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In utero exposure to fluoride and cognitive development delay in infants

L. Valdez Jiménez[a], O.D. López Guzmán[b], M. Cervantes Flores[b], R. Costilla-Salazar[c], J. Calderón Hernández[a], Y. Alcaraz Contreras[d], D.O. Rocha-Amador[d,*]

[a] Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología (CIACYT), Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico
[b] Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango (Unidad Durango), Victoria de Durango, Mexico
[c] División de Ciencias de la Vida, Universidad de Guanajuato, Guanajuato, Mexico
[d] División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato, Mexico

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ABSTRACT

The objective of this study was to evaluate the association between *in utero* exposure to fluoride (F) and Mental and Psychomotor Development (MDI and PDI) evaluated through the Bayley Scale of Infant Development II (BSDI-II) in infants. The sample included 65 mother-infant pairs. Environmental exposure to F was quantified in tap and bottled water samples and F in maternal urine was the biological exposure indicator; samples were collected during the 1st, 2nd and 3rd trimester of pregnancy. The mean values of F in tap water for the 1st, 2nd and 3rd trimester were 2.6 ± 1.1 mg/l, 3.1 ± 1.1 mg/l and 3.7 ± 1.0 mg/l respectively; above to 80% of the samples exceeded the reference value of 1.5 mg/l (NOM-127-SSA1-1994). Regarding F in maternal urine, mean values were 1.9 ± 1.0 mg/l, 2.0 ± 1.1 mg/l and 2.7 ± 1.1 mg/l for the 1st, 2nd and 3rd trimester respectively. The infants with MDI and PDI scores less than 85 points were 38.5% and 20.9% respectively. After adjusting for potential confounding factors (gestational age, age of child, marginalization index and type of water for consumption), the MDI showed an inverse association with F levels in maternal urine for the first ($\beta = -19.05$, $p = 0.04$) and second trimester ($\beta = -19.34$, $p = 0.01$). Our data suggests that cognitive alterations in children born from exposed mothers to F could start in early prenatal stages of life.

1. Introduction

Fluorides are naturally-occurring components in rocks and soil and are also found in air, water, plants, and animals. The general population is exposed to fluoride (F) through the consumption of drinking water, foodstuff, and dental products. Populations living in areas with naturally high F levels in water and soil may be exposed to high levels of F in water, especially if drinking water is provided from wells (ATSDR, 2003; Vineet Dhar1, 2009). In the central and north areas of Mexico there are groundwater with elevated levels of F (Ortega-Guerrero, 2009). In this areas, almost 90% of the population has the practice of use tap water for food preparation and direct consumption as drinking water (Jarquín-Yañez et al., 2015). The bioavailability of F through ingestion 80–100% (ATSDR, 2003).

Epidemiological research conducted in school age children living in endemic hydrofluorosis areas have evaluated the influence of exposure of F on cognitive development assessed as intelligence quotient scores (IQ). Different intelligence tests have been used (RAVEN-Chinese version, Wechsler Intelligence Scales, Stanford-Binet Intelligence Scale) and have reported lower IQ points associated with F exposure at concentrations of 2.20–3.94 mg/l compared with residents from control areas (concentrations of F in water <0.41 mg/l). The lack of biomarkers of exposure and control of potential confounders is an issue that has to be considered in these studies (Karimzade et al., 2014; Trivedi et al., 2012). Other well conducted research papers also reported that F decreases IQ scores (Ding et al., 2011; Rocha-Amador et al., 2007).

Cognitive development alterations associated with F exposure could start in early prenatal stages of life and come up later at school age; and likely continue into adulthood. Few studies have explored this hypothesis and the evidence is inconclusive. For

* Corresponding author at: Departamento de Farmacia, División de Ciencias Naturales y Exactas, Universidad de Guanajuato Noria Alta S/N, C.P. 36050 Guanajuato, Guanajuato, México.
E-mail address: drochaa@ugto.mx (D.O. Rocha-Amador).

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example, Chinese newborns; scored lower in the Standard Neonatal Behavioral Neurological Assessment (NBNA) test in the high exposure group (F in urine 3.58 ± 1.47) compared with the control group (1.74 ± 0.96 mg/l); 36.48 ± 1.09 vs 38.28 ± 1.10, $p < 0.05$, respectively (Li et al., 2008). Another study in aborted fetuses of mothers living in an endemic hydrofluorosis area (4.3 ± 2.9 mg/l of F in urine) reported changes in neurotransmitters compared with levels of aborted fetuses of mothers living in non-endemic areas (F in urine 1.67 ± 0.8 mg/l) (Yu et al., 2008). Regarding experimental studies, data shows that F accumulates in the brain, specifically in the hippocampus; a region associated with memory, attention and learning (Shivarajashankara et al., 2001; Bhatnagar et al., 2006; Basha et al., 2011). About gestational exposure, some experimental studies, indicated that F alters learning and memory (Mullenix et al., 1995; Bera et al., 2007; Basha et al., 2011).

In endemic hydrofluorosis areas millions of people consume contaminated water daily, including pregnant women and there is evidence that support the F capacity to cross the placental and the blood-brain barriers and accumulate in critical areas of the brain related to cognitive development. The objective of this research was to evaluate the influence of *in utero* exposure to F in Mexicans infants born from mothers living in endemic hydrofluorosis areas on the Mental and Psychomotor Development (MDI and PDI) through the Bayley Scale of Infant Development II (BSDI-II).

2. Methodology

2.1. Participants and recruitment

Authorizations from the authorities of the Ministry of Health (SSA) of the selected municipalities included for the study were obtained. The project protocol was conducted in accordance with the Declaration of Helsinki and was approved by the bioethics committees of the SSA from the municipalities. Follow up was conducted in pregnant women recruited from 2013 to 2014 who received prenatal care in health centers located in Durango City and Lagos de Moreno, Jalisco, Mexico. Both are endemic hydrofluorosis areas (Hurtado-Jiménez and Gardea-Torresdey, 2005; Rocha-Amador et al., 2007). Inclusion criteria were: ≤12 weeks of gestation, with no history of thyroid disease, without clinically diagnosed diabetes, and a minimum 5 years of residence in the study area; 182 potential participants were identified. Each woman was visited at home to explain the objective of the study, the risks and the benefits of their participation. A written informed consent was obtained from those who agree to participate (n = 90) and 65 women approved the participation of their infants in the neuropsychological evaluation. Water and urine samples were obtained at three periods through pregnancy; 1st trimester (between the 8 to 12 week), 2nd trimester (between the 24 to 28 week) and the 3rd trimester (after the 30 week); 65, 46 and 29 women provided water and urine samples for the 1st, 2nd and 3rd trimesters, respectively.

2.2. Maternal interviews

In the 1st trimester of pregnancy a questionnaire was applied to obtain information about sociodemographic, prenatal history, mother's health status before pregnancy (use of drugs, vaccines, diseases, etc.) and the type of water for drinking and cooking. The marginalization index (MI) was obtained from the National Population Council (CONAPO). The mother's address was geographically referenced to identify the basic geostatistical area (AGEB) to which she belonged. The marginalization index integrates information from educational level variables to health services and social assistance, living and deceased children, housing conditions, number of rooms and bedrooms, type of floor, toilet, and availability of goods. Two additional surveys were applied during the 2nd and 3rd trimester of pregnancy to get information about the mother's health, pregnancy evolution and sources of water consumption.

2.3. Fluoride exposure assessment

Tap and bottled water samples were collected at participant's home. First morning voided urine samples were collected in plastic bottles at each trimester of pregnancy (as described in participants and recruitment). All samples were kept refrigerated at −4 °C until processed.

Fluoride in water (FW) was quantified by adding a TISAB buffer to the samples just prior to the analysis with a specific ion sensitive electrode. As an internal quality control, primary standard reference material "Fluoride standard solution" (NIST SRM 3183; National Institute of Standards and Technology, United States) was analyzed. The accuracy was 98 ± 3%. F in maternal urine (FU) was analyzed according to the method 8308 (Fluoride in urine) from the National Institute of Occupational Safety and Health (NIOSH, 1984). As quality control "Urine Control Lyophilized for Trace Elements" ClinChek® of Iristech Co was analyzed. The accuracy was 97 ± 6%. The F urine levels were corrected by specific gravity and were reported as mg/l (AIHA, 2004).

2.4. Maternal interview about child's health

A survey was conducted to get information about childbirth (type of birth, week of birth, weight and length at birth, Apgar and health conditions of the baby during the first month of life). This information was corroborated with the birth certificate. The gestational age of the babies was calculated and classified into three categories: immature (21–27 weeks of gestation and birth weight <1.0 kg); preterm (28–37 weeks of gestation and birth weight between 1.0 to 2.5 kg); and term (37–41 weeks and birth weight >2.5 kg) according to the Official Mexican Norm 007 (NOM-007-SSA2-1993).

2.5. Neurodevelopmental and behavioral evaluation in young children

Neurodevelopment was assessed with the Bayley Scales of Infant Development II (BSDI-II) (Bayley, 1993). This test has good reliability and validity: it is applied to evaluate developmental delay in children between 3 months to 5 years in Mexico by the SSA (CNPSS, 2013). The Mental Development Index scale (MDI) of the Bayley test evaluate aspects of functioning such as eye-hand coordination, manipulation, understanding of object relation, imitation and early language development whereas the Psychomotor Development Index (PDI) scale assesses gross motor development. A trained psychologist who was blinded about mother's F exposure evaluated the infants at the participant's home; the application lasted 30–45 min. All young children were healthy at the time of the test application and accompanied by the mother. The raw scores of MDI and PDI were standardized by age with an average of 100 and a standard deviation of 15. To standardize the raw scores for children who were born prematurely, the number of months of prematurity was subtracted from their chronological age. The scores below 85 points indicated a possible developmental delay.

2.6. Statistical analysis

The sample size was calculated with data from a previous study of decreased IQ and F exposure in school age children. In this study near to 60% of the children consumed contaminated water and the

prevalence of children with IQ below 90 points was 25% in the control group (F urine 1.5 mg/g creatinine) in comparison with the 58% of children in the exposed group (F urine >5 mg/g creatinine) (OR = 4.1, CI 95% 1.3–13.2) (data unpublished). A meta-analysis of F and children's intelligence also reported a similar OR (Tang et al., 2008). The two-side confidence level and power were 95% and 80% respectively. The method followed was the X-Sectional, Cohort, & Randomized Clinical Trials. The final sample was 86, in our study 90 women accepted to participate in the pregnancy follow up. The calculation was done with Open Epi.

Regarding statistical analysis, first we examined the univariate (mean, standard deviation, and proportions) characteristics of demographic, maternal and child factors. The variables, concentration of F in tap water, F in bottled water and F in maternal urine were log10-tranformed and reported as geometric mean and standard error. Normality, homoscedasticity and linearity assumption were satisfied for MDI and PDI scores and were modeled as continuous variables. For qualitative variables, frequency and percentage (education, marital status, occupation, parity, type of water used for drinking or cooking) are reported. F levels in tap water, bottled water and maternal urine across trimesters were compared with paired *t*- test (1st and 2nd, 2nd and 3rd and 1st and 3rd). The relationship between education, occupation and marital status of the mother, the type of water for drinking and food preparation, child sex, type of birth, breastfeeding, birth weight, number of antenatal care visits and MDI and PDI scores were analyzed using bivariate tests as applied (correlations, t-student, ANOVA). Previous to run multivariable regression linear models we explored the goodness of fit between the dependent variables (MDI or PDI) and the F in maternal urine exposure by each trimester with the curve fitting method to find the best-fit line. Also, scatterplots were conducted as a visual exploratory tool between the F exposure-outcome. To examine the association between BSID II scores and maternal F exposure, we performed separate adjusted multiple regression linear models (MRLM) for MDI and PDI by the 1st and 2nd trimesters of pregnancy. All models were adjusted for the same set of covariates (gestational age, children age, MI and type of drinking water) even if they were not significant in the bivariate analysis. Finally, we reported two MRLM including only children with data of F in maternal urine for both the 1st and the 2nd trimester (n = 46). The stepwise method was used to select the best-fit model. To assess the validity model, residual analysis was performed. All statistical analysis was performed with SPSS 20 (SPSS Inc., Chicago, IL, USA) the significance level was set at $p < 0.05$.

3. Results

Table 1 shows the comparison of general characteristics of the pregnant women between participants in the biological monitoring and the women who agreed to their child's cognitive evaluation. The average age of the participants was 22.4 ± 4.0, in education 41.5% of the participants had completed high school. Regarding marital status, 69.7% of the participants were married, 70.7% of the participants reported being housewives, and for 44.6% of the participants it was their first birth. About marginalization index 52.3% of participants were classified in the mid-level. Almost 64.6% of the participants reported tap water for drinking and 56.6% use tap water for prepare food (data not shown). No statistical significant differences (p > 0.05) in education level, marital status, occupation, parity, MI or type of drinking water were observed between pregnant women whose children participated in the study and those who were did not participate in the neuropsychological evaluation (n = 25).

Table 2 shows information about F levels in tap water. The values ranged from 0.5 to 12.5 mg/l, 81.5%, 86.7% and 92.2% of samples in each trimester exceeded the NOM-127-SSA1-1994 (1.5 mg/l). No statistically significant differences were observed between trimesters (1st vs 2nd $p = 0.35$; 1st vs 3rd $p = 0.28$ and 2nd

Table 1
Comparison of general characteristics between the pregnant women who consented to their children's cognitive evaluation and participants that only participated in the biological monitoring.

	(n = 65)	(n = 25)	$p^{a,b}$
Age[c]			
Years	22.0 ± 4.0 (15–30)	20.7 ± 5.0 (20–28)	0.09
Education[d]			
Illiterate	10.7 (7)	12.0 (3)	
Incomplete elementary	12.3 (8)	4.0 (1)	0.13
school	16.9 (11)	8.0 (2)	
Elementary school	41.5 (27)	44.0 (11)	
High school	18.4 (12)	32.0 (8)	
Marital status[d]			
Married	69.7 (45)	80.0 (20)	0.72
Single	30.2 (20)	20.0 (5)	
Occupation[d]			
Housewife	70.8 (46)	44.0 (11)	0.72
Works	23.1 (15)	32.0 (8)	
Student	6.1 (4)	8.0 (2)	
Search works	–	16.0 (4)	
Parity[d]			
First pregnancy	44.6 (29)	64.0 (16)	0.76
Drinking water[d]			
Bottled water	64.6 (43)	48.0 (12)	0.20
Tap water	33.8 (22)	52.0 (13)	
Cooking water[d]			
Bottled water	33.8 (22)	16.0 (4)	0.46
Tap Water	66.1 (43)	84.0 (21)	
Marginalization Index[d]			
Very high	–	–	
High	12.3 (8)	12.0 (3)	0.90
Medium	52.3 (34)	48.0 (12)	
Low	24.6 (16)	28.0 (7)	
Very Low	10.8 (7)	12.0 (3)	

[a]Continuous variables were compared through *t*-test.
[b]Categorical variables were analyzed by chi-square test.
[c]Arithmetic mean ± standard deviation (minimun-maximun).
[d]Percentage (n).

Table 2
Fluoride concentration (water, bottled and urine) by trimester of pregnancy.

F concentration (mg/l) Trimester	GM ± SE*	(min- max.)	
Tap water			% ≤ NOM127[a]
First	2.6 ± 1.1	(0.5 – 10.8)	81.5
Second	3.1 ± 1.1	(0.7 – 10.0)	86.7
Third	3.7 ± 1.0	(0.6 – 12.5)	92.2
Bottled Water			% ≤ NOM41[b]
First	2.3 ± 1.1	(0.01 – 5.7)	52.0
Second	2.0 ± 1.1	(0.02 – 8.1)	50.0
Third	2.9 ± 1.0	(0.02–7.9)	66.7
Urine%>0.65[c]			
First	1.9 ± 1.0	(0.16 – 4.9)	96.0
Second	2.0 ± 1.1	(0.7 – 6.0)	100.0
Third	2.7 ± 1.1**	(1.3 – 8.2)	100.0

[a]NOM-127-SSA1-1994 (1.5 mg/l); [b]NOM-041-SSA1-1993 (0.7 mg/l); [c]0.65 mg/l level in pregnant women determined by ion selective electrode (Opydo-Szymaczek and Borysewicz-Lewicka, 2005b); 1st trimester n = 65; 2nd trimester n = 46; 3rd trimester n = 29; * Geometric mean ± standard error; ** Differences were observed with the 1st, 2nd vs the 3rd trimester ($p = 0.004$ and $p = 0.009$ respectively trought paired *t* test; n = 29).

vs 3rd $p = 0.79$). Regarding bottled water 52.0%, 50.0% and 66.7% of samples in each of the trimesters exceeded the NOM-041-SSA1-1993 (0.7 mg/l). The mean concentration of F in the urine of the mothers was 1.9 ± 1.0 mg/l (0.16–4.9 mg/l) for the 1 st trimester, 2.0 ± 1.1 mg/l (0.7–6.0 mg/l) for the 2nd and 2.7 ± 1.1 mg/l (1.3–8.2 mg/l) for the 3rd trimester. There is not a biological exposure index for F in urine in pregnant women. However, we compared our data with the mean value of F in urine (0.65 mg/l) of pregnant women residents in areas with low levels of F in drinking water (0.4–0.8 mg/l) and 96% to 100% of the samples for the three trimesters exceeded this value (Opydo-Szymaczek and Borysewicz-Lewicka, 2005). No statistically significant differences were observed between the levels of F in urine of the mother between the 1st and the 2nd trimester ($p > 0.05$). Only differences were observed with the 1st, 2nd vs the 3rd trimester ($p = 0.004$ and $p = 0.009$ respectively; n = 29). Nevertheless, when we correlate F in urine levels into trimesters we found a relationship between the 1st and 2nd trimester r = 0.40, $p = 0.05$ (n = 46) and 2nd and 3rd r = 0.37, p = 0.05 (n = 29).

In Table 3 pregnancy and childbirth characteristics are listed. The average age of children assessed was 8 months (3–15 months) and almost 70% were girls. Only 66.2% of the babies were at term. From this 66.2% were born vaginally and weighed and measured on average 3.1 ± 0.5 kg and 50 ± 6.0 cm, respectively. We found higher levels of F in urine across trimester in premature compared with full term 2.4 vs 1.6 mg/l (1st); 2.3 vs 1.8 mg/l (2nd); and 4.1 vs 2.8 mg/l (3rd) (data not shown). During pregnancy, women reported having an average of 8 prenatal control visits and 92.3% reported that they breastfed their children (with a duration between 3 to 7 months). Regarding, BSID-II the mean of MDI score was 91.6 ± 14.3 (ranged from 60 to 135 points), the normal range of MDI is between 85 to 115, 38.5% of the newborns scored below 85 while 11.9% scored above 115. Regarding PDI, the average was 90.9 ± 13.5 (ranged from 54 to 131 points). The proportion of infants scored less than 85 was 20.9% and 6% scored over 115 points.

Table 4 shows results of MRLM between F in urine and MDI and PDI. After adjusting for potential confounders, an inverse association was observed between levels of F in urine in the 1 st and 2nd trimesters with MDI scores ($\beta = -19.05$; $p = 0.04$; $\beta = -19.35$; $p = 0.013$ respectively). No differences in general characteristics and F in urine levels (2.2 vs 2.1 mg/l, p = 0.47) were observed between the 46 women who provided environmental and biological samples in the 1st and 2nd trimester with the 19 women who do not provided samples for the 2nd trimester (data no shown).

Table 3
Characteristics of pregnancy, childbirth and development indices of infant evaluated.

Variable	
	% (n)
Gender	
Male	30.7 (20)
Female	69.2 (45)
Gestational age (NOM 007)[a]	
Immature	–
Premature	33.8 (22)
Term	66.2 (43)
Type of delivery	
Vaginal	66.2 (43)
Caesarean section	33.8 (22)
Feeding type	
Breast feeding	92.3 (60)
Formula	7.6 (5)
	$\bar{x} \pm SD$[d] (min- max.)
Birth weight (kg)	3.1 ± 0.5 (1.8 – 4.0)
Size at birth (cm)	50.0 ± 6.0 (27.0 – 54.0)
Number of antenatal care visits	8.0 ± 3.0 (4.0 – 20.0)
MDI[b]	91.6 ± 14.3 (60.0 – 135.0)
MDI[c] % ≤ 85	38.5
MDI% ≥ 115	11.9
PDI[b]	90.9 ± 13.5 (54.0 – 131.0)
PDI[c] % ≤ 85	20.9
PDI% ≥ 115	6.0

[a]NOM007 Immature (21–27 weeks, weight ≥ 1.0 kg; Premature 28–37 weeks gestation weight between 1.001 to 2.5 kg); term 37–41 weeks gestation, weight ≥ 2.5 kg).
[b]Reference value 100 ± 15.
[c]Possible developmental delay (n = 65).
[d]Arithmetic mean ± standard deviation.

4. Discussion

The objective of this research was to evaluate the influence of *in utero* exposure to F on Mental and Psychomotor Development evaluated through the BSDI II in Mexican children born from mothers living in endemic hydrofluorosis areas. The proportion of children with values of MDI less than minus one standard deviation (SD) was 38.5%; scores under 85 points are considered as an indicator of possible developmental delay. This test evaluates psychological processes such as attention, memory, sensory processing, exploration and manipulation, and concept formation (Bayley, 1993). F in maternal urine (log_{10}) for the 1st and the 2nd trimester were negatively associated with MDI ($\beta = -19.05$;

Table 4
Multiple Regression Linear Models of MDI and maternal fluoride exposure after adjustments for gestational age, age of child, marginality index and type of drinking water (1st and 2nd trimester).

1st trimester	β	Standard error	Standard β	p
(Constant)	95.9	3.3		0.00
Maternal exposure to F[a]	−15.8	9.07	−0.27	0.08
Adjusted Model				
(Constant)	46.1	19.36		0.022
Gestational age[b]	−10.0	6.59	−0.23	0.13
Age of child	0.55	0.76	0.11	0.47
Marginality index	1.5	2.49	0.09	0.54
Type of drinking water[c]	−13.3	5.4	0.38	0.02
Maternal exposure to F[a]	−19.05	8.9	−0.32	0.04
2nd trimester	**β**	**Standard error**	**Standard β**	**p**
(Constant)	97.0	3.1		0.00
Maternal exposure to F[a]	−18.1	7.6	−0.34	0.02
Adjusted Model				
(Constant)	47.31	18.31		0.014
Gestational age[b]	−10.51	6.23	−0.24	0.1
Age of child	0.57	0.73	0.11	0.43
Marginality index	2.22	2.35	0.14	0.35
Type of drinking water[c]	−11.39	4.88	0.34	0.025
Maternal exposure to F[a]	−19.34	7.46	−0.36	0.013

[a]Log of F in urine.
[b]Gestational age was coded like 1 = rerm, 2 = preterm and 3 = immature.
[c]Type of drinking water was coded like 1 = bottled and 2 = tap; 1st trimester: R = 0.26; $R^2 = 0.07$; 1st trimester adjusted model: R = 0.50; $R^2 = 0.25$; Durbin-Watson = 2.2, $p = 0.05$; 2nd trimester: R = 0.34; $R^2 = 0.11$; 2nd trimester adjusted model: R = 0.53; $R^2 = 0.28$; Durbin-Watson = 1.88, $p = 0.02$; n = 46 for both MLRM.

p = 0.04; β = −19.34; p = 0.01, respectively) and the R-squared was 24%. These coefficients indicate that for every additional F in maternal urine (log_{10}) we can expect MDI to decrease by an average of 19.5 scores. In previous studies conducted by our group we reported similar results between F levels in urine in school age children and IQ scores (R^2 = 25%; β = −16.9; p = 0.05) (Rocha-Amador et al., 2007). In the present study the highest value quantified of F in maternal urine was 8.2 mg/l for the 3rd trimester; in other endemic hydrofluorosis areas of Mexico it is possible that women have similar levels of F in urine. It is particularly important because critical periods of development are most likely to occur in utero, the development of the CNS begins in the third week of gestation and continues maturation into adolescence (Selevan et al., 2000); thus F exposure in utero could possible explain the cognitive alterations observed in school age children chronically exposed to this element (Rocha-Amador et al., 2007; Poureslami et al., 2011). Some authors mention that attention, memory and visuospatial organization could be impacted by F exposure (Calderón et al., 2003; Jiang et al., 2014) and recent reports have linked F exposure to attention problems and hyperactivity in adolescents (Malin and Till, 2015).

The BSID-II has been used in other risk settings to test pesticides exposure or low socioeconomic status contribution to children cognitive development (Eskenazi et al., 2006; Kolobe 2004). In this stud, we did not find any differences between MDI and MI (p > 0.05), nevertheless this variable was included in the final models. Regarding PDI and F in maternal urine we did not find any association with F in urine in the 1st and 2nd trimesters before and after performing multivariable modeling (1st β = 6.28, p = 0.48; 2nd β = 5.33, p = 0.48, respectively) (data not shown).

Regarding F levels in tap water mean concentrations for each trimester were: 2.6 ± 1.1 mg/l, 3.1 ± 1.1 mg/l and 3.7 ± 1.0 mg/l respectively. It is worthy to note that over 81.5% of the samples of tap water were above 1.5 mg/l (NOM-127- SSA1-1994) with the highest value of 12.5 mg/l. These higher values are consistent with reports of F in groundwater in the study sites (Hurtado-Jiménez and Gardea-Torresdey, 2005; Rocha-Amador et al., 2007). About maternal exposure to F, the data indicate that over 96.0% had levels of F in urine above 0.65 mg/l (reported in pregnant women exposed to low levels of F in water; Opydo- Szymaczek and Borysewicz-Lewicka, 2005) in the 1st trimester, while in the 2nd and 3rd trimester were 100% and 100% respectively. The F levels in urine were similar as expected in endemic hydrofluorosis areas (F in water 1.7–6.0 mg/l; F in urine 3.58 ± 1.47 mg/l) (Li et al., 2008). Comparing the average values of F in urine by each trimester, we noted that the first two trimesters remained constant (1.9 ± 1.0 mg/l vs 2.0 ± 1.1 mg/l), except for the 3rd trimester the average values were 1.5 times higher (2.7 ± 1.1 mg/l). Some authors mention that metabolism of F change with gestation, decreasing the absorption of F in calcified tissues and decreasing fetal bone calcification at the end of pregnancy (Opydo- Szymaczek and Borysewicz-Lewicka, 2005). It is possible that the observed increment in F in urine for the 3rd trimester could be explained by this mechanism. However, we only get samples for 41.5% of participants. In our study 92.3% of women reported breastfeed their babies and F is poorly transferred from plasma to milk (Ekstrand et al., 1981). However, it is remarkable that 33.8% of women reported drink tap water and 78.4% use it for cooking. The practice of use tap water for drink or cooking is crucial because exposure to F could be increased when the infant change to bottle feeding and starts solid foods. Regarding, bottled water it is important to mention that we have analyzed several brands of this water and 65% of the samples exceeded the 0.7 mg/l value (NOM-041-SSA1-1993) and 22.9% had values over 1.5 mg/l (NOM-127-SSA1-1994) data not shown.

In our study, 33.8% of children were born premature i.e. between weeks 28–36 and had a birth weight lower than 2.5 kg. The incidence of prematurity according to the National Institute of Perinatology is 19.7%. The World Health Organization (WHO) in Mexico reported a rate of 7.3 cases per 100 births (Zamudio et al., 2013); compared with 33.8% of cases per 100 births that we observed in our study. We have 26.5% more cases than expected. A study conducted in and endemic hydrofluorosis area (F water levels on average 4.7 mg/l) reported 25.9% of newborns weighing less than 2.5 kg compared with 6.9% in the control area (F water <0.009 mg/l). A risk of 1.99 (95% CI 1.3-3.67) of having babies with low birth weight (Diouf et al., 2012). None of the mothers involved in this research, reported drink alcohol or smoke during pregnancy; thus the high proportion of low birth weight babies is not explained by these factors. Another fact that stands out, is the high proportion of girls born in the cohort (69.2%) compared to the 51.2% reported by CONAPO for Mexican population. In the present study we did not find differences between gender (p > 0.05).

This study has some limitations including the small sample size of evaluated children, the low participation to provide biological and environmental samples in the last trimester and it was conducted in residents from endemic areas. However, in Mexico F in water remains as the main source of F exposure. In endemic hydrofuorosis areas of Mexico only non-fluorinated salt is distributed according to the NOM-040-SSA1-1993.

5. Conclusion

Due the importance of cognitive development in children and the amount of people (millions) exposed daily; more studies need to be conducted to support the *in utero* exposure of F and effects in young children. Considering previous data supporting the potential neurotoxicity of F in school-age children, preventive measures in affected communities should be implemented (communication programs, treatments water methods and a continuous monitoring to guarantee water quality) to decrease the F intake through drinking water along with an early intervention cognitive program in infants who are at risk of developmental delay or disabilities.

Conflict of interest

None.

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