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In utero exposure to fluoride and cognitive development delay in infants

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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

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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 $98 \pm 3\%$. 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 $97 \pm 6\%$. 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