Reproductive Toxicology 69 (2017) 68–74

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Low-level environmental metals and metalloids and incident pregnancy loss

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ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form
20 December 2016
Accepted 23 January 2017
Available online 2 February 2017

Keywords:
Arsenic
Cadmium
Epidemiology
Lead
Mercury
Pregnancy
Miscarriage
Spontaneous abortion

ABSTRACT

Environmental exposure to metals and metalloids is associated with pregnancy loss in some but not all studies. We assessed arsenic, cadmium, mercury, and lead concentrations in 501 couples upon trying for pregnancy and followed them throughout pregnancy to estimate the risk of incident pregnancy loss. Using Cox proportional hazard models, we estimated hazard ratios (HR) and 95% confidence intervals (CIs) for pregnancy loss after covariate adjustment for each partner modeled individually then we jointly modeled both partners' concentrations. Incidence of pregnancy loss was 28%. In individual partner models, the highest adjusted HRs were observed for female and male blood cadmium (HR = 1.08; CI 0.81, 1.44; HR = 1.09; 95% CI 0.84, 1.41, respectively). In couple based models, neither partner's blood cadmium concentrations were associated with loss (HR = 1.01; 95% CI 0.75, 1.37; HR = 0.92; CI 0.68, 1.25, respectively). We observed no evidence of a significant relation between metal(loids) at these environmentally relevant concentrations and pregnancy loss.

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1. Introduction

Contemporary human populations remain at risk for exposure to various environmental metals and metalloids through geographical [1], occupational [2,3] or accidental contamination pathways [4]. Published reviews have focused on the reproductive and developmental toxicity (RADT) of individual metals and metalloids for human populations, including cadmium [5,6], lead [7], mercury [8], and arsenic [9,10]. Pregnancy loss is often considered when assessing the RADT of metal(loid)s, given that it represents embryonic or fetal mortality and has a high (25%–31%) incidence when measured prospectively [11–14]. A recent review evaluated the collective evidence on the RADT of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and mercury (Hg) and concluded that all but Hg were associated with spontaneous abortion [15]. Another review focusing specifically on low levels of metal(loid) exposures and male fertility concluded that evidence was strongest for Cd, Hg and Pb with less certainty for As [16]. Both reviews noted key methodologic limitations that impact the weighing of evidence, particularly very few prospective cohorts with exposures quantified during critical windows of human reproduction and development and adequate attention to potential confounders.

To date, we are unaware of research focusing on male and female partners' exposures and pregnancy loss despite the couple dependent nature of pregnancy. In contrast to the ubiquitous nature of environmental metals and their designation as endocrine disrupters (EDCs) [17], or exogenous agents that can interfere with any aspect of hormone action [18], there are few epidemiologic studies focusing on metals at environmentally relevant concen-

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http://dx.doi.org/10.1016/j.reprotox.2017.01.011
0890-6238/Published by Elsevier Inc.

trations. To date, much of our understanding about their RADT stems from an important literature focusing on higher exposed populations, such as occupational workers or residents of endemic geographic areas. Moreover pregnancy loss, *per se*, is not directly measured in most animal research, but rather relies on proxies such as resorption or late fetal deaths. Timing of demise is challenging to determine without prospective measurement. This data gaps precludes our understanding of the impact of metal(loids) on pregnancy loss among populations with environmentally relevant concentrations. Such concentrations are typical of those for most populations without unique occupational or geographical exposures, such as residence in an endemic region. In light of the importance of metal(loid) exposures during critical and sensitive windows of development for pregnancy outcomes, we sought to empirically assess these associations. Motivated by this gap, we sought to explore the individual relationships between environmentally relevant concentrations of three metals (cadmium, lead, mercury), one metalloid (arsenic) and incident pregnancy loss in a cohort of couples prospectively followed across sensitive windows of human reproduction.

2. Materials and methods

2.1. Study population and participants

The study's referent cohort is the LIFE Study that comprised 501 reproductive aged couples who were recruited in 2005–2009 upon discontinuing contraception with the intent of becoming pregnant. Using population-based sampling frameworks, we recruited couples from 16 counties in Michigan and Texas of which 42% of eligible individuals enrolled in the study. Complete details are provided elsewhere [19]. By design, inclusion criteria were minimal: female partners aged 18–40 and male partners aged ≥18 years who were in a committed relationship; no physician diagnosis of infertility/sterility; off contraception <2 months; and an ability to communicate in English or Spanish. Female partners also had to have menstrual cycles ranging between 21 and 42 days as required by the fertility monitor and without the use of injectable hormonal contraceptives in the past year given the uncertain timing for ovulation return. This interval captures the majority of menstruating women in light of its 21-day span. Of the 501 participating couples, the study cohort was restricted to the 344 (68%) pregnant couples (n = 3 multiple gestation pregnancies were excluded), since pregnancy is a necessary criterion for loss. All pregnant women were followed through delivery.

2.2. Data collection

Data collection was multi-faceted and included in-person interviews with each partner, standardized anthropometric assessment for the estimation of measured body mass index (BMI), daily preconception journals that captured couples' lifestyles and home pregnancy test results for female partners. In addition, female partners completed daily early pregnancy journals through 7 weeks post-conception then monthly journals until a loss or delivery. The preconception and early pregnancy (7 post-conception weeks) journal data on lifestyle were used to estimate female partners' daily cigarette smoking and alcohol consumption during these sensitive windows of human reproduction and development. Male partners only completed daily journals during the preconception window. Approximately 80% of women completed daily journals for the first 7-weeks post-conception and monthly, thereafter, until delivery. All participating research sites received full institutional review board approval, and couples gave written informed consent at enrollment and before any data collection.

2.3. Estimation of conception, pregnancy and loss

Female partners used the Clearblue® Fertility Monitor (Inverness Medical Innovations, Waltham, MA), a urinary based home kit that is intended to help women time intercourse to ovulation to maximize their chances of becoming pregnant. When prompted, the women tested their urine for the detection of estrone-3-glucuronide and luteinizing hormone (LH), which gave a 'high' or 'peak' fertility prompt on the monitor's display, respectively. The accuracy of the fertility monitor for detecting the LH surge is 99% [20]. Pregnancy was prospectively captured by women's use of the Clearblue® digital home pregnancy test, which is sensitive in detecting 25 mIU/mL of human chorionic gonadotropin (hCG) and accurately used by women [21]. This pregnancy test kit has a low (0–0.3%) false positive rate [22]. Depending upon timing of loss, it was detected by conversion to a negative pregnancy test, clinical confirmation or return of menses. We use the term pregnancy loss rather than spontaneous abortion or stillbirth, terms typically defined by estimated gestation, to remove assumptions about the etiologic role of metals in embryonic/fetal mortality, the uncertain timing of actual pregnancy demise, and in light of no standardized endocrine criteria for defining loss [23].

2.4. Trace element analyses

At the enrollment home visit, the research nurse obtained whole blood specimens from all participants using supplies pre-screened and certified for trace element analysis. All blood specimens were analyzed for cadmium (Cd), lead (Pb) and mercury (Hg) in the Inorganic and Radiation Analytical Toxicology Branch, at the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC). The analytical method for Pb, Cd and Hg in blood performed at CDC is based on inductively coupled plasma mass spectrometry (ICP-MS), and has been fully validated for use in establishing reference values for the US population via the National Health and Nutrition Examination Surveys [24]. In brief, whole blood is diluted with an alkaline reagent containing appropriate internal standards and analyzed for Pb, Cd and Hg using a PerkinElmer ELAN DRC II ICP-MS (PerkinElmer, Shelton, CT) calibrated with NIST-traceable standards. Method accuracy is assured via analysis of NIST SRM 955c Toxic Elements in Caprine Blood, which is certified for Cd, Pb and Hg at 4 concentration levels. Ongoing laboratory performance is monitored via satisfactory participation in numerous proficiency testing (PT) programs for trace elements in blood, including those operated by Centre de toxicolgie du Québec, and The New York State Department of Health. Typical limits of detection are 0.25 μg/dL for blood Pb, and 0.16 μg/L for both Cd and Hg in blood. In addition to blood, participants also provided a urine specimen that was analyzed for As, Cd and Pb in the Laboratory of Inorganic and Nuclear Chemistry at the Wadsworth Center, New York State Department of Health (NYSDOH) using well-established methods based on ICP–MS [25]. In brief, the Wadsworth Center lab also uses a PerkinElmer ELAN DRC II ICP-MS calibrated with NIST-traceable standards. Method accuracy is assured via analysis of NIST 2668 Toxic Elements in Frozen Human Urine, which is certified for As, Cd and Pb at two mass concentrations. Ongoing laboratory performance is monitored via satisfactory participation in numerous proficiency testing (PT) programs for trace elements in blood, including those operated by Centre de toxicolgie du Québec, the UK Trace Elements External Quality Assessment Scheme, and The New York State Department of Health's PT program for Trace Elements. Typical limits of detection for As, Cd and Pb in urine are 1 μg/L, 0.02 μg/L, and 0.7 μg/L respectively. For both blood and urine, all instrument derived data, including zeros and negative values, were used for statistical anal-