Journal of Exposure Science and Environmental Epidemiology (2015) 25, 639–648

www.nature.com/jes

ORIGINAL ARTICLE

Maternal–infant biomarkers of prenatal exposure to arsenic and manganese

Ema G. Rodrigues[1], Molly Kile[2], Christine Dobson[1], Chitra Amarasiriwardena[3], Quazi Quamruzzaman[4], Mahmuder Rahman[4], Mostofa Golam[4] and David C. Christiani[1]

Because arsenic (As) and manganese (Mn) are able to pass the placenta, infants among exposed populations may be exposed to considerable levels *in utero*. The main objective of this paper is to evaluate infant toenails, hair, and cord blood as biomarkers of prenatal exposure to As and Mn and determine the relationship between maternal and infant As and Mn concentrations in these biomarkers. Of the 1196 pregnant women in Bangladesh who were monitored throughout pregnancy until 1 month post-partum and completed all study visits, we included 711 mother–infant pairs who had at least one maternal and one infant biomarker of exposure available for analysis. Toenail and hair samples were collected from the women during the first trimester and 1 month post-partum and from the infants at the age of 1 month. Cord blood was collected at the time of delivery. Maternal toenail concentrations were correlated with infant toenail concentrations for As and Mn ($n = 258$, $r = 0.52$, 95% CI: 0.43–0.60, $P < 0.0001$ and $r = 0.39$, 95% CI: 0.28–0.49, $P < 0.0001$), respectively. Similarly, maternal hair concentrations were correlated with infant hair As ($n = 685$, $r = 0.61$, 95% CI: 0.56–0.65, $P < 0.0001$) and infant hair Mn ($n = 686$, $r = 0.21$, 95% CI: 0.14–0.28, $P < 0.0001$). Cord blood As was correlated with infant toenail and hair As, although cord blood Mn was only correlated with infant toenail. Toenails and cord blood appear to be valid biomarkers of maternal–fetal transfer of As and Mn, whereas hair may not be a suitable biomarker for *in utero* exposure to Mn.

Journal of Exposure Science and Environmental Epidemiology (2015) **25,** 639–648; doi:10.1038/jes.2015.45; published online 26 August 2015

Keywords: arsenic; cord blood; fetal transfer; manganese; toenails

INTRODUCTION

Concentrations of arsenic (As) and manganese (Mn) in Bangladesh's drinking water have been of great public health concern with ~31% of water samples exceeding 50 μg/l of arsenic (WHO's guideline is 10 μg/l) and 42% exceeding WHO's former health-based guideline of 400 μg/l of manganese (as of 2011, there is no WHO guideline for Mn).[1–4] Several studies have reported health effects associated with exposure to As and Mn in drinking water among children in Bangladesh. Specifically, As exposure via drinking water was negatively associated with intellectual function, measured as Performance and Processing speed raw scores among 6-year-old children[5] and Performance and Full-Scale raw scores among 10-year-old children.[6] High water Mn concentrations (>400 μg/l) have also been associated with infant mortality,[7] and lower mathematics achievement test scores among children 8- to 11-year old.[8] In addition, significant associations between water Mn and negative classroom behaviors, measured as Teacher's Report Form externalizing and internalizing scores, have been reported.[9]

Although many studies have investigated the effects of childhood exposures to As and Mn, prenatal exposure to these metals is also of concern because As and Mn readily pass the placenta.[10,11] Data collected in epidemiological studies show that maternal blood and cord blood As concentrations are highly correlated ($r = 0.85$ and $r = 0.84$) with stronger associations observed among those exposed to higher levels of As compared with those exposed to lower levels ($r = 0.86$ *vs* 0.60).[11,12] Conversely, correlations between maternal blood and cord blood Mn concentrations are weaker ($r = 0.28$ and $r = 0.38$).[10,13,14] It is unclear if As concentrations are higher in maternal or cord blood but studies consistently show that cord blood Mn concentrations are higher than maternal blood Mn concentrations.[10,13–15] Unlike As, Mn is an essential nutrient that is required for fetal development and may impact various biomarker concentrations.

With the use of cord blood metal concentrations, several studies have shown that infants are exposed to As and Mn *in utero* and that such exposures are associated with newborn and child health including but not limited to neurodevelopment outcomes.[16–18] In addition to cord blood, infant hair and nails may serve as good biomarkers of *in utero* exposure. Hair and nails are keratinous tissues, which have a greater affinity for As than any other tissue, and their growth begins in the third month of gestation, making it a suitable biomarker of *in utero* exposure.[19] In comparison to blood and urine, nails and hair provide a better measure of long-term exposure, as once these tissues are formed, they are removed from the body's blood stream and metabolism.[20] Significant correlations have been observed between hair and

[1]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; [2]Department of Public Health, College of Public Health and Human Sciences, Oregon State University, Corvallis, Oregon, USA; [3]Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA and [4]Dhaka Community Hospital, Dhaka, Bangladesh. Correspondence: Dr. Ema G. Rodrigues, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Avenue, Building 1, Room 1404F, Boston, MA 02115, USA.
Tel.: +508 264 5445. Fax: +617 432 0219.
E-mail: emarod@hsph.harvard.edu
Received 19 December 2014; accepted 30 June 2015; published online 26 August 2015

toenail As concentrations among adults exposed to As-contaminated drinking water in Bangladesh.[21] Previous studies of mother–infant pairs have reported statistically significant correlations between maternal and infant toenail As concentrations, concluding that infant toenails may be a reliable biomarker of *in utero* exposure to As.[22] Hair has been used as a biomarker of exposure to Mn among children exposed to ferroalloy plant emissions.[23] In addition, maternal hair Mn concentrations have been positively associated with drinking water Mn concentrations during pregnancy[24] and both As and Mn hair concentrations have been quantified in mother–infant pairs in Iran showing that hair may also serve as a reliable biomarker of *in utero* exposure.[25]

The main objectives of this paper are to (1) characterize *in utero* exposure to arsenic and manganese using cord blood, infant hair and toenails as biomarkers of exposure, (2) assess the relationship between maternal and infant biomarkers of exposure to arsenic and manganese, and (3) compare the use of cord blood and infant hair and toenails as biomarkers of *in utero* exposure to arsenic and manganese among a population in Bangladesh.

MATERIALS AND METHODS

Study Design and Population

A prospective birth cohort study was conducted in the Sirajdikhan and Pabna regions of Bangladesh from 2008 to 2011 to investigate the effects of chronic arsenic exposure via drinking water and reproductive health outcomes. Specific recruitment methods are described by Kile et al.[26] Briefly, this study was conducted in collaboration with Dhaka Community Hospital clinics in the two regions where pregnant women were invited to participate in the study if they were 18 years of age or older, had a confirmed singleton pregnancy of less than 16 weeks gestation, used a tubewell as their primary drinking water source, planned to live at their current residence for the duration of the pregnancy, planned to continue prenatal health care with DCH, and agreed to deliver at DCH or at home with a DCH-trained midwife.

The study included four scheduled visits throughout for each participant. The first visit (V1), the time of enrollment into the study, occurred at $\leq$16 weeks of gestation. The second visit (V2), third visit (V3), and fourth visit (V4) were at 28 weeks gestational age, delivery, and 1-month postpartum, respectively. All women received free prenatal care from DCH and prenatal vitamins throughout their pregnancy.

This study was approved by the Human Research Committees at the Harvard School of Public Health (HSPH), Dhaka Community Hospital, and informed consent was obtained from each participant before any data collection.

Questionnaires and Medical Forms

Detailed questionnaires were administered to each participant during V1, V2, and V4. We collected information regarding demographics, medical history, pregnancy history, pregnancy symptoms, diet, and drinking water history. In addition, sonograms were performed at V1 and V2 and medical forms were completed documenting the gestational age and status of the fetus. At V3, information regarding the birth, such as infant sex, birth weight and length, and type of delivery, were recorded.

Water Samples

Arsenic and manganese were measured in personal drinking water samples collected at V1 and V4. Approximately 50 ml of water was collected in a polyethylene tube and preserved with ultra-pure nitric acid. Samples were shipped at room temperature to HSPH where they were aliquoted and sent to Environmental Laboratory Services for analysis by inductively coupled plasma-mass spectrometry following US EPA method 200.8 (Environmental Laboratory Services, North Syracuse, New York, NY, USA). Nineteen percent of the water samples were below the limit of detection (LOD) of 1 μg As/l and were re-assigned a value equal to the LOD divided by $\sqrt{2}$.

Hair and Toenail Samples

Hair and toenail samples were collected from the pregnant participants at V1 and V4, and they were collected from the infants at V4. Stainless steel scissors were used to clip all toenails, and they were placed in a labeled paper envelope and stored at room temperature. Titanium nitride scissors were used to cut ~50 strands of hair from the nape of the neck of each participant, as close to the scalp as possible. Hair samples were similarly placed in a labeled paper envelope and stored at room temperature until shipped to HSPH for analysis.

Samples were processed using a microwave-assisted (GE 1.5 KW microwave oven, General Electric) acid digestion method described by Chen et al.[27] Briefly, samples were placed in glass beakers with 1% Triton X-100 solution (Sigma-Aldrich, St. Louis, MO, USA) and washed for 20 min in an ultrasonic bath and then rinsed several times with deionized water to remove the Triton solution. Once cleaned, samples were dried overnight in a 60 °C drying oven (Fisher Scientific Model 650G Isotemp oven). Once cooled, the dried samples were weighed, using an analytical scale with capacity of up to 210 g and readability of 0.1 mg (Explorer E10640, OHaus Corporation, Parsippany, NJ, USA), into 23-ml PTFE sample cups (Parr Instrument Company, Moline, IL, USA) and were digested using ultra-high-purity nitric acid (BDH Aristar Ultra, VWR International) and then diluted with deionized water to achieve ~8% (vol/vol) in nitric acid. To minimize the effect of static electricity when weighing the hair samples, we used sterile disposable nylon forceps (Tradewinds Direct) to transfer the hair sample to the sample cup.

Samples were digested in batches that included a method blank, certified human hair reference material (CRM Hair; Shanghai Institute of Nuclear Research, Academia Sinica, China), and 21 samples. The 21 samples in each batch included the maternal sample at V1, the maternal sample at V4, and the infant sample for seven mother–infant pairs. Digested samples were analyzed for As and Mn using a Perkin Elmer Model Elan DRC-II 6100 Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Perkin Elmer, Shelton, CT, USA). All sample analytical values were blank-corrected. To correct for inter-batch differences in instrument performance, sample analytical values were further adjusted by a factor equal to the inverse of the batch-specific Hair CRM % recovery (mean % recovery for As: 76%, mean % recovery for Mn: 90%). The sample LODs were dependent on both the instrument LOD and the sample weight, and mean LODs were 0.04 μg/g for toenail As, 0.10 μg/g for toenail Mn, 0.03 μg/g for hair As, and 0.09 μg/g for hair Mn. The percent relative standard deviation (%RSD) for the samples were 5.7%, 6.1%, 1.7%, and 1.9% for hair As, toenail As, hair Mn, and toenail Mn, respectively. Hair and toenail samples with a mass $<$5 mg were excluded from all statistical models because of unreliable measurement. For sample concentrations that were below the LOD, the value was re-assigned as the LOD divided by $\sqrt{2}$.

Cord Blood

Cord blood was collected at the time of delivery into a trace metal-free Vacutainer tube containing K2EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and stored at 4 °C until shipped to HSPH for storage and analysis. Cord blood was analyzed by ICP-MS following an acid digestion method. Approximately 1 g of blood was dissolved in 2 ml of ultra-high-purity nitric acid (HNO_3; BDH Aristar Ultra, VWR International) at room temperature for 48 h. The samples were then treated with 1 ml of ultra-high-purity hydrogen peroxide (H_2O_2; BDH Aristar Ultra, VWR International), allowed to digest over 2–3 days before being diluted to 10 ml with deionized water, and then analyzed for As and Mn via ICP-MS.

Standard reference material water (NIST 1643e, Trace Elements in Water, National Institute of Standards and Technology, Gaithersburg, MD, USA) and 1 ng/ml mix standard were used as the initial calibration verification standards and continuous calibration verification standards. Certified reference material GBW 07601 human hair (RT Corporation, Laramie, WY, USA) was used as the QC sample as there is no certified reference material for both Mn and As in human blood. We also used a large preparation of pooled acid-digested blood sample (in house blood, IHB) to monitor daily variation. Above-mentioned all QC samples were analyzed with every batch. Recoveries for As and Mn recoveries ranged from 90 to 110%. The daily variation for the IHB was measured as %RSD of 2–9% for As, and % RSD of 1–10% for Mn. The precision for the analysis is given as %RSD $<$10% for the samples. The mean LODs were 0.36 μg/l for As and 0.21 μg/l for Mn. For sample concentrations that were below the LOD, the value was re-assigned as the LOD divided by $\sqrt{2}$.

Statistical Analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Wilcoxon signed rank tests were used to compare water

Table 1. Participant demographics.

Variable	*N* = 711
Infant characteristics	
Sex, n *(%)*	
Male	350 (49.2)
Female	349 (49.1)
Missing	12 (1.7)
Birth gestational age in weeks, mean (SD)	38.0 (1.9)
Missing	14
Birth weight in kg, mean (SD)	2.85 (0.4)
Missing	12
Maternal characteristics	
Age at enrollment, mean (SD)	23.0 (4.2)
BMI at enrollment in kg/m^2, mean (SD)	20.3 (3.2)
Parity, n *(%)*	
0	274 (38.5)
1	208 (29.3)
≥ 2	229 (32.2)
Education, n *(%)*	
≤ Primary education	324 (45.6)
> Primary education	387 (54.4)
Monthly income level, n *(%)*	
0–3000 Taka	176 (24.8)
3001–5000 Taka	388 (54.6)
>5001 Taka	141 (19.8)
Missing	6 (0.8)
Household ETS exposure, n *(%)*	
Yes	326 (45.9)
No	385 (54.1)
Chew betel nuts, n *(%)*	
Yes	8 (1.1)
No	700 (98.5)
Missing	3 (0.4)
Chew tobacco leaves, n *(%)*	
Yes	3 (0.4)
No	707 (99.4)
Missing	1 (0.1)
Region, n *(%)*	
Sirajdikhan	307 (43.2)
Pabna	404 (56.8)

concentrations at V1 and V4. Spearman correlations were calculated to assess the strength of the relationship among maternal and infant biomarkers. Owing to the lognormal distributions of the sample measurements (water, hair, toenail, and cord blood), metal concentrations were natural log transformed for use in the linear regression models. Linear regression was used to assess the relationship between maternal biomarker concentrations of As and Mn and infant biomarker concentrations. Several maternal and infant characteristics, such as maternal age, body mass index (BMI), income, education, exposure to environmental tobacco smoke, and betel nut use, were assessed as potential confounders, defined as changing the effect estimates by 10% or more, of the maternal–infant biomarker relationship. Although these additional predictors of infant biomarker concentrations, such as education, parity, and baby gender, were not confounders of the maternal–infant biomarker relationship, we have presented models adjusting for these covariates. A percent change in infant biomarkers was reported for a doubling of concentration in maternal biomarkers. To investigate whether the maternal–fetal transfer of As and Mn was dependent on maternal exposure, the ratios of maternal biomarker concentrations to infant biomarker concentrations were calculated and linear regression was used to assess the relationship between maternal exposure and these ratios. Quartiles of maternal exposure were used for these analyses for ease of interpretability of results.

RESULTS

Of the 1196 participants who completed all study visits, we limited this present analysis to mother–infant pairs who had at least one maternal and one infant biomarker of exposure available for analysis ($n = 711$). The participant demographics at the time of enrollment are presented in Table 1. About 57% of the participants were from Pabna and 43% from Sirajdikhan. The participants' mean age was 23.0 years and the mean gestational age of the infants was 38.0 weeks. More than half of the infant toenail samples (58%) were excluded from further analyses because of insufficient toenail mass resulting in a sample size of 260. Infants who were excluded due to insufficient toenail mass had a lower mean birth weight compared with those with sufficient toenail mass (2.81 *vs* 2.89 kg, $P = 0.01$), but no other differences were observed. Further analyses focused only on biomarker samples collected at V4 as these measures would better represent the time of pregnancy and infant samples were available for this time point.

Arsenic

The As concentrations in water, hair, toenail, and cord blood samples are presented in Table 2. Although the median water As concentration at study enrollment (V1) was relatively low (6.05 μg/l) for this study population (i.e., < 10 μg/l), 181 (25%) of the water samples exceeded the drinking water standard in Bangladesh of 50 μg/l. The median water As concentrations were considerably higher in Pabna compared with Sirajdikhan (27.1 *vs* 1.3 μg/l), and 84% of the water samples that exceeded 50 μg/l, were from Pabna. Water As concentrations were statistically different between V1 and 1 month postpartum (V4) in each region, although the As concentrations decreased over time in Sirajdikhan ($P = 0.004$) and increased in Pabna ($P < 0.0001$). Unlike the water concentrations, median maternal toenail As concentrations were higher at V1 compared with V4 for both regions, and there were no differences in median maternal hair concentrations. Correlations between the sample arsenic concentrations at V1 and V4 are shown in Figures 1, 2 and 3. Although the water As concentrations were strongly correlated in Pabna ($\rho = 0.84$), there was no strong correlation in Sirajdikhan ($\rho = 0.06$). The correlations were slightly higher among those in Pabna compared with Sirajdikhan for both maternal toenail As ($\rho = 0.78$ *vs* 0.55) and hair As concentrations ($\rho = 0.85$ *vs* 0.68).

Correlations between maternal and infant biomarkers of exposure are presented in Table 3. Although the correlations between water As and infant toenail As ($\rho = 0.36$), hair As ($\rho = 0.39$), and cord blood As ($\rho = 0.47$) were moderate, the associations between the maternal and infant biomarkers were stronger (maternal–infant toenail As $\rho = 0.52$, maternal–infant hair As $\rho = 0.61$). Cord blood As was similarly correlated with infant toenail ($\rho = 0.43$) and hair ($\rho = 0.43$). The maternal–infant biomarker correlations for As are shown by site in Supplementary Figures. Although the correlations were similar for both sites, the maternal–infant toenail As correlation was higher in Pabna compared with Sirajdikhan ($\rho = 0.78$ *vs* 0.55).

We assessed maternal and infant variables as significant predictors of infant biomarker concentrations (Table 4) and found that several maternal and infant characteristics were associated with infant biomarker concentrations. Although the statistically significant predictors varied by infant biomarker, higher education and monthly incomes were generally associated with a decrease in biomarker As concentrations, whereas parity was positively associated with biomarker As concentrations.

In Table 5, the percent changes in infant biomarkers are presented in relation to a doubling of exposure in the maternal biomarkers while adjusting for the statistically significant predictors described in Table 4. Region modified the associations between maternal–infant toenail and hair As. Subsequently, the

Table 2. Concentrations of arsenic and manganese in water and biomarker samples.

Sample	Sirajdikhan							Pabna						
	n	>5 mg	< LOD	Mean (std)	P25	Median	P75	n	>5 mg	< LOD	Mean (std)	P25	Median	P75
Arsenic														
Water, μg/l														
As, V1	306	NA	101	18.9 (61.8)	0.5	1.3	2.1	404	NA	36	79.6 (135.1)	6.0	27.1	91.5
As, V4	305	NA	139	8.4 (31.4)	0.5	0.9	1.6	403	NA	33	101.6 (171.6)	8.3	32.0	126.0
Mn, V1	306	NA	33	752.3 (865.8)	35.5	455.0	1300.0	404	NA	3	731.5 (670.3)	290.0	530.0	970.0
Mn, V4	305	NA	16	694.0 (758.5)	39.1	690.0	780.0	396	NA	1	776.9 (677.4)	320.0	550.0	1060.0
Toenail, μg/g														
Maternal As, V1	253	250	0	2.3 (3.2)	0.8	1.2	2.5	362	358	0	5.1 (5.8)	1.6	2.8	6.5
Maternal As, V4	253	252	1	2.0 (3.4)	0.5	0.9	1.9	362	358	1	4.0 (4.7)	1.2	2.2	5.2
Infant As, V4	253	101	10	1.0 (2.1)	0.2	0.5	0.9	362	159	5	1.6 (3.0)	0.4	0.7	1.4
Maternal Mn, V1	253	250	1	10.0 (7.5)	4.8	8.1	13.1	362	358	2	14.7 (12.5)	7.2	11.8	17.2
Maternal Mn, V4	253	252	2	8.3 (9.6)	2.3	5.3	10.7	362	358	6	11.6 (9.9)	5.3	9.0	14.4
Infant Mn, V4	253	101	10	8.4 (15.2)	0.8	3.3	8.9	362	159	11	11.0 (15.7)	2.2	6.6	13.6
Hair, μg/g														
Maternal As, V1	302	302	1	1.3 (2.2)	0.4	0.6	1.3	402	402	2	1.8 (2.0)	0.6	1.0	2.3
Maternal As, V4	302	302	1	1.4 (2.7)	0.4	0.6	1.2	402	402	3	1.8 (2.2)	0.5	1.0	2.1
Infant As, V4	302	297	35	0.6 (3.4)	0.1	0.2	0.4	402	389	19	0.6 (0.8)	0.1	0.3	0.7
Maternal Mn, V1	302	302	4	44.6 (38.7)	18.2	34.7	59.4	402	402	5	33.6 (25.8)	17.9	27.1	41.0
Maternal Mn, V4	302	302	4	48.2 (44.5)	20.2	35.8	59.3	402	402	4	34.2 (27.3)	17.4	27.3	43.1
Infant Mn, V4	302	297	3	14.0 (16.4)	3.4	8.4	18.6	402	389	4	13.0 (11.9)	4.2	9.5	17.8
Cord blood, μg/dl														
As, V3	300	NA	12	0.5 (0.6)	0.2	0.3	0.5	341	NA	0	1.4 (2.0)	0.6	0.8	1.5
Mn, V3	300	NA	0	6.8 (7.5)	4.1	5.2	6.7	341	NA	0	15.8 (26.7)	4.6	6.5	15.9

Abbreviations: LOD, limit of detection; std, standard deviation. V1:1st visit at enrollment, V3: birth, V4: 4th visit at 1 month postpartum.

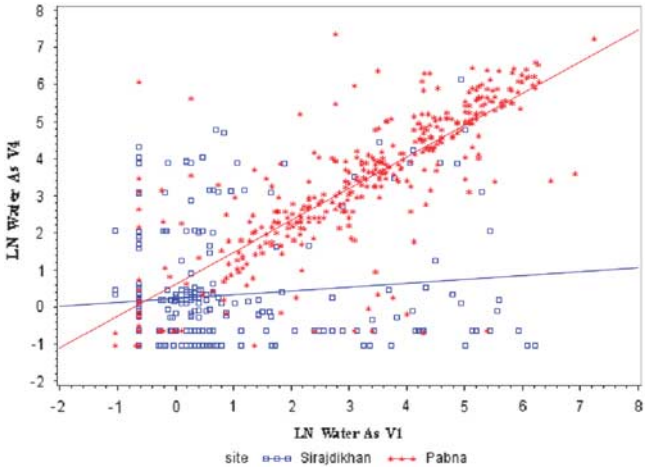

Figure 1. Correlations between water As concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

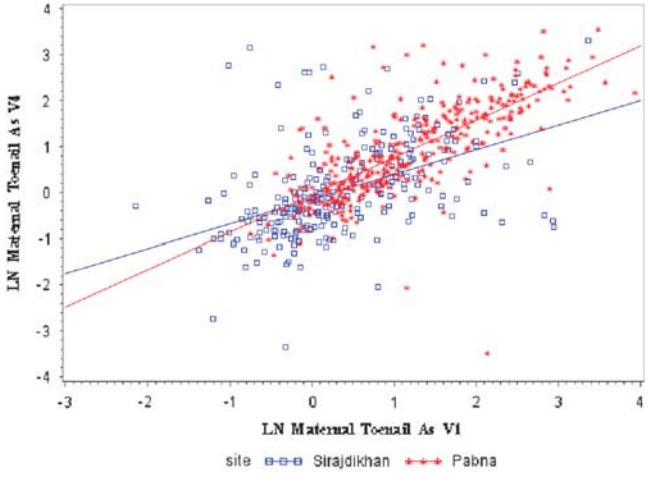

Figure 2. Correlations between maternal toenail As concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

results are presented by site in addition to the overall results. Overall, infant toenail As concentration increased by 58.2% for a doubling of maternal toenail As concentration with a statistically significant interaction with region ($P < 0.0001$). When examined by region, this percent change in infant toenail As concentration was greater among participants in Pabna (81.1%) compared with participants in Sirajdikhan (27.0%). A similar result was observed for the maternal–infant hair As concentrations, where a greater percent change in infant hair As was observed for those in Pabna compared with Sirajdikhan (74.8% *vs* 61.1%, *P*-value for interaction = 0.06). There was a significant relationship between maternal toenail As, maternal hair As and cord blood As. Yet, the percent increase in cord blood As in relation to maternal toenail and hair As concentrations was somewhat lower than the percent change observed for infant toenail and hair As concentrations (Table 5).

The ratios of maternal to infant biomarkers indicated that As concentrations were generally higher in maternal hair and nail compared with infants'. The median maternal–infant ratios for As biomarkers were 2.98 and 3.22 for toenail and hair, respectively, indicating that maternal As concentrations were, on average, three times higher than infant concentrations in both toenail and hair samples. Although the maternal–infant ratios were statistically higher for the As biomarkers in the highest quartile of maternal exposure compared with the lowest quartile, there were differences by region, with higher maternal–infant ratios in Sirajdikhan compared with Pabna (Table 6).

Manganese

The Mn concentrations in water, hair, and toenail samples are presented in Table 2. Overall, the median water Mn concentration at V1 (512 μg/l) was above the former WHO guideline of 400 μg/l in drinking water with 50 (7%) of the samples having more than 2000 μg/l. The median water Mn concentrations at V1 were

considerably higher in Pabna compared with Sirajdikhan (530 *vs* 455 μg/l), and 61% of the water samples that exceeded 400 μg/l were from Pabna. Water Mn concentrations were statistically significantly higher at V4 compared with V1 in Pabna ($P < 0.0001$), although there was no statistically significant difference in Mn concentrations over time in Sirajdikhan ($P = 0.73$).

Correlations between the sample manganese concentrations at V1 and V4 are shown in Figures 4, 5 and 6. Water Mn concentrations were strongly correlated in Pabna ($\rho = 0.78$), but not in Sirajdikhan ($\rho = -0.02$). The correlations for maternal toenail Mn ($\rho = 0.41$ *vs* 0.37) and hair Mn concentrations ($\rho = 0.63$ *vs* 0.64) were similar for Pabna and Sirajdikhan, respectively.

The relationship between maternal–infant biomarkers was substantially weaker for Mn with the strongest correlations being maternal–infant toenail ($\rho = 0.39$) and maternal–infant hair ($\rho = 0.21$; Table 3). Cord blood Mn concentrations were weakly correlated with infant toenail Mn concentrations ($\rho = 0.14$).

Similar to the As concentrations, increased parity was associated with higher biomarker Mn concentrations, whereas higher education and monthly incomes were associated with lower biomarker Mn concentrations (Table 4).

For Mn the greatest change in infant biomarkers was observed for infant toenails, with a 54.1% increase in infant toenail Mn for a doubling of exposure in maternal toenail Mn concentrations while adjusting for the statistically significant predictors (Table 5). Although there was no statistically significant difference by site, the % increases in infant biomarker Mn concentrations were generally higher in Sirajdikan compared with Pabna.

The median maternal–infant ratios for Mn biomarkers were 1.57 and 3.29 for toenail and hair, respectively, indicating that maternal Mn concentrations were, on average, higher than infant concentrations in both toenail and hair samples. Unlike As, there was no statistically significant dose–response relationship between these ratios and maternal biomarker Mn concentrations, although the maternal–infant ratios increased with increasing quartile of maternal biomarker concentration, with the exception of toenail Mn in Pabna (Table 7).

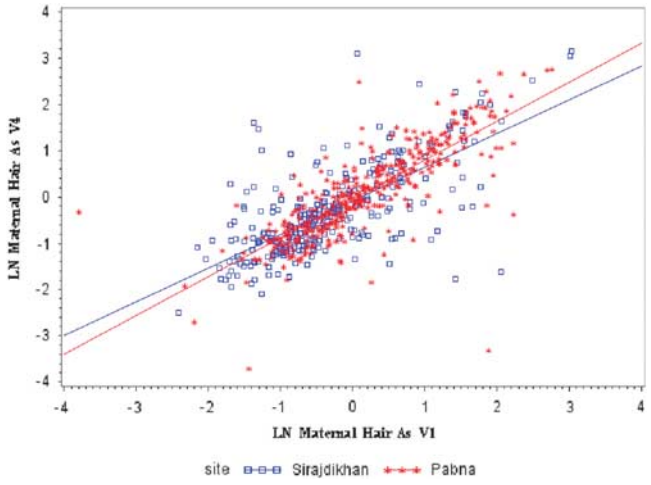

Figure 3. Correlations between maternal hair As concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

DISCUSSION

This large, population-based birth cohort study in Bangladesh quantified As and Mn concentrations in maternal (toenail and hair) and infant (toenail, hair, and cord blood) biomarkers representing *in utero* exposure. Our results showed that pregnant women and their infants are exposed to both As and high concentrations of Mn from their drinking water.

Arsenic

Infant toenail, hair, and cord blood As concentrations were positively associated with maternal biomarkers as well as with drinking water As exposure. This suggests that infant hair, toenail, and cord blood are useful quantitative biomarkers of prenatal arsenic exposure. The different matrices, however, would reflect different critical windows of development based on the tissue. Among this population that is chronically exposed to As, cord blood As concentrations may reflect a steady-state exposure. Infants begin to develop hair and nails in the first trimester, however, and subsequently As concentrations in infant hair and nails would provide cumulative As exposure throughout fetal development. Although the median maternal As concentrations were lower in toenails collected at V4 compared with V1, no such

Table 3. Spearman correlations (95% CI) for As and Mn biomarkers.

	Maternal toenail V4	*Maternal hair V4*	*Infant toenail V4*	*Infant hair V4*	*Cord blood V3*
Arsenic					
Water, V4	0.54* (0.48, 0.60) $n = 607$	0.45* (0.39, 0.51) $n = 700$	0.36* (0.25, 0.46) $n = 258$	0.39* (0.32, 0.45) $n = 683$	0.47* (0.41, 0.53) $n = 640$
Maternal toenail, V4	1.00 $n = 610$	0.68* (0.64, 0.72) $n = 602$	0.52* (0.43, 0.60) $n = 258$	0.64* (0.59, 0.68) $n = 585$	0.55* (0.48, 0.60) $n = 546$
Maternal hair, V4	0.68* (0.64, 0.72) $n = 602$	1.00 $n = 703$	0.41* (0.30, 0.50) $n = 253$	0.61* (0.56, 0.65) $n = 685$	0.44* (0.38, 0.50) $n = 634$
Cord blood, V3	0.55* (0.48, 0.60) $n = 546$	0.44* (0.38, 0.50) $n = 634$	0.43* (0.32, 0.53) $n = 229$	0.43* (0.36, 0.49) $n = 619$	1.00 $n = 641$
Manganese					
Water, V4	0.17* (0.09, 0.24) $n = 600$	0.15* (0.08, 0.22) $n = 694$	0.06 (−0.06, 0.18) $n = 255$	0.04 (−0.04, 0.11) $n = 676$	0.13† (0.05, 0.20) $n = 634$
Maternal toenail, V4	1.00 $n = 610$	0.14† (0.06, 0.22) $n = 603$	0.39* (0.28, 0.49) $n = 258$	0.07 (−0.01, 0.15) $n = 585$	0.14† (0.06, 0.22) $n = 546$
Maternal hair, V4	0.14† (0.06, 0.22) $n = 603$	1.00 $n = 704$	0.14‡ (0.02, 0.26) $n = 253$	0.21* (0.14, 0.28) $n = 686$	−0.01 (−0.09, 0.07) $n = 635$
Cord blood, V3	0.14† (0.06, 0.22) $n = 546$	−0.01 (−0.09, 0.07) $n = 635$	0.14‡ (0.01, 0.26) $n = 229$	0.05 (−0.03, 0.13) $n = 619$	1.00 $n = 641$

Note: V3: birth, V4: 4th visit at 1 month postpartum. *$P < 0.0001$, †$P = 0.001$, ‡$P < 0.05$.

Table 4. Significant predictors of infant biomarker concentrations.

	Hair As		*Hair Mn*		*Toenail As*		*Toenail Mn*		*Cord As*		*Cord Mn*	
	β *(SE)*	P-*value*	β *(SE)*	P-*value*	β *(SE)*	P-*value*	β *(SE)*	P-*value*	β *(SE)*	P-*value*	β *(SE)*	P-*value*
Infant characteristics												
Sex, n *(%)*												
Male	Ref		Ref		Ref		Ref		Ref		Ref	
Female	−0.10 (0.09)	0.30	−0.20 (0.09)	0.03	−0.06 (0.16)	0.72	0.05 (0.22)	0.83	−0.04 (0.07)	0.63	−0.06 (0.06)	0.39
Birth gestational age in weeks, mean (SD)	−0.04 (0.03)	0.15	0.08 (0.02)	0.003	−0.02 (0.05)	0.70	0.003 (0.06)	0.96	−0.12 (0.02)	< 0.0001	−0.04 (0.02)	0.02
Birth weight in kg, mean (SD)	0.007 (0.11)	0.95			−0.20 (0.20)	0.32	−0.64 (0.26)	0.01	−0.02 (0.09)	0.84	−0.10 (0.08)	0.24
Maternal characteristics												
Age at enrollment, mean (SD)	−0.02 (0.01)	0.05	−0.0004 (0.01)	0.97	0.01 (0.02)	0.57	0.05 (0.02)	0.04	0.004 (0.009)	0.68	0.0003 (0.008)	0.97
BMI at enrollment,mean (SD)	−0.02 (0.14)	0.14	0.02 (0.01)	0.25	0.02 (0.02)	0.32	0.006 (0.03)	0.85	−0.02 (0.01)	0.16	−0.008 (0.01)	0.46
Parity, n *(%)*												
0	Ref		Ref		Ref		Ref		Ref		Ref	
1	0.13 (0.11)	0.23	0.18 (0.11)	0.12	0.16 (0.20)	0.41	0.31 (0.26)	0.24	0.17 (0.09)	0.06	0.05 (0.08)	0.50
≥2	−0.008 (0.11)	0.94	0.13 (0.11)	0.25	0.39 (0.19)	0.04	0.72 (0.25)	0.004	0.34 (0.09)	0.0002	0.19 (0.08)	0.01
Education, n *(%)*												
≤Primary education	Ref		Ref		Ref		Ref		Ref		Ref	
>Primary education	0.006	0.95	−0.0003 (0.09)	1.00	−0.37 (0.16)	0.02	−0.44 (0.21)	0.04	−0.01 (0.07)	0.87	−0.02 (0.07)	0.74
Monthly income level, n *(%)*												
0–3000 Taka	Ref		Ref		Ref		Ref		Ref		Ref	
3001–5000 Taka	−0.009 (0.11)	0.93	0.06 (0.11)	0.60	−0.12 (0.19)	0.53	−0.48 (0.25)	0.05	−0.10 (0.09)	0.27	−0.19 (0.08)	0.02
>5001 Taka	−0.27 (0.14)	0.05	−0.07 (0.14)	0.59	−0.42 (0.23)	0.08	−0.86 (0.31)	0.006	−0.44 (0.11)	< 0.0001	−0.38 (0.10)	0.0002
Household ETS exposure, n *(%)*												
No	Ref		Ref		Ref		Ref		Ref		Ref	
Yes	−0.02	0.83	−0.10 (0.09)	0.28	−0.26 (0.16)	0.11	0.10 (0.22)	0.66	0.01 (0.07)	0.87	−0.03 (0.07)	0.68
Chew betel nuts, n *(%)*												
No	Ref		Ref		Ref		Ref		Ref		Ref	
Yes	0.22 (0.42)	0.60	0.09 (0.43)	0.83	0.73 (0.65)	0.26	1.49 (0.86)	0.08	−0.43 (0.34)	0.20	−0.48 (0.30)	0.11
Chew tobacco leaves, n *(%)*												
No	Ref		Ref		Ref		Ref		Ref		Ref	
Yes	0.31 (0.33)	0.34	0.44 (0.33)	0.19	0.10 (1.3)	0.94	0.32 (1.72)	0.85	−0.12 (0.26)	0.64	−0.33 (0.23)	0.16
Clinic, n *(%)*												
Sirajdikhan	Ref		Ref		Ref		Ref		Ref		Ref	
Pabna	0.55 (0.09)	< 0.0001	0.02 (0.09)	0.82	0.56 (0.16)	0.0006	0.64 (0.21)	0.003	1.07 (0.06)	< 0.0001	0.49 (0.06)	< 0.0001

Note: All biomarker concentrations are LN-transformed.

Table 5. Predictors of infant biomarkers of exposure to arsenic and manganese adjusted for significant covariates.[a]

Predictor	LN (infant toenail As concentration)[b]								LN (infant hair As concentration)[c]								LN (cord blood As concentration)[d]							
	All			Sirajdikhan			Pabna		All			Sirajdikhan			Pabna		All			Sirajdikhan			Pabna	
	n	% Change (95% CI)		n	% Change (95% CI)		n	% Change (95% CI)	n	% Change (95% CI)		n	% Change (95% CI)		n	% Change (95% CI)		n	% Change (95% CI)		n	% Change (95% CI)		n	% Change (95% CI)
LN (Arsenic concentration																								
Maternal toenail (μg/g)	258	58.2 (45.8, 70.6)		101	27.0 (4.7, 49.2)		157	81.1 (65.8, 96.4)																
Maternal hair (μg/g)		—			—			—																
Cord blood (μg/dl)	229	51.2 (35.4, 67.1)		99	50.0 (20.0, 80.1)		130	49.6 (24.6, 74.5)	679	71.6 (64.5, 78.7)		291	61.1 (48.2, 74.1)		388	74.8 (66.7, 82.9)		539	43.9 (37.7, 50.1)		241	25.1 (16.6, 33.5)		298	37.6 (29.2, 45.9)
									614	53.9 (44.9, 62.8)		286	58.9 (40.6, 77.2)		328	54.6 (41.5, 67.6)		627	37.6 (31.0, 44.3)		290	26.7 (18.5, 35.0)		337	32.9 (24.6, 41.1)
										—			—			—			—			—			—
	LN (infant toenail Mn concentration)[e]								LN (infant hair Mn concentration)[f]								LN (cord blood Mn concentration)[d]							
LN (manganese concentration)																								
Maternal toenail (μg/g)	249	54.1 (33.2, 74.9)		99	66.8 (34.9, 98.6)		150	38.3 (9.5, 67.0)																
Maternal hair (μg/g)		—			—			—	672	17.1 (10.9, 23.3)		296	18.8 (10.3, 27.3)		376	15.5 (6.3, 24.7)		539	8.7 (2.2, 15.3)		241	3.4 (−2.6, 9.3)		298	8.1 (−2.9, 19.0)
Cord blood (μg/dl)	228	17.6 (−8.3, 43.5)		98	31.9 (−40.1, 103.9)		130	8.1 (−18.2, 34.3)	617	6.9 (−4.5, 18.4)		290	5.4 (−17.1, 28.0)		327	3.0 (−11.0, 16.9)		627	−4.5 (−8.9, −0.1)		290	−1.4 (−6.0, 3.1)		337	−5.4 (−12.9, 2.0)
										—			—			—			—			—			—

[a]The percent changes reported are for a doubling of exposure as a result of LN-transformed predictor and outcome in linear regression analysis. [b]Adjusted for parity and education. [c]Adjusted for maternal age and monthly income. [d]Adjusted for gestational age, parity, and monthly income. [e]Adjusted for birth weight, parity, education, and monthly income. [f]Adjusted for gender and gestational age.

difference was observed in hair. This inconsistent finding may be due to exogenous arsenic contamination as it has been shown that hair can adsorb more inorganic arsenic than nails (21–29% *vs* 2–3.5%).[28] With increased arsenic methylation in late gestation, it has been shown that the majority of As excreted in urine during pregnancy is of the methylated form, dimethylarsinic acid (DMA), and it is the major form that is transferred to the fetus,[16] with 43% DMA in cord blood[11] but it has also been shown that DMA content in hair is relatively low compared with nails (3.6% *vs* 12.2%),[28] but it is unclear if this is the case in pregnant women. This may indicate that toenails are a better biomarker of arsenic exposure among pregnant women.

The higher maternal–infant ratios in Sirajdikhan compared with Pabna are most apparent in the highest quartile of exposure. This may indicate that the transfer of As to the infant is greatest among those in Pabna where the As concentrations are substantially higher. Of the covariates tested, we did not find other significant predictors of the maternal–infants concentration ratios, but future work will investigate the effects of other factors on the transfer of As such as methylation capacity and nutritional factors, which may change substantially during pregnancy. It has been shown that methylation capacity measured as urinary As metabolites (i.e., DMA, MMA, DMA/MMA) is associated with the correlations between infant and maternal As concentrations.[22]

Manganese

Infant toenail and hair Mn concentrations were positively associated with maternal Mn concentrations but not with drinking water concentrations. Specifically, only cord blood Mn was related to maternal exposure to Mn in drinking water, but infant toenail appears to be a better biomarker of *in utero* Mn exposure as it increased proportionally with maternal dose, measured as toenail Mn. However, the half-life of whole blood Mn is estimated to be between 13 and 74 days, which would indicate that Mn measured in cord blood would only be reflective of the last trimester. Although we did not have maternal blood samples collected, it has been shown that cord blood serum Mn concentrations are higher than maternal serum Mn concentrations suggesting active transfer to the fetus.[13,29] Although Mn concentrations in adult humans are typically tightly regulated through absorption and excretion rates, this homeostasis may not be fully developed in infants, leading to higher cord blood Mn concentrations and possibly higher Mn concentrations in other organs and tissues.[30]

As of the latest edition of the WHO guidelines for drinking water quality, the health-based guideline for Mn of 400 μg/l was revoked as Mn was "Not of health concern" at levels found in drinking water.[3] The results from this study show that more than half of the water samples collected contained concentrations of Mn higher than the previous health-based guideline and that this is contributing to exposure in pregnant women and the developing fetus. Others have also documented high Mn exposures in this vulnerable population. A study of 408 pregnant women living in rural Bangladesh, reported that 25% were drinking water with Mn concentrations above 1000 μg/l and maternal blood Mn (erythrocyte cell portion) concentrations were higher than expected ranging from 7 to 53 μg/kg.[31]

Some have reported that there were no strong correlations between the Mn concentrations in blood and hair of adults exposed occupationally or via drinking water.[32,33] Similarly, our study showed no correlation between cord blood Mn and infant hair Mn, but there was a significant relationship between cord blood and infant toenail Mn concentrations indicating that toenails may be a better biomarker of exposure for Mn than hair. In addition, there was a stronger relationship between maternal and infant toenail Mn concentrations. Because Mn and iron share a transport system via the gastrointestinal tract, we considered the effect of maternal iron status on the maternal–infant biomarker

Table 6. Maternal–infant ratios of biomarker As concentrations.

Maternal biomarker As	*Biomarker As ratio, maternal/infant*								
	All			*Sirajdikhan*			*Pabna*		
	N	β (*SE*)	P-*value*	n	β (*SE*)	*P-value*	n	β (*SE*)	*P-value*
Toenail As, μg/g									
≤0.79	67	Ref	—	49	Ref	—	18	Ref	—
0.80–1.49	59	0.83 (2.6)	0.75	18	0.57 (4.1)	0.89	41	1.04 (4.0)	0.79
1.50–3.84	70	4.19 (2.5)	0.09	21	8.82 (3.9)	0.03	49	2.31 (3.9)	0.55
>3.84	62	6.87 (2.6)	0.008	13	14.34 (4.7)	0.003	49	4.99 (3.9)	0.20
P-value for linear trend			0.02			0.01			0.18
Hair As, μg/g									
≤0.43	175	Ref	—	105	Ref	—	70	Ref	—
0.44–0.81	170	1.73 (2.0)	0.38	84	2.06 (3.9)	0.60	86	1.64 (0.9)	0.07
0.82–1.80	170	2.06 (2.0)	0.30	56	2.95 (4.4)	0.50	114	2.06 (0.8)	0.01
>1.81	170	8.28 (2.0)	<0.0001	51	22.50 (4.5)	<0.0001	119	2.66 (0.8)	0.002
P-value for linear trend			0.001			<0.0001			0.19

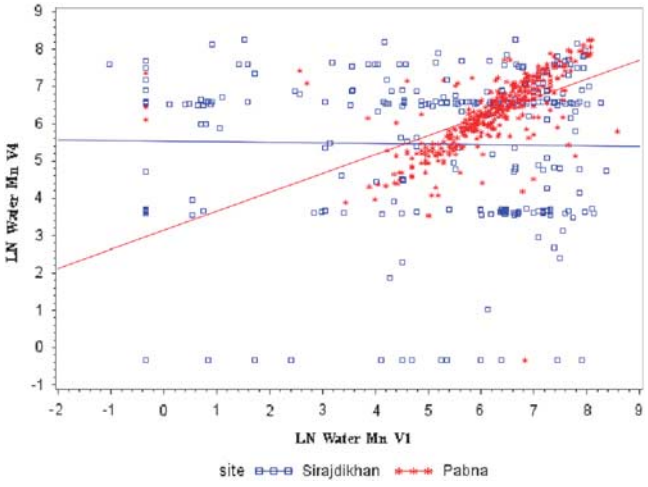

Figure 4. Correlations between water Mn concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

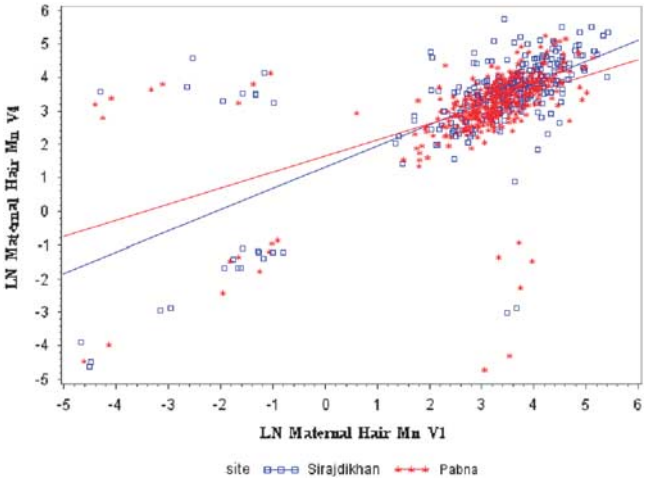

Figure 6. Correlations between maternal hair Mn concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

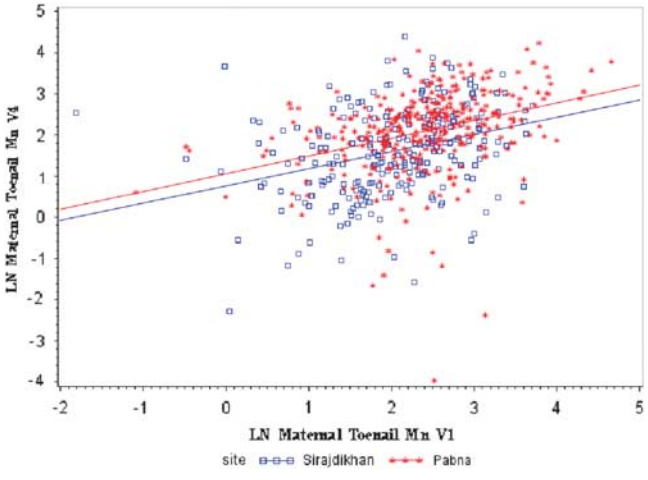

Figure 5. Correlations between maternal toenail Mn concentrations at study enrollment (V1) and 1 month postpartum (V4) by site.

relationships. It has been shown that serum ferritin levels are highest during the first trimester of pregnancy and the levels decrease during the second and third trimesters.[34] This decrease was observed among women who did not receive iron supplements. Although we had maternal serum ferritin levels at study enrollment during the first trimester, we did not analyze maternal ferritin levels at other times during this study and all women in our study received prenatal vitamins throughout their pregnancy. Given this limitation of one measurement, we analyzed the maternal–infant biomarker relationships among women with a normal serum ferritin level of 18 ng/ml or higher.[35] In general, the maternal–infant biomarker relationships were not different, with the exception of toenail Mn. When considering only women with normal ferritin levels, the percent change in infant toenail Mn concentrations in relation to maternal toenail Mn concentrations was less when compared with all the women in the study (Supplementary Table 1). This may indicate less transfer of Mn to the fetus in the presence of high iron status, but further research is needed. Mora et al. has shown that hair Mn concentrations were not associated with iron supplementation during pregnancy.[24]

Table 7. Maternal–infant ratios of biomarker Mn concentrations.

Maternal biomarker Mn	*Biomarker Mn ratio, maternal/infant*								
	All			*Sirajdikhan*			*Pabna*		
	N	β (*SE*)	P-*value*	n	β (*SE*)	P-*value*	n	β (*SE*)	P-*value*
Toenail Mn, μg/g									
≤3.66	66	Ref	—	40	Ref	—	26	Ref	—
3.67–7.35	64	5.35 (8.5)	0.53	27	0.09 (17.0)	1.00	37	11.15 (7.5)	0.14
7.36–12.93	53	9.10 (9.0)	0.31	16	9.22 (20.2)	0.65	37	11.71 (7.5)	0.12
>12.93	75	11.98 (8.2)	0.15	18	33.71 (19.4)	0.09	57	8.07 (7.0)	0.25
P-value for linear trend			0.42			0.10			0.62
Hair Mn, μg/g									
≤18.2	169	Ref	—	64	Ref	—	105	Ref	—
18.3–30.8	173	4.37 (11.65)	0.71	58	4.44 (10.1)	0.66	115	4.37 (18.2)	0.81
30.9–48.3	169	21.16 (11.72)	0.07	76	14.94 (9.5)	0.12	93	26.15 (19.2)	0.17
>48.3	175	25.79 (11.65)	0.03	99	15.70 (8.9)	0.08	76	38.63 (20.3)	0.06
P-value for linear trend			0.06			0.22			0.08

Although more work is needed to determine the health effects associated with prenatal Mn exposure measured in toenails and hair, significant negative associations between performance on neuropsychological tests and Mn concentration in hair and fingernails have been reported in adults.[36]

Overall

Our results show that although there are some statistically significant associations between maternal and infant biomarkers of exposure to As and Mn, these associations are not uniform across the two regions of Bangladesh studied. Although the sites in this study may be different in several ways, such as exposure levels, education, and nutrition, it is unclear which factors may be responsible for the inter-site differences observed.

In addition, the maternal–infant ratio of biomarker concentrations (e.g., toenail and hair) generally increased with increasing quartile of maternal biomarker concentration. Thus, while the infant toenail and hair concentrations increased with increasing maternal toenail and hair concentrations on average, the increase appears to be less dramatic in infants compared with their mothers. Although we did not have maternal blood samples, several studies have shown that cord blood concentrations of As and Mn are higher than maternal blood concentrations,[10,11,13,15] but we did not observe this with toenails and hair, as maternal concentrations were higher than infant concentrations.

These data show that the developing fetus is exposed to environmental sources of As and Mn. Some advantages of using nails as biomarkers of exposure include ease of collection, storage, transport, and they represent long-term exposure (e.g., *in utero*) rather than recent changes in exposure.[20] Unfortunately, one limitation of this study was that we excluded the majority of the infant toenail samples from the statistical analyses because of insufficient mass (<5 mg). It can be difficult to collect sufficient toenail mass from young infants. When comparing the infants, we found that the infants with insufficient toenail mass had lower birth weights compared with those with sufficient toenail mass (2.81 *vs* 2.89, $P=0.01$), but no other differences were observed. Although there was no difference in the number of hair and toenail samples excluded because of insufficient mass by site, the infant hair samples included in the analyses had a greater mean mass in Pabna compared with Sirajdikhan (73.2 *vs* 61.1 mg, $P<0.0001$), but no difference was observed for infant toenails (8.8 *vs* 8.4 mg, $P=0.70$). Another limitation is the potential exposure misclassification when measuring concentrations in toenails and hair. Although Chen et al.[27] reports that the washing method used effectively removes exogenous contaminating material, we did not compare this to other methods that used different reagents. Therefore, it is possible that some sample concentrations, particularly maternal samples, may be overestimated because of exogenous contamination, leading to non-differential misclassification, which may bias the results toward the null and provide wide confidence intervals. An additional limitation is that we only collected water samples on two occasions, enrollment and 1 month postpartum. The variability in water concentrations may have been better characterized if we had collected additional water samples throughout pregnancy.

Future work is ongoing to investigate the possible effect of arsenic methylation capacity and possible nutritional factors that may contribute to the *in utero* transfer of arsenic and manganese. Additional work on the use of infant toenails and hair as biomarkers of exposure is also needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge the contributions from Li Su, Nicola Lupoli, Ivan Pantic, Shangzhi Gao, Jongeun Rhee, Pi-I (Debby) Lin, Sakila (Joya) Afroz, and Hafiza (Suchanda) Sultana. This work was supported by grants from the US National Institute of Environmental Health Sciences (R01ES015533 and T42OH008416).

REFERENCES

1 Mukherjee A, Sengupta MK, Hossain MA, Ahamed S, Das B, Nayak B *et al.* Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. *J Health Popul Nutr* 2006; **24**: 142–163.
2 Sengupta MK, Mukherjee A, Hossain MA, Ahamed S, Rahman MM, Lodh D *et al.* Groundwater arsenic contamination in the Ganga-Padma-Meghna-Brahmaputra plain of India and Bangladesh. *Arch Environ Health* 2003; **58**: 701–702.
3 (WHO) WHO. Guidelines for Drinking-water Quality, 2011.
4 Hasan S, Ali MA. Occurence of manganese in groundwater of Bangladesh and its implications on safe water supply. *Journal of Civil Engineering* 2010; **32**: 121–128.
5 Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline J *et al.* Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. *Environ Health Perspect* 2007; **115**: 285–289.

6 Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A *et al.* Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. *Environ Health Perspect* 2004; **112**: 1329–1333.
7 Hafeman D, Factor-Litvak P, Cheng Z, van Geen A, Ahsan H. Association between manganese exposure through drinking water and infant mortality in Bangladesh. *Environ Health Perspect* 2007; **115**: 1107–1112.
8 Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V *et al.* Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology* 2012; **33**: 91–97.
9 Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F *et al.* Manganese exposure from drinking water and children's classroom behavior in Bangladesh. *Environ Health Perspect* 2011; **119**: 1501–1506.
10 Guan H, Wang M, Li X, Piao F, Li Q, Xu L *et al.* Manganese concentrations in maternal and umbilical cord blood: related to birth size and environmental factors. *Eur J Public Health* 2014; **24**: 150–157.
11 Hall M, Gamble M, Slavkovich V, Liu X, Levy D, Cheng Z *et al.* Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. *Environ Health Perspect* 2007; **115**: 1503–1509.
12 Guan H, Piao F, Zhang X, Li X, Li Q, Xu L *et al.* Prenatal exposure to arsenic and its effects on fetal development in the general population of Dalian. *Biol Trace Elem Res* 2012; **149**: 10–15.
13 Krachler M, Rossipal E, Micetic-Turk D. Trace element transfer from the mother to the newborn--investigations on triplets of colostrum, maternal and umbilical cord sera. *Eur J Clin Nutr* 1999; **53**: 486–494.
14 Zota AR, Ettinger AS, Bouchard M, Amarasiriwardena CJ, Schwartz J, Hu H *et al.* Maternal blood manganese levels and infant birth weight. *Epidemiology* 2009; **20**: 367–373.
15 Osada H, Watanabe Y, Nishimura Y, Yukawa M, Seki K, Sekiya S. Profile of trace element concentrations in the feto-placental unit in relation to fetal growth. *Acta Obstet Gynecol Scand* 2002; **81**: 931–937.
16 Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. Exposure to inorganic arsenic metabolites during early human development. *Toxicol Sci* 1998; **44**: 185–190.
17 Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH *et al. In utero* exposure to environmental lead and manganese and neurodevelopment at 2 years of age. *Environ Res* 2013; **123**: 52–57.
18 Parajuli RP, Fujiwara T, Umezaki M, Watanabe C. Association of cord blood levels of lead, arsenic, and zinc with neurodevelopmental indicators in newborns: a birth cohort study in Chitwan Valley, Nepal. *Environ Res* 2013; **121**: 45–51.
19 Hopps HC. The biologic bases for using hair and nail for analyses of trace elements. *Sci Total Environ* 1977; **7**: 71–89.
20 He K. Trace elements in nails as biomarkers in clinical research. *Eur J Clin Invest* 2011; **41**: 98–102.
21 Rakib MA, Huda ME, Hossain SM, Naher K, Khan R, Sultana MS *et al.* Arsenic content in inactive tissue: human hair and nail. *J Sci Res Rep* 2013; **2**: 522–535.
22 Davis MA, Li Z, Gilbert-Diamond D, Mackenzie TA, Cottingham KL, Jackson BP *et al.* Infant toenails as a biomarker of in utero arsenic exposure. *J Expo Sci Environ Epidemiol* 2014; **24**: 467–473.
23 Eastman RR, Jursa TP, Benedetti C, Lucchini RG, Smith DR. Hair as a biomarker of environmental manganese exposure. *Environ Sci Technol* 2013; **47**: 1629–1637.
24 Mora AM, van Wendel de Joode B, Mergler D, Cordoba L, Cano C, Quesada R *et al.* Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. *Environ Sci Technol* 2014; **48**: 3467–3476.
25 Savabieasfahani M, Hoseiny M, Goodarzi S. Toxic and essential trace metals in first baby haircuts and mother hair from Imam Hossein Hospital Tehran, Iran. *Bull Environ Contam Toxicol* 2012; **88**: 140–144.
26 Kile ML, Rodrigues EG, Mazumdar M, Dobson CB, Diao N, Golam M *et al.* A prospective cohort study of the association between drinking water arsenic exposure and self-reported maternal health symptoms during pregnancy in Bangladesh. *Environ Health* 2014; **13**: 29.
27 Chen KL, Amarasiriwardena CJ, Christiani DC. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. *Biol Trace Elem Res* 1999; **67**: 109–125.
28 Mandal BK, Ogra Y, Suzuki KT. Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. *Toxicol Appl Pharmacol* 2003; **189**: 73–83.
29 Takser L, Lafond J, Bouchard M, St-Amour G, Mergler D. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. *Environ Res* 2004; **95**: 119–125.
30 Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. *Mol Aspects Med* 2005; **26**: 353–362.
31 Ljung KS, Kippler MJ, Goessler W, Grander GM, Nermell BM, Vahter ME. Maternal and early life exposure to manganese in rural Bangladesh. *Environ Sci Technol* 2009; **43**: 2595–2601.
32 Gil F, Hernandez AF, Marquez C, Femia P, Olmedo P, Lopez-Guarnido O *et al.* Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. *Sci Total Environ* 2011; **409**: 1172–1180.
33 Kondakis XG, Makris N, Leotsinidis M, Prinou M, Papapetropoulos T. Possible health effects of high manganese concentration in drinking water. *Arch Environ Health* 1989; **44**: 175–178.
34 Kaneshige E. Serum ferritin as an assessment of iron stores and other hematologic parameters during pregnancy. *Obstet Gynecol* 1981; **57**: 238–242.
35 WebMD. Ferritin [updated August 6, 2012; cited 2015 May 13, 2015]. Available from http://www.webmd.com/a-to-z-guides/ferritin?page = 2.
36 Viana GF, de Carvalho CF, Nunes LS, Rodrigues JL, Ribeiro NS, de Almeida DA *et al.* Noninvasive biomarkers of manganese exposure and neuropsychological effects in environmentally exposed adults in Brazil. *Toxicol Lett* 2014; **231**: 169–178.

Supplementary Information accompanies the paper on the Journal of Exposure Science and Environmental Epidemiology website (http://www.nature.com/jes)