Environmental Research 150 (2016) 489–495

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Urinary and plasma fluoride levels in pregnant women from Mexico City

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form
16 June 2016
Accepted 28 June 2016
Available online 14 July 2016

Keywords:
Exposure assessment
Biomarkers
Prenatal exposures
Pregnancy
Fluoride
Public health

ABSTRACT

Background: There is need to assess the developmental neurotoxicity of fluoride. Our knowledge of prenatal fluoride exposure is challenged as few population-based studies have been conducted and these generally date back several decades, provide incomplete data on sociodemographic variables, and have methodological limitations.
Objective: To measure urinary and plasma fluoride levels across three time points in pregnant mothers who were enrolled in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) birth cohort study.
Methods: Fluoride levels were characterized in archived urine and plasma from 872 pregnant mothers sampled from the ELEMENT cohort. Various statistical methods were used to analyze the fluoride data with particular consideration for changes across three stages of pregnancy and against sociodemographic variables.
Results: All samples had detectable levels of fluoride. The mean urinary and plasma fluoride levels were 0.91 and 0.0221 mg/L respectively, and these were not statistically different across three stages of pregnancy. Fluoride levels correlated across the stages of pregnancy studied, with stronger correlations between neighboring stages. Urinary fluoride changed as pregnancy progressed with levels increasing until ~23 weeks and then decreasing until the end of pregnancy. For plasma fluoride, there was a decreasing trend but this was not of statistical significance. Creatinine-adjusted urinary fluoride levels did not associate consistently with any of the sociodemographic variables studied.
Conclusions: This study provides the most extensive characterization to date of fluoride exposure throughout pregnancy. These results provide the foundation to explore exposure-related health outcomes in the ELEMENT cohort and other studies.

1. Introduction

Community water fluoridation has been used for nearly 60 years to reduce the prevalence of dental caries. An estimated 210 million people in the United States (CDC, 2013) and millions more across 30+ countries drink water with fluoride levels currently considered optimal for caries prevention (Fawell et al., 2006). In addition to water, population-level exposures in some areas are achieved via the fluoridation of salt (e.g., certain regions of Mexico; Martinez-Mier et al. (2009) and Jimenez-Farfan et al. (2011)) or milk (Petersen et al., 2015; Weitz et al., 2015). Despite clear benefits in relation to dental caries, there remains an intense debate over the safety of fluoridation because of evidence demonstrating that excessive fluoride intake is associated with adverse effects on teeth (DenBesten and Li, 2011), bones (Chachra et al.,

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http://dx.doi.org/10.1016/j.envres.2016.06.046
0013-9351/© 2016 Published by Elsevier Inc.

2008), and childhood cognition (Tang et al., 2008). This controversy, in part, spurred a review of the health literature by the U.S. National Research Council (NRC, 2006). The resulting NRC report called for more research to address the potential impacts of population level fluoride exposure, particularly among vulnerable populations including pregnant women and children.

Based on epidemiological and animal evidence, there is growing concern that fluoride is a developmental neurotoxicant. For example, a systematic review by Choi et al. (2012) that included 27 epidemiological studies found reduced children's intelligence associated with high fluoride exposures. Reports of developmental impacts are plausible given that maternally ingested fluoride has been shown to reach the fetus through the umbilical cord or placenta. Maternal blood fluoride, for example, is moderately correlated with cord blood, indicating that at least some fluoride moves from the maternal compartment to the fetus (Gedalia et al., 1961; Shimonovitz et al., 1995). Fluoride levels in the fetal brain (Du et al., 2008; Narayanaswamy and Piler, 2010) and teeth (Parker and Bawden, 1986) have been shown to increase in parallel with maternal exposures. Taken together, these findings suggest that the fetus is exposed to fluoride, and that maternal fluoride levels can be used as a proxy for fetal exposure. However, very few studies have characterized prenatal exposures to fluoride.

Fluoride exposure in pregnant women, to our knowledge, has been reported in very few population-based studies (Caldera et al., 1988; Gardner et al., 1952; Gedalia et al., 1959; Malhotra et al., 1993). These studies are limited in that, for example, they generally date back several decades, provide incomplete data on socioeconomic or demographic variables that could help interpret exposures, and have methodological limitations (e.g., they lack robust sample sizes, multiple biomarkers, and repeated measures). Given the limitations of the aforementioned studies and a need to better resolve prenatal exposures, the current study aimed to increase our understanding of developmental fluoride exposures by measuring maternal levels of fluoride over the course of pregnancy. To achieve this, we measured urinary and plasma fluoride levels across three time points in pregnant mothers who were enrolled in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) birth cohort study.

2. Methods

2.1. Study population

The institutional review boards of the National Institute of Public Health of Mexico, University of Michigan, Indiana University, the University of Toronto, and participating clinics approved the study procedures. Study participants were recruited between 1997 and 2006 from three clinics of the Mexican Institute of Social Security in Mexico City (Mexico) as part of the ELEMENT study. Pertinent details of the three cohorts that make up the ELEMENT study, including for example recruitment, collection of demographic information such as maternal age, education, marital status and smoking status during pregnancy, and collection and archival of pregnancy urine and plasma can be found elsewhere (Hu et al., 2006; Afeiche et al., 2011). Of particular note is that all urine samples consisted of early morning 2nd voided specimens collected at our study clinic; and all plasma samples were collected using special methods to prevent contamination and hemolysis (Smith et al., 2002).

The ELEMENT population consists of 2161 mothers (Fig. 1). Here, the study population was drawn from women who had prenatal visits associated with archived prenatal urine or plasma sample with adequate volume for additional analyses (i.e., they were drawn from two of the three ELEMENT cohorts). Of the 997

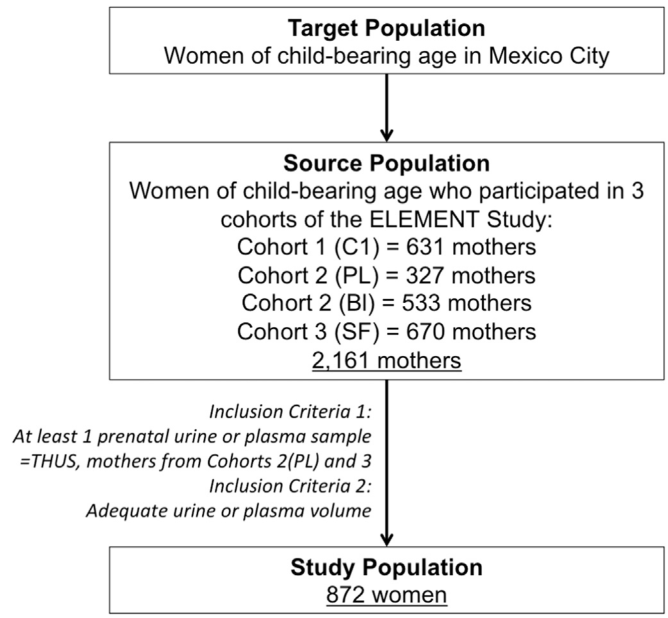

Fig. 1. Flow chart of study population drawn from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort.

women in the source population, 872 contributed at least one urine or plasma sample during the early, mid, and late stages of pregnancy; 825 women contributed a total of 1520 urine samples, and 330 women contributed a total of 627 plasma fluoride samples. The gestational week (i.e., self-reported date of last menstrual period) the samples were donated and the child's sex were recorded.

2.2. Fluoride in urine samples

The free ionic form of fluoride was measured using a micro diffusion method (Martínez-Mier et al., 2011) at the University of Michigan School of Public Health (UMSPH). Briefly, urine was diluted with equal parts Milli-Q water in a petri dish, and allowed to react for 20–24 h with 3 M sulfuric acid saturated with hexamethyldisiloxane (HMDS). The diffused fluoride was collected in 0.05 M of sodium hydroxide that was spotted on the interior of the petri dish cover. Following neutralization with 0.25 M of acetic acid, the concentration of fluoride in this solution was determined using an ion-selective electrode (Orion Fluoride Combination Electrode).

Urinary reference materials obtained from the Institut National de Santé Publique du Québec (INSPQ) were used to gauge analytical accuracy and precision. In addition, each batch run contained procedural blanks and replicate runs. The average recovery rate (analytical accuracy) for the reference materials was 100%. The mean relative standard deviation (analytical precision) for all samples was below 20% for samples containing less than or equal to 0.2 mg/L of fluoride and below 10% for samples with a fluoride concentration above 0.2 mg/L. Note, samples that had a coefficient of variation $>20\%$ were re-analyzed if there was urine remaining. The analytical detection limit (mean: 0.00656 mg/L) was calculated as the mean blank value plus two times the standard deviation.

An additional 390 urine samples were measured for fluoride content at the Indiana University Oral Health Research Institute (OHRI) using a similar procedure detailed in Martínez-Mier et al. (2011) with similar outcomes. Further, a validation study was conducted to determine the degree of similarity between samples measured at UMSPH versus at OHRI. Forty-eight samples were