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Maternal and infant inflammatory markers in relation to prenatal arsenic exposure in a U.S. pregnancy cohort

Shohreh F. Farzan[a,*], Elizabeth B. Brickley[b], Zhigang Li[b], Diane Gilbert-Diamond[b], Anala Gossai[b], Caitlin G. Howe[a], Thomas Palys[b], Yu Chen[c], Margaret R. Karagas[b]

[a] Department of Preventive Medicine, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA
[b] Children's Environmental Health & Disease Prevention Research Center at Dartmouth, Hanover, NH, USA and Department of Epidemiology, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA
[c] Department of Population Health, New York University School of Medicine, New York, NY, USA

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ABSTRACT

Introduction: Accumulating evidence indicates that arsenic (As), a potent environmental toxicant, may increase cardiovascular disease risk and adversely affect endothelial function at high levels of exposure. Pregnancy is a vulnerable time for both mother and child; however, studies examining the association between prenatal As exposure and plasma biomarkers of inflammation and endothelial function in mothers and newborns are lacking.
Methods: We examined maternal urinary As levels at gestational weeks 24–28 and levels of inflammatory biomarkers in plasma from 563 pregnant women and 500 infants' cord blood. We assessed a multiplexed panel of circulating inflammatory and endothelial function markers, including tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein 1 (MCP1), intercellular adhesion molecule (ICAM1) and vascular cell adhesion molecule (VCAM1).
Results: Compared with the bottom tertile, the highest tertile of maternal urinary As during pregnancy was associated with a 145.2 ng/ml (95% CI 4.1, 286.3; p = 0.04) increase in cord blood ICAM1 and 557.3 ng/ml (95% CI −56.4, 1171.1; p = 0.09) increase in cord blood VCAM1. Among mothers, the highest tertile of maternal urinary As during pregnancy was related to a 141.8 ng/ml (95% CI 26.1, 257.5; p = 0.02) increase maternal plasma VCAM1 levels. Urinary As was unrelated to MCP1 or TNFα in maternal plasma and cord blood. In structural equation models, the association between maternal urinary As and infant VCAM was mediated by maternal levels of VCAM ($\beta_{mediation}$: 0.024, 95% CI: 0.002, 0.050).
Conclusion: Our observations indicate that As exposure during pregnancy may affect markers of vascular health and endothelial function in both pregnant women and children, and suggest further investigation of the potential impacts on cardiovascular health in these susceptible populations.

1. Introduction

Arsenic (As) exposure continues to be a major public health concern across the globe. Worldwide, the main source of As exposure is contaminated groundwater, with an estimated 200 million individuals exposed to levels exceeding the World Health Organization safety standard and US EPA maximum contaminant level (MCL) of 10 μg/L. (World Health Organization (WHO), 2011; International Agency for Research on Cancer IARC, 2004; Naujokas et al., 2013) In the US, nearly 44.5 million people rely on private wells as their primary water source and an estimated 7% of these systems exceed the As MCL. (National Research Council, 2014; Ayotte et al., 2011; Maupin et al., 2014) Furthermore, a growing number of studies have raised concerns about certain foods, including rice and rice products, as sources of As exposure. (Cottingham et al., 2013; Davis et al., 2012; Gilbert-Diamond et al., 2011; Nachman et al., 2013; Wong et al., 2013).

Arsenic's toxicity at high levels of exposure is well documented. Known primarily for its role as a potent carcinogen of the bladder, skin, kidney, liver and lung, As has also been associated with a multitude of adverse health effects, including respiratory disease, diabetes, neurological impairment, and immune dysfunction. (Naujokas et al., 2013; National Research Council, 2014) Epidemiological evidence further supports a relationship between As exposure and cardiovascular effects and pregnant women and children may be especially vulnerable to

* Correspondence to: Division of Environmental Health, Department of Preventive Medicine, Keck School of Medicine of University of Southern California, 2001 N. Soto Street, MC 9237, Los Angeles, CA 90089, USA.
E-mail address: sffarzan@usc.edu (S.F. Farzan).

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these effects. (Abhyankar et al., 2012; Chen et al., 2011, 2013; Farzan et al., 2015a, 2015b, 2013; Moon et al., 2013; Wu et al., 2014) We previously reported that women with higher urinary As levels during pregnancy had greater increases in blood pressure over the course of pregnancy. Farzan et al. (2015b) In children, early life As exposure has been associated with early indicators of cardiovascular risk, including increased blood pressure, as well as carotid intima media thickness (cIMT). (Hawkesworth et al., 2012; Osorio-Yanez et al., 2013, 2015).

Arsenic-related cardiovascular dysfunction may occur by a number of pathways, such as increased inflammation, cytokine induction and production of reactive oxygen species, which can each affect endothelial activation and function. (Wu et al., 2014; States et al., 2009) Cellular damage or toxic insults to the vascular endothelium activate a signaling cascade, triggering expression of pro-inflammatory mediator tumor necrosis factor alpha (TNFα). TNFα induces inflammatory responses by promoting secretion of cytokines and activating endothelial cells by promoting expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM1) and vascular adhesion molecule-1 (VCAM1) on their surface. (Modur et al., 1996; Gerszten et al., 1999) Adhesion molecules allow mononuclear cells to attach to the endothelium, a key step in atherosclerotic initiation. In experimental studies, As elevates circulating vascular inflammatory marker levels, including ICAM1 and VCAM1 (Hou et al., 2005; Soucy et al., 2005; Lemaire et al., 2015), increases monocyte adhesion to the vascular endothelium via increased binding to VCAM1 (Lemaire et al., 2015) and increases atherosclerotic lesion formation, along with expression of inflammatory mediators, such as monocyte chemoattractant protein 1 (MCP1), at vascular lesion sites. Srivastava et al. (2009) Levels of these circulating markers may predict future cardiovascular disease risk (Blankenberg et al., 2001; Haim et al., 2002; Hwang et al., 1997; Ridker et al., 1998) and of these, soluble VCAM1 and ICAM1 have been consistently related to chronic As exposure in studies among adults in Bangladesh. (Wu et al., 2012; Chen et al., 2007).

However, very little is known about whether arsenic may affect these particular markers, which may indicate cardiovascular health, in either children or pregnant women. A recent study from an Iranian birth cohort found positive associations between ambient air pollutants and cord blood ICAM1, VCAM1 and endothelian-1, suggesting that these markers may be impacted by environmental exposures. Poursafa et al. (2016) We therefore hypothesized that As exposure may be associated with increases in plasma and cord blood levels of markers of endothelial dysfunction (VCAM1, ICAM1), and mediators of the endothelial inflammatory response (MCP1, TNFα) among mother-infant pairs enrolled in the New Hampshire Birth Cohort Study.

2. Methods

2.1. The New Hampshire Birth Cohort

The New Hampshire Birth Cohort is an ongoing study that began in January 2009, recruiting 18–45 year old pregnant women receiving prenatal care at study clinics, as previously described. Gilbert-Diamond et al. (2011) Women were enrolled at 24–28 weeks gestation if they reported using water from a private well at their residence since their last menstrual period and were not planning to move prior to delivery. Only singleton births were included in the study. All protocols were approved by the Dartmouth College Institutional Review Boards. Participants provided written, informed consent upon enrollment.

2.2. Medical record review

Participants completed a detailed medical history and lifestyle questionnaire upon enrollment, which ascertained sociodemographic factors (age, race/ethnicity, marital status, education), reproductive history (previous pregnancies, complications, birth outcomes), and health history. Women were asked about habits, including tobacco and alcohol use, along with their home water source and consumption. At two weeks postpartum, mothers were sent a follow-up questionnaire to obtain additional information about pregnancy, delivery and changes in key exposures. Participants also consented to a medical record review, which allowed additional information to be recorded about prenatal infections, medication use, birth outcomes and delivery details, and general health of the women and their infants after birth.

2.3. Arsenic exposure assessment

Women provided a spot urine sample upon enrollment at 24–28 weeks gestation, which was collected and stored, as previously described. Gilbert-Diamond et al. (2011) Urine samples were analyzed for levels of arsenite (iAs^{III}), arsenate (iAs^{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine by high-performance liquid chromatography (HPLC) inductively coupled plasma mass spectrometry (ICP-MS) at the University of Arizona Hazard Identification Core. (Le et al., 2000; Wei et al., 2001; Larsen et al., 1993) Total urinary As was calculated by summing inorganic ($iAs = iAs^{III} + iAs^{V}$) and organic (DMA, MMA) metabolites. Gilbert-Diamond et al. (2011) Arsenobetaine, a form of As found in fish and seafood was excluded, as it is thought to pass through the body unmetabolized. Tseng (2009) Urine samples that registered below the As detection limit (ranging from 0.10 to 0.15 μg/L for individual urine species; 14.0%, 20.8%, and 40.3% of the study population were below the detection limit for DMA, MMA and iAs, respectively) were assigned a value equal to the detection limit divided by the square root of two. Participants were given instructions and prepaid mailing materials upon enrollment to collect samples of their home tap water and return the samples to the study office, which were analyzed by high resolution inductively coupled plasma mass spectrometry (ICP-MS) at the Dartmouth Trace Element Analysis Core, as previously described. Gilbert-Diamond et al. (2011) Water arsenic detection limits ranged from 0.005 to 0.074 μg/L.

2.4. Plasma and cord blood inflammatory markers testing

We selected the first 564 mothers with adequate plasma sample volumes for testing and an available maternal urine sample for As assessment. A paired infant cord blood sample was available for 500 women. A total of 563 mothers and 500 infants were successfully assayed for one or more markers.

Maternal and infant circulating protein marker levels were measured using MILLIPLEX-MAP human magnetic bead multiplexed panels (Millipore, Billerica, MA), according to manufacturers instructions. MCP1 and TNFα were assayed using undiluted plasma samples and had minimum assay sensitivities of 1.9 and 0.7 pg/ml for MCP1 and TNFα, respectively. Plasma samples assayed for soluble ICAM1 and VCAM1 were diluted 1:100 and had minimum assay sensitivities of 0.019 and 0.024 ng/ml for ICAM1 and VCAM1, respectively. Calibration curves of recombinant standards were prepared with three-fold dilution steps in the same matrix as the samples. Standards were measured in triplicate, samples were measured once, and blank values were subtracted from all readings. All assays were carried out directly in a 96-well filtration plate (Millipore) at room temperature and protected from light. Briefly, wells were pre-wet with 100 μl PBS containing 1% BSA. Then, 100 μl volume of beads and either a standard, sample, spikes, or blank were added and incubated at room temperature for 30 min with continuous shaking. Beads were washed with 100 μl PBS containing 1% BSA and 0.05% Tween 20. A cocktail of biotinylated antibodies (50 μl/well) was added to beads for a further 30 min incubation with continuous shaking. Beads were washed, then streptavidin-phycoerythrin was added for 10 min. Beads were again washed and resuspended in 125 μl of PBS containing 1% BSA and 0.05% Tween 20. The fluorescence intensity of the beads was measured using a Bio-Plex Array Reader (Bio-Rad Laboratories, Hercules, CA) and analyzed using Bio-Plex Manager software with five-parametric curve