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Associations between blood metals and fecundity among women residing in New York State

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ABSTRACT

Trace exposures to metals may affect female reproductive health. To assess the relation between trace concentrations of blood metals and female fecundity, 99 non-pregnant women discontinuing contraception for the purpose of becoming pregnant were prospectively followed. Participants completed a baseline interview and daily diaries until pregnant, or up to 12 menstrual cycles at risk for pregnancy; home pregnancy test kits were used. For 80 women, whole blood specimens were analyzed for arsenic, cadmium, lead, magnesium, nickel, selenium and zinc using inductively coupled plasma mass spectrometry (ICP–MS). Time to pregnancy was estimated using Cox proportional hazards models for discrete time. Metal concentrations were generally within population reference intervals. Adjusted models suggest a 51.5% increase in the probability for pregnancy per 3.60 μg/L increase in Mg ($P = 0.062$), and a 27.7% decrease per 0.54 μg/L increase in Zn ($P = 0.114$). Findings indicate that Mg and Zn may impact female fecundity, but in varying directions.

1. Introduction

Environmental factors such as metals and polychlorinated biphenyls (PCBs) may interfere with reproduction in women [1]. Non-essential metals including arsenic (As), cadmium (Cd) and lead (Pb) are widely distributed in the environment and are recognized reproductive toxicants [2]. Exposure to high concentrations of these non-essential toxic trace metals may diminish fecundity, defined as the biologic capacity for reproduction, which is frequently assessed as time-to-pregnancy (TTP) [3–5]. Other metals, including magnesium (Mg), nickel (Ni), selenium (Se), and zinc (Zn) are essential for human reproduction in trace concentrations [6,7]; however, exposure to excess quantities of these elements may also be hazardous [8].

Several metals including As, Cd, Mg, Pb, and Zn have been detected in human follicular fluid underscoring the proximity of these agents to reproductive organs and tissues [9–12]. Studies indicate associations between clinical pregnancy loss and exposure to high concentrations of As (reviewed by [13]); however, there appear to be no data regarding fecundity and As exposure. Among women undergoing *in vitro* fertilization (IVF), inverse associations have been reported between Pb and Cd concentrations and pregnancy rates [14–18]. In contrast, additional studies suggest positive associations between Cd concentrations, in follicular fluid or urine, and successful IVF outcomes [12,14,19]. A recent, small pregnancy cohort study reported no relation between Pb and retrospectively reported TTP [20]. While equivocal, much of the available data relies upon retrospectively reported TTP whose validity is reported to be low [21], or incomplete quantification of metal exposures, representing a critical data gap in our understanding of human reproduction.

Another critical data gap is the absence of information focusing on the relation between the essential trace metals (Mg, Ni, Se and Zn) and female fecundity, despite longstanding recognition of the deleterious reproductive effects associated with under-nutrition [22]. Several authors have reported significant associations between clinical pregnancy loss and Se deficiencies [6,23–26] or excess [8], as well as Zn deficiency (reviewed by [27]).

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Using a prospective pregnancy cohort design with preconception enrollment of women and longitudinal data collection, we sought to address the above data gaps.

2. Materials and methods

2.1. Study cohort

One-hundred thirteen women were recruited from a larger cohort of anglers who participated in a survey about species-specific fish consumption and knowledge of New York State fish consumption advisories in 1991–1992 [28]. Women aged 18–34 years, who reported having not completed childbearing, were re-contacted in 1996–1997 to request participation in a prospective pregnancy study. Two-hundred forty-four eligible women were located, including the 113 women who enrolled in this study. However, 14 women were determined to be pregnant prior to study enrollment and were excluded leaving 99 women in the study cohort. A total of 83 women completed the study of which 80 had sufficient blood volume available for the analysis of metals. Previous analysis demonstrated no difference between excluded women and study participants with regard to reproductive endpoints [29], and we have no reason to suspect differences in blood metals concentrations between these groups.

2.2. Longitudinal data collection

Study participation included completion of a standardized in-person interview administered by a research nurse in the participant's home and completion of daily diaries designed to capture menstruation, sexual intercourse and use of cigarettes and alcoholic and caffeinated beverages. Prior to the study, nurses described and reviewed the 'fertile window' with all women and instructed them in the use of home pregnancy kits. Kits were capable of detecting at least 50 mIU/mL of human chorionic gonadotropin (hCG) with sensitivity and specificity >99%, according to the manufacturer, corresponding to levels expected on the first anticipated day of menstruation following conception. A more in-depth description of this study is provided elsewhere [29]. Upon completion of the interview and instructional session, the nurse obtained a 25 mL non-fasting blood specimen that was processed within hours by the participating toxicology laboratory. The nurses followed universal precautions when collecting blood. All equipment was approved by our laboratory as free from contamination. Participants were censored on the day following their first positive pregnancy test or upon completion of 12 menstrual cycles at risk for pregnancy in the absence of pregnancy. An 'at-risk' cycle denoted at least one act of sexual intercourse in the fertile window, which included the five days before through two days after the day of ovulation as estimated by the Ogino–Knaus method [30,31]. Institutional Review Board approval was granted by the University at Buffalo, State University of New York; women provided informed consent prior to enrollment.

2.3. Toxicologic analysis

Five frozen 1 mL aliquots of whole blood per study participant were analyzed for As, Cd, Mg, Ni, Pb, Se, and Zn concentrations according to previously published standardized operating procedures [32–34]. Our laboratory was unable to assess blood Hg due to insufficient sample availability. Briefly, specimens were analyzed by inductively coupled plasma mass spectrometry (ICP–MS) in a modular class 1000 clean laboratory at the University of Notre Dame (Notre Dame, IN). Specimens were pooled by subject in a pre-cleaned and weighed Teflon-Bomb and recorded accordingly. Specimens were analyzed using external calibration and an internal standardization procedure with monitoring for isotopes: ^{75}As, ^{111}Cd, ^{114}Cd, ^{25}Mg, ^{60}Ni, ^{62}Ni, ^{206}Pb, ^{207}Pb, ^{208}Pb, ^{77}Se, ^{82}Se, ^{63}Zn, and ^{65}Zn. Aliquots of caprine serum spiked with known concentrations of analytes were included with each batch to assess measurement reliability (i.e., quality control (QC) specimens). A procedural blank was included in each run to monitor contamination from reagents and laboratory equipment. A bovine liver standard reference material (National Institute of Standards and Technology (NIST) SRM 1577b) was also analyzed with each run to evaluate the accuracy and precision of the technique. Measured values were within the recommended error for the certified values from the NIST in 10 independent digestions and analyses.

Limits of detection (LODs) for metal analytes were calculated as the concentration equivalent to three times the standard deviations of the measured intensities for calibration blanks. These were expressed as μg/L whole blood; 0.005 for As, 0.003 for Cd, 0.006 for Pb, 0.06 for Mg, 0.003 for Ni, 0.012 for Se and 0.03 for Zn. Mean relative standard deviations across and within batches were less than 10% for most analytes including: 6.6% and 5.9% for As, 2.7% and 2.4% for Cd, 5.3% and 8.3% for Pb, 3.0% and 5.7% for Mg, 12.8% and 14.8% for Ni, 5.1% and 5.9% for Se, and 4.9% and 2.4% for Zn, respectively. Concentrations were reported as μg/L whole blood.

2.4. Statistical analysis

2.4.1. Descriptive and bivariate analyses

Descriptive statistics were utilized to inspect the data and to aid in specifying models. All covariates captured by the daily diaries were standardized to a 28-day cycle to address the inherent variability in menstrual cycle length within and across women, and to account for varying TTPs. Distributions were characterized and metals summarized as geometric means and standard deviations and, subsequently, log-transformed to meet normality assumptions. For metals with an available population reference interval, proportions of values above and/or below the interval were compared for participants with a positive pregnancy test and participants without a positive pregnancy test. For metals with no available population reference interval, proportions of values above the 90th percentile and below the 10th percentile were assessed for participants with and without positive pregnancy tests. Statistical significance was defined as $P < 0.05$ for a two-tailed test based upon the χ^2-test for categorical variates, ANOVA for continuous variables including metals and PCBs, or the Kruskall–Wallis test for ordinal variables as appropriate. Correlations among metals and PCBs were assessed using Pearson correlation coefficients. All analyses were conducted using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC).

2.4.2. Multivariable analysis

A Cox proportional-hazards regression model for discrete-time data [35] was used to estimate the effect of individual metal concentrations on TTP. Models included metals following log transformation and potential confounders selected for inclusion using a model fitting algorithm intended to maximize fit inclusive of age (continuous), parity (0 vs. 1+), groupings of PCB congeners (estrogenic PCBs, anti-estrogenic PCBs and 'other' PCBs in μg/L serum), serum lipids (mg/dL serum), frequency of intercourse during the fertile window, and use of cigarettes and alcohol standardized to a 28-day cycle. A previously published comprehensive analysis of PCB data suggested that biologic activity groupings [36] of PCB congeners with estrogenic properties (i.e., IUPAC #s 4_10, 5_8, 15_17, 18, 31, 44, 47, 48, 52, 70, 99, 101, 136, 153 and 188) and anti-estrogenic properties (i.e., IUPAC #s 77_110, 105, 114, 126, 171_156 and 169) were inversely and independently associated with TTP [29]. Effects were expressed in terms of model coefficients and their corresponding 95% confidence intervals (CI) in which a positive value for β indicates a shorter TTP, whereas a negative value for β indicates a longer TTP. To facilitate interpretation of these effect estimates in the context of the range of values in the study sample, model coefficients were 'back-transformed' to an arithmetic scale, and expressed as the percent change in the conditional probability for a positive pregnancy test, in any given at risk cycle, corresponding to a single interquartile range (IQR) increase in the value of a predictor. The IQR was defined as the difference between the 75th percentile and the 25th percentile for the sample distribution of a variate.

3. Results

3.1. Characteristics of the study sample

The 80 participating women with blood metals data available contributed 387 'at risk' cycles for analysis including 27 women who contribute ≤1 cycle, 10 who contribute 2 cycles, 7 contributing 3 cycles, 16 women contributing 4–6 cycles, and 20 women contributing >6 cycles. Fifty-nine (74%) of these women became pregnant while under observation; 46 (78%) pregnancies resulted in live births while 13 (22%) ended in losses at a median of 7 days gestation. Ten (12.5%) women did not conceive during the study period, and 11 women withdrew without pregnancy after contributing a mean (±SD) of 4.7 (±4.4) cycles.

Overall, few differences are observed in either the baseline or daily diary characteristics of the cohort between women with a positive pregnancy test and those without as shown in Table 1. A difference is detected for cigarette smoking ($P = 0.007$) and suggested for parity ($P = 0.060$). First, women with positive pregnancy tests reported significantly more cigarettes smoked per cycle while attempting pregnancy than women not achieving pregnancy (i.e., approximately 187 and 22, respectively). Second, restricted to the 60 study participants who reported a pregnancy prior to the inception of this study, 96% of women with a positive pregnancy test had one or more prior births compared with only 4% of women without a positive pregnancy test.

3.2. Concentrations of blood metals

With the exception of Mg, no differences are observed in the geometric means for the metals and pregnancy despite all concentrations having been measured above the limits of detection. In addition, again with the exception of Mg, no statistically significant differences are detected in the distribution of values above or below the reference limits, the 90th percentiles, or the 10th