 (0.06 - 55.60) p = 0.212, mild OR = 3.402 (0.73 - 31.74) p = 0.095 and very mild OR = 1.73 (0.031 - 22.661) p = 0.638 were always adjusted; the OR was above 1 but the values of the superior interval confidence were < 1, therefore, there was no statistical significance.

DISCUSSION

In Mexico, in the states of Coahuila and Durango, there is a chronic hydrofluorosis endemic. A "fluoride belt" has been reported in the states of Coahuila, Zacatecas, San Luis Potosi, Guanajuato and Queretaro (Ortega, 2009). Even though the State of Hidalgo is not located in this area, the geological characteristics in Tula of non mineral deposits such as sedimentary, hydrothermal, metamorphic and volcanic rock determine the nature of the aquifer. Moreover, in the area, there are deposits of limestone, kaolin, clay, quartz and silicium, which are associated with the findings of naturally present F- in water (Geological Monograph, 1992; Gaciri and Davies, 1993).

Some reports in the states of San Luis Potosi (Grimaldo et al., 1995), Jalisco (Hurtado and Gardea-Torresdey,

2005) and Queretaro (Sánchez et al., 2004), where the water for human consumption is above 1.5 mg/L of F-, show a prevalence of dental fluorosis of 98, 98 and 89.5%, respectively which is in accordance with the findings in this work and of Pontigo-Loyola et al. (2008) who reported an overall prevalence of dental fluorosis of 83.8% and a CFI of 1.85 in three communities in Tula de Allende. The selected prevalence for the calculation for the sample size used in the work reported by Irigoyen et al. (1995) of 57%, increased by approximately 1.5 compared with the 85% of SMV.

Even though the quantity of F- in SMV means it is under the standard (1.41 mg/L) it is important to note that for half of the year, monthly measurements were over the norm NMX-AA-077-SCFI-2001). In addition to this, considering the mean air temperature of 21.4°C (38.52°F) the level of F- should be under 0.8 mg/L according to the Centers for Disease Control (1991). The exposed population (SMV) had a positive correlation with teeth pigmentation, and a CFI of 1.67. If the levels of F- concentrations are conserved or increased and chronic exposure continues, it is probable that the population could be susceptible to develop other severe diseases like skeletal fluorosis, renal disease, and cancer (WHO, 2004).

In La Malinche, F- had a mean of 0.62 mg/L. The values were under the norm of 0.78 mg/L. The mean air temperature of 25.8°C (46.44°F), means the F- concentrations should be under 0.8 mg/L (Centers for Disease Control, 1991). The prevalence of dental fluorosis in this community was 4% and CFI was determined to be 0.2. According to Dean´s criteria (1942), this should not present a public health problem. These results have been statistically verified.

The gender of the students of SMV and LaM showed no significant differences with the criteria of Dean´s Index. From the epidemiological point of view, females and males have the same probability of suffering dental fluorosis. There were similar outcomes with age, most likely due to the fact that the critical period for the development of fluorosis is during the training of the enamel of permanent teeth, and this occurs between 11 months and 7 years old (Ishii and Suckling, 1991). Age susceptibility was reviewed by Baden (1991), Evans and Stamm (1991), Ismail and Messer, (1996) and Ripa and Clark (2001). But investigators have focused only on the aesthetic problem of the central incisors' maxillaries.

Few studies had been carried out to examine fluorotic enamel lesions histologically and biochemically due to the ingestion of F- in water. Chronic exposure to high levels of systemic F- results in the retention of enamel protein during maturation, but prior to dental eruption. This is associated with poor crystal formation, hypocalcification, low fluoride incorporation and "softer" subsurface tissue during the inorganic phase of enamel. These subsurface lesions can be appreciated as white spots, commonly found in mild to moderate fluorosis. Therefore, it is a high risk that a large proportion of enamel surface could be affected (Limeback, 1994).

It is important to mention that the present work has internal validity since it is representative of the study population. A simple random probabilistic sample was carried out in order to avoid a bias selection. The error of measurement was diminished through a person previously standardized avoiding the bias of information. The design of this work was cross-sectional, which, beside the descriptive analysis, allowed us to carry out an analytical phase in order to evaluate the statistical associations of the different variables.

It is important to mention the limitations of the study: It lacks external validity, has temporary ambiguity and did not allow for formulating hypothesis (Hernández and Velasco-Mondragón, 2000; Hernández-Ávila et al., 2000).

Conclusions

Even though the two communities studied were located in the same municipality, with similar geographic and socioeconomic conditions, the quality of water seems to be the most important factor for the presence of dental fluorosis. In San Miguel Vindhó, the presence of F- follows a geological pattern making it an important contaminant of ground water and constituting a hazard for students' health.

Hydrogeological and geochemical studies of groundwater quality and monitoring in aquifers can help identify safe aquifer zones of water for human consumption. Changing the source of drinking water to a well with lower levels of F-, ensuring the defluoration of natural water, and performing more studies to determine the impact of F- in health can provide solutions to the quality problems in groundwater.

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