A prospective cohort study of in utero and early childhood arsenic exposure and infectious disease in 4- to 5-year-old Bangladeshi children

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Background: Previous research found that infants who were exposed to high levels of arsenic in utero had an increased risk of infectious disease in the first year of life. This prospective study examined the association between arsenic exposures during gestation, and respiratory, diarrheal, and febrile morbidity in children 4–5 years of age.

Methods: A cohort of pregnant women was recruited in 2008–2011 in Bangladesh. Their children (N = 989) were followed, and household drinking water samples were collected during pregnancy, toddlerhood (12–40 months of age), and childhood (4–5 years of age). We actively surveyed mothers every 2 weeks regarding their children’s infectious diseases symptoms from 4 to 5 years of age. Poisson regression models were used to estimate the association between arsenic exposure and respiratory or diarrheal illness.

Results: Median drinking water arsenic was 4.6, 8.8, and 4.2 μg/L in pregnancy, toddlerhood, and childhood, respectively. We observed 0.01, 1.2, and 1.0 cases per 100 person-days of diarrhea, respiratory, and febrile illness, respectively. The incident rate ratios (IRRs) for each doubling of drinking water arsenic during pregnancy were 1.10 (95% confidence interval [CI] = 1.00, 1.22) and 0.93 (95% CI = 0.82, 1.05) for respiratory and febrile illness, respectively, after adjusting for covariates. The association between arsenic exposure measured during toddlerhood and childhood was attenuated and not significantly associated with either outcome. Diarrheal disease was too infrequent to assess.

Conclusions: Drinking water arsenic exposure during pregnancy was associated with a higher risk of acute respiratory infections in children 4–5 years old in Bangladesh.

Keywords: Arsenic; Bangladesh; Respiratory infection; Pregnancy; Windows of susceptibility; Developmental immunotoxicity

Introduction

Inorganic arsenic is immunotoxic. Yet few epidemiologic studies have examined the effect of environmental exposure to arsenic and immune-related outcomes in pediatric populations. Bangladesh presents a unique opportunity to examine the association between arsenic exposure from drinking water and acute infectious diseases. This is because arsenic exposure from drinking contaminated groundwater remains widespread in this country even though there have been tremendous efforts to remediate this hazard.1,2 Drinking water surveys conducted in 2012–2013 reported one-eighth of sampled wells had arsenic concentrations above the Bangladeshi national guideline of 50 μg/L,3 and one-quarter were above the World Health Organization (WHO) guideline of 10 μg/L.4,5 These data indicate that there are approximately 57 million people exposed to arsenic concentrations from drinking water that exceed WHO recommendations.

Furthermore, infectious diseases remain an important cause of morbidity and mortality. In Bangladesh, infectious diseases in children under 5 years of age account for 17%, 6%, 4.5%, and 2% of deaths from lower respiratory infections, intestinal infections, neonatal sepsis and other infections, and vaccine-preventable illnesses, respectively (2015 estimate6). Furthermore, infectious diseases remain an important cause of morbidity and mortality. In Bangladesh, infectious diseases in children under 5 years of age account for 17%, 6%, 4.5%, and 2% of deaths from lower respiratory infections, intestinal infections, neonatal sepsis and other infections, and vaccine-preventable illnesses, respectively (2015 estimate6).

The potential for inorganic arsenic to influence immunological functioning is well established in experimental models. In vitro studies demonstrate that arsenic selectively inhibits T-cell proliferation, has dose-dependent effects on natural T regulatory lymphocytes, causes apoptosis of monocytic and lymphoid cells, impairs macrophages,7–15 and causes higher morbidity and mortality from influenza.16–18 Additionally, inorganic arsenic can readily cross the placenta. Thus, gestational exposure may also be immunotoxic.19–21 Epidemiologic studies have shown that arsenic exposure from drinking water contributes to altered DNA methylation of leukocytes in expectant mothers and their fetuses.7,22–24

Data collected in a large prospective study in Bangladesh also reported a positive dose–response between gestational arsenic exposure and increased risk of lower respiratory infection and diarrhea in children during their first year of life.14 In the same cohort, arsenic was also associated with decreased bacillus Calmette-Guerin (BCG, anti-Tb) vaccine response;16 increased

What this study adds

This prospective study observed an exposure–response relationship between gestational arsenic exposure and a modest increased risk of acute respiratory illness in children 4–5 years old. However, arsenic exposure during toddlerhood and childhood was not associated with an increased risk of respiratory illness suggesting that fetal development may be the most susceptible lifestyle to the immunotoxic effects of arsenic.
total IgG and IgE, and decreased mumps-specific IgG.37 Another prospective study conducted in the United States reported a dose–response association between gestational arsenic exposure and a higher risk of respiratory infections requiring prescription medicine in children during the first year of life.25,29 These studies document an association between gestational arsenic exposure and disease in infancy. However, it is unclear if gestational arsenic exposure is also associated with diseases later in childhood or whether arsenic exposure that occurs after fetal development influences susceptibility to infectious diseases.22,26,30

Therefore, we conducted a follow-up study of an existing prospective birth cohort recruited in Bangladesh. This cohort had repeated arsenic exposure measurements available from pregnancy, when the children were 1–2 years of age and through age 5. We also conducted active disease surveillance among the children when they were 4–5 years old. We hypothesized that higher arsenic exposure, as measured in household drinking water, would be associated with an increased incidence of infectious disease symptoms.

Methods

Data and study population

Pregnant women were enrolled in a birth cohort study between January 2008 and June 2011 in Sirajdikhan and Pabna Sadar Upazila, Bangladesh. Details about cohort formation are described elsewhere.33–35 Briefly, women were ≥18 years old, had an ultrasound-confirmed singleton pregnancy ≤16 weeks gestational age, and used the same drinking water source for at least 6 months before enrollment. Dhaka Community Hospital (DCH) provided prenatal and primary care to participants. DCH notified participants of the arsenic concentrations in the water from their household’s well after enrollment. If arsenic levels were above 50 μg/L, the Bangladesh national standard, DCH gave participants arsenic awareness training. After the initial enrollment visit, women completed two additional study visits to the clinic and DCH staff provided monthly home visits. Initially, 1,608 eligible pregnant women were enrolled, of which 224 experienced a fetal loss or neonatal death, and 263 withdrew or were lost to follow-up by 1-month postpartum, leaving 1,121 children remaining for study after 1-month postpartum. A subset of these maternal–child pairs were reenrolled to participate in a follow-up study (2010–2013) after 1-month postpartum. A subset of these maternal–child pairs were reenrolled to participate in a follow-up study (2010–2013) that collected samples at 12 months of age and/or between 20 and 40 months of age to explore the effects of metals on neurodevelopment.46 In 2015–2017, children and their caregivers were reenrolled into another follow-up study to explore the relationship between metals and immune function.16 In this follow-up study, children needed to be at least 4 years (±3 months) of age to be eligible for the active disease surveillance which ended at their fifth birthday. The active disease surveillance involved a field staff member visiting participants homes every 2 weeks for the duration of time the child was eligible for observation. Of the 1,121 children eligible from the initial birth cohort, we excluded children if they were unable to be contacted or refused to participate (n = 47), were reported dead (n = 1), were too old (n = 78), or were missing outcome data (n = 6), which left a total sample size of 989. Due to the rolling enrollment of the initial cohort and the active disease surveillance follow-up study, children were observed for varying amounts of time, ranging from a single visit to 29 visits over a year (eTable 1; http://links.lww.com/EE/A72).

Consent was obtained from each participant before initiating any study activity. Consent documents were in Bengali and read aloud by study staff to account for varying literacy rates in this population. This study was approved by the Institutional Review Boards at Dhaka Community Hospital, Oregon State University, and Harvard T.H. Chan School of Public Health.

Arsenic exposure assessment

The exposure of interest was arsenic measured in the family’s primary drinking water source. Drinking water samples were collected up to 6 times throughout the cohort study: at ≤16 completed weeks gestational age, within 1 month of birth, at 12 months, 20–40 months, 4 years, and 5 years of age. Concentrations were averaged to summarize these observations into three distinct time periods corresponding to distinct developmental exposure windows: pregnancy, which encompasses transplacental exposure (enrollment and <1-month postpartum); toddlerhood, where breastfeeding provides protection from arsenic ingestion (12 and 20–40 months); and childhood, where exposure results from food and water ingestion (4 and 5 years).37 Water arsenic measurements were available for all children in pregnancy and childhood, but 117 (12%) were missing in toddlerhood. Thus, analyses were conducted using imputed measurements for the missing water samples and using only complete data.

All water samples were collected following the same protocol. Briefly, 50 ml of water was collected and preserved with ultrapure nitric acid. Samples were analyzed following United State Environmental Protection Agency Method 200.8 by Environmental Laboratory Services, as described previously.18 Quality control procedures demonstrated that the average percentage recovery of arsenic from PlasmaCAL multielement Quality Control standard #1 solution (SCP Science, Canada) was 101% (range: 92%–110%). The limit of detection (LOD) was 1 μg As/l, and 2%, 19%, and 28% of samples in pregnancy, toddlerhood, and childhood were below the LOD. These samples were subsequently assigned LOD/2 for analysis.

Infectious disease surveillance and outcomes

As part of the active disease surveillance, caregivers were contacted every 2 weeks between children’s fourth and fifth birthdays and administered a structured questionnaire that asked about symptoms experienced by their children in the past 3 days or past 2 weeks. Symptoms included diarrhea (defined as 3 or more loose stools in a 24-hour period), acute respiratory infection (defined as cough or difficulty breathing), and fever (not medically confirmed). Given that 3-day recall is more accurate and less biased than 2-week recall,35 only 3-day recall outcomes were analyzed in this study. This resulted in 8,592 days of observation. Outcomes were analyzed as incidence rates (number of instances of illness per person-time at risk) to account for the variable at-risk observation period of each child that resulted from the rolling enrollment in this follow-up study and that not all children were able to be enrolled on their fourth birthday.

Covariates

Covariates of interest were selected a priori after reviewing literature on arsenic exposure and infectious disease outcomes to improve our ability to replicate previous research findings.31,40 Information on covariates was collected via questionnaires with responses supplied by either caregivers or medical personnel. Demographic variables included child sex, maternal age at time of initial enrollment into the birth cohort (categorized as above or below the median age of 22 years because exact birthdays are often unknown to rural residents of Bangladesh), monthly family income at time of child’s birth (as reported by husband or father), self-reported maternal education, and gravidity (the number of previous pregnancies reported by the mother regardless of the outcome of those pregnancies). Information from birth outcomes was collected by skilled birth attendants: delivery type (vaginal versus Cesarean) and preterm birth (defined as <37 weeks gestation). Information about environmental and behavioral exposures included maternal smoking (defined as any smoking around the mother during pregnancy or around the child during childhood, ever/never), the number of hours the
mother spent a day cooking over an open fire during pregnancy (continuous), biomass fuel use during pregnancy and childhood (defined as kerosene versus dung/crop residue and wood), sanitary latrine (yes/no), and duration of breastfeeding (continuous). Maternal protein consumption was measured using a validated dish-based food-frequency questionnaire collected at 28 weeks gestational age and analyzed as tertiles of protein consumption including fish.

Statistical analysis

Graphical and numerical descriptive statistics were used to examine the distributions of water arsenic exposure, infectious disease outcomes, and selected covariates. Water arsenic levels were natural-log transformed for regression analysis. Multicollinearity of drinking water arsenic exposure measurements and covariates was explored by condition indices and variance decomposition proportions in the “perturb” and “car” packages in R. Variance inflation factors (VIF) were all <2.5 indicating no multicollinearity. Poisson regression was used to estimate the association between water arsenic level and incidence of infectious symptoms using the equation \( \ln(\theta_i) = \beta_0 + \beta_1 \ln(\text{As}_{\text{pregnancy}}) + \beta_2 \ln(\text{As}_{\text{toddlerhood}}) + \beta_3 \ln(\text{As}_{\text{childhood}}) + \text{covariates}, \) where \( \theta_i \) is the incidence rate of disease for child \( i \) during the person-time at risk for child \( i \) and covariates included child sex, maternal age, household income, maternal education, sanitation, maternal protein consumption, preterm birth, Cesarean birth, biomass fuel burning, environmental tobacco smoke, gravidity, duration of breastfeeding, and hours spent over open fire during pregnancy.

We used multiple imputation by chained equations (MICE) to impute the missing predictor variables in the fully adjusted models using all other predictor variables (12% of toddlerhood drinking water arsenic values and <1.5% of all other variables). We used predictive mean matching (PMM) for continuous variables, logistic regression for binary variables, and multinomial logistic regression for categorical variables. A total of 20 imputed datasets were produced. We also reran the analysis using complete case (nonimputed) data and explored effect measure modification by including interaction terms for child sex and each time period of arsenic exposure.

Results

A description of the selected characteristics of the 989 children who were included in this analysis is presented in Table 1. Child sex was evenly distributed between girls and boys. For most mothers (70%), this was their first or second pregnancy, and half of mothers were under 23 years of age. Most mothers received at least a primary education and breastfed for an average of 27 months, and a majority of households had sanitary latrines. Households that never reported illnesses tended to be lower income, have higher maternal protein consumption during pregnancy, have a higher proportion of preterm births but fewer Cesarean births, and more biomass fuel burning in the home. A majority of pregnant women and children were exposed to environmental tobacco smoke, and women spent on average 2.5 hours a day over a cooking fire during pregnancy. Infectious disease symptoms experienced in the last 3 days are reported in Table 1. Symptoms that could indicate acute respiratory illness (ARI) was most frequently observed with 16.5% of children reporting respiratory symptom during the active disease follow-up (4–5 years of age) yielding an incidence rates of 1.2 per 100 person-days. Fever was the next most common symptom with an incidence rate of 1.0 cases per 100 person-days. Only 1 case of diarrhea was reported corresponding to incidence rates of 0.01 cases per 100 person-days. Given the scarcity of diarrhea cases, this outcome was not analyzed further in this study. Arsenic levels in drinking water during pregnancy ranged from 1 to 1,400 µg/L. The majority of participants (77%) used wells that met the Bangladesh drinking water action level (Table 1). Generally, drinking water arsenic levels were low. The median arsenic concentration in the household’s drinking water well was 4.6 µg/L when the mother was pregnant with the child (pregnancy), 8.8 µg/L when the child was between the ages of 12 and 40 months (toddlerhood), and 4.2 µg/L when the child was 4–5 years of age (childhood) (Table 2). Water arsenic values were correlated between all three follow-up visits \((r = 0.57 \text{ to } r = 0.61)\), with an intraclass correlation coefficient of 0.57.

The associations between household drinking water arsenic concentrations at each life stage and disease incidence are presented in Table 3. The association between arsenic exposure during pregnancy and the incidence rate ratio (IRR) for respiratory symptoms in crude models was 1.03 (95% confidence interval \([CI] = 0.94, 1.14\) only controlling for toddlerhood and childhood arsenic exposures. However, adjusting for covariates strengthened this association, and we observed that each doubling in drinking water arsenic during pregnancy was associated with a 10% increase in ARI \((\text{IRR} = 1.10, 95\% \text{ CI} = 1.00, 1.22)\) after controlling for arsenic exposure during toddlerhood and childhood, child sex, maternal age, household income, maternal education, sanitation, maternal protein consumption, preterm birth, Cesarean birth, biomass fuel burning, environmental tobacco smoke, gravidity, duration of breastfeeding, and hours spent over open fire during pregnancy. The association between drinking water arsenic exposure in toddlerhood and childhood and ARI was attenuated \((\text{As}_{\text{toddlerhood}} \cdot \text{As}_{\text{childhood}} \cdot \text{RR} = 0.96, 95\% \text{ CI} = 0.88, 1.04)\). Arsenic exposure during pregnancy was associated with a lower incidence rate ratio for fever symptoms in models that only adjusted for arsenic exposures at different life stages \((\text{IRR} = 0.88, 95\% \text{ CI} = 0.79, 0.99)\). However, after adjusting for other covariates, the associations became null \((\text{As}_{\text{pregnancy}} \cdot \text{IRR} = 0.93, 95\% \text{ CI} = 0.82, 1.05)\). Null associations were also observed in adjusted models between fever and drinking water arsenic in toddlerhood \((\text{IRR} = 1.06, 95\% \text{ CI} = 0.98, 1.14)\) and drinking water arsenic in childhood \((\text{IRR} = 0.94, 95\% \text{ CI} = 0.86, 1.04)\). These analyses with the complete data produced similar conclusions (eTable 2; http://links.lww.com/EE/A72). The interaction terms for child sex and drinking water arsenic were null at all three time periods of exposure (eTable 3; http://links.lww.com/EE/A72).

Discussion

In this prospective cohort study in Bangladesh, we observed that exposure to arsenic from drinking water during pregnancy was marginally associated with higher incidence of respiratory systems but not fever in children between the fourth and fifth year of life. However, exposure to arsenic from drinking water during toddlerhood or childhood was not associated with infectious disease symptoms. This attenuation of effect could be due to exposure misclassification from using household water arsenic levels instead of using a biomarker of internal dose. Alternatively, it could also be a function of the timing of the arsenic exposure and the developing fetus being more sensitive to the immunotoxic effects of arsenic.

Our observation that gestational arsenic exposure is associated with higher incidence of respiratory disease symptoms is consistent with other prospective birth cohort studies that showed that higher maternal urinary arsenic levels were associated with more infectious diseases in the first year of life. The Maternal and Infant Nutrition Interventions in Matlab (MINIMat) study in Bangladesh measured arsenic exposure in maternal urine twice during pregnancy and field staff conducted weekly home visits to interview caregivers about disease symptoms during children’s first year of life.25 The authors reported an increased risk of lower respiratory infection among children with highest versus lowest arsenic exposure levels after controlling for mother’s education, asset index, parity, body mass index, gestational age, and infant’s sex. The New Hampshire Birth Cohort Study (NHBCS),
another prospective birth cohort study conducted in the United States, also used maternal urinary biomarkers to assess arsenic exposure during pregnancy.\textsuperscript{28,29} This study also reported that higher arsenic exposure during pregnancy was associated with an increased risk of respiratory infection based on parent recall of infections and doctor’s diagnoses during the first 4 months of life. Our study provides a useful addition to these studies by continuing follow-up into the fifth year of life. Like the MINIMat and NHBCS studies, we also observed a positive exposure–response between arsenic exposure and respiratory infectious disease symptoms, although the magnitude of effect observed in our study is smaller than the studies that utilized urinary arsenic biomarkers. However, our study did differ from the NHBCS and MINIMat study regarding fever. The NHBCS reported a positive association between arsenic exposure in drinking water and fever in the first year of life,\textsuperscript{29} and the MINIMat study reported a positive association between arsenic exposure and maternal fever during pregnancy.\textsuperscript{31} However, we observed a null association between arsenic exposure in drinking water at all three time points and fever.

Table 1. Descriptive statistics of selected characteristics by illness status using a 3-day recall period during 12 months of active disease surveillance in a cohort of children age 4–5 years recruited in Bangladesh (N = 989)

| Outcomes | No Illness | Diarrhea | Respiratory (ARI) | Fever |
|----------|------------|----------|-------------------|-------|
| No. events (cases) | 0 | 1 | 107 | 84 |
| Total time at risk (days) | 8,592 | 8,592 | 8,592 | 8,592 |
| Incidence rate (cases/100 person-days) | 0.01 | 1.2 | 1.0 | 1.0 |
| No. children, n | 826 | 103 | 78 | 78 |
| Water As tertile in pregnancy | | | | |
| Median (IQR) | | | | |
| 1 (0.8, 1.2) µg/L | 212 (25.7) | 0 (0) | 24 (23.3) | 15 (19.2) |
| 4.6 (3.1, 11.3) µg/L | 180 (21.8) | 1 (100) | 34 (33) | 43 (55.1) |
| 90.4 (39.4, 188.1) µg/L | 213 (25.8) | 0 (0) | 21 (20.4) | 12 (15.4) |
| Child sex | | | | |
| Female | 403 (48.8) | 1 (100) | 55 (53.4) | 40 (51.3) |
| Male | 423 (51.2) | 0 (0) | 48 (46.6) | 38 (48.7) |
| Maternal age (years) | | | | |
| 18–22 | 446 (54) | 0 (0) | 62 (60.2) | 38 (48.7) |
| 23+ | 380 (46) | 1 (100) | 41 (39.8) | 40 (51.3) |
| Monthly household income (Taka, Tk) | | | | |
| 0–4,000 | 368 (44.6) | 1 (100) | 35 (34) | 23 (29.5) |
| 4,001–5,000 | 248 (30) | 0 (0) | 27 (26.2) | 21 (26.9) |
| 5,001–6,000 Tk | 115 (13.9) | 0 (0) | 19 (18.4) | 17 (21.8) |
| 6,001 + Tk | 91 (11) | 0 (0) | 21 (20.4) | 17 (21.8) |
| Maternal education | | | | |
| No school to primary | 380 (46) | 0 (0) | 57 (55.3) | 41 (52.6) |
| Secondary + | 446 (54) | 1 (100) | 46 (44.7) | 37 (47.4) |
| Sanitary latrine in pregnancy and childhood | | | | |
| No | 17 (2.1) | 0 (0) | 4 (3.9) | 3 (3.8) |
| Yes | 799 (96.7) | 1 (100) | 97 (94.2) | 73 (93.6) |
| Protein consumption | | | | |
| First tertile | 255 (30.9) | 0 (0) | 42 (40.8) | 40 (51.3) |
| Second tertile | 276 (33.4) | 1 (100) | 37 (35.9) | 22 (28.2) |
| Third tertile | 294 (35.6) | 0 (0) | 24 (23.3) | 16 (20.5) |
| Preterm birth | | | | |
| No | 640 (77.5) | 0 (0) | 94 (91.3) | 66 (84.6) |
| Yes | 186 (22.5) | 1 (100) | 9 (8.7) | 12 (15.4) |
| Cesarean birth | | | | |
| No | 551 (66.7) | 0 (0) | 59 (57.3) | 37 (47.4) |
| Yes | 275 (33.3) | 1 (100) | 44 (42.7) | 41 (52.6) |
| Biomass fuel burning in pregnancy and childhood | | | | |
| No | 59 (7.1) | 0 (0) | 7 (6.8) | 11 (14.1) |
| Yes | 766 (92.7) | 1 (100) | 99 (92.2) | 65 (83.3) |
| Environmental tobacco smoke | | | | |
| No | 359 (43.5) | 0 (0) | 51 (49.5) | 27 (34.6) |
| Yes | 467 (56.5) | 1 (100) | 52 (50.5) | 50 (64.1) |
| Mean (SD) Mean (SD) Mean (SD) Mean (SD) | | | | |
| Gravida | 1.1 (1.2) | 2 (NA) | 0.8 (1) | 1 (1) |
| Duration of breastfeeding (months) | | | | |
| 26.7 (10.9) | 18 (NA) | 26.6 (12.6) | 27.9 (11.2) |
| Hours spent cooking over open fire during pregnancy | | | | |
| 2.5 (0.7) | 2 (NA) | 2.4 (0.8) | 2.5 (0.7) |

*Percentages do not always match column totals due to missing covariate data. IQR, interquartile range; Water As, water arsenic.
Table 2.
Description of arsenic exposure in participants’ household drinking water during pregnancy, toddlerhood, and childhood

|          | n   | Mean (SD) | Median (IQR) | Minimum–maximum | Percent detectable |
|----------|-----|-----------|---------------|-----------------|-------------------|
| Pregnancy| 989 | 49.4 (105.8) | 4.6 (1.2–39.2) | 0.5–1,400       | 97.9              |
| Toddlerhood| 872 | 60.86 (117.96) | 8.8 (7.5–62.03) | 0.04–1,470      | 80.7              |
| Childhood | 989 | 50.56 (119.89) | 4.19 (5.4–47.14) | 0.5–1,440       | 71.6              |
| Overall  | 989 | 53.3 (114.7) | 5.4 (8.9–48.5) | 0.04–1,470      | 83.5              |

|          | Pregnancy | Toddlerhood | Childhood |
|----------|-----------|-------------|-----------|
| Correlation for ln(As) | 1 | 0.57 | 0.58 |

IQR, interquartile range.

Table 3.
Poison models estimated incident rate ratio for respiratory and fever symptoms in children 4–5 years of age per doubling in household drinking water arsenic concentration at the three life stages, Bangladesh, 2008–2017 (N = 989, missing data imputed)

|          | Crude IRR (95% CI) | Adjusteda IRR (95% CI) |
|----------|--------------------|------------------------|
| ARI, 3-day recall |                     |                        |
| Pregnancy | 1.03 (0.94, 1.14)  | 1.10 (1.00, 1.22)     |
| Toddlerhood| 1.03 (0.95, 1.11)  | 1.00 (0.93, 1.08)     |
| Childhood  | 0.93 (0.86, 1.01)  | 0.96 (0.88, 1.04)     |
| Fever, 3-day recall |                 |                        |
| Pregnancy | 0.88 (0.79, 0.99)  | 0.93 (0.82, 1.05)     |
| Toddlerhood| 1.07 (0.99, 1.16)  | 1.06 (0.98, 1.14)     |
| Childhood  | 0.93 (0.85, 1.02)  | 0.94 (0.86, 1.04)     |
| Diarrhea, 3-day recall |                 |                        |
| Models not fit |                |                        |

aAdjusted for child sex, maternal age, household income, maternal education, sanitary latrine, maternal protein consumption, preterm birth, Cesarean birth, biomass fuel burning, environmental tobacco smoke, gravity, duration of breastfeeding, and hours spent over open fire during pregnancy.

households in this cohort during 5 years of repeated clinic and research staff home visits and training community health care worker. Also, the levels of arsenic exposure in our study were relatively modest, and perhaps there is a threshold related to immunological effects. Finally, it should be noted that almost everyone in this population had sanitary latrines which has been shown to reduce childhood diarrheal illness, although it should be noted that the high prevalence of sanitary latrines in this sample is not representative of all families in rural Bangladesh.

It is biologically plausible that gestational arsenic exposure can increase the risk of respiratory illnesses. Toxicology studies in humans and animals have shown that arsenic accumulates in the lungs, leading to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues. Arsenic also leads directly to the generation of reactive oxygen species, leading to lipid peroxidation and increased oxidative stress and damage. Arsenic exposure leads to chronic inflammation, including in lung tissues.

Our study had several weaknesses that could explain our inability to replicate the association between arsenic exposure and fever, as well as the attenuation of the effect between postnatal arsenic exposure and respiratory symptoms. Primarily, our analysis relied on household drinking water arsenic levels to assign children's exposures which could result in exposure misclassification. Arsenic is not readily excreted in breastmilk; thus, arsenic exposure would be reduced during breastfeeding periods. Subsequently, household water arsenic levels would overestimate children’s arsenic exposure during breastfeeding and possibly underestimate their exposure after being weaned because it would not reflect dietary arsenic intake. Additionally, we do not account for seasonal variations in groundwater arsenic levels which could contribute to exposure misclassification. Although previous studies in this cohort have observed that household drinking water arsenic exposure is positively correlated with maternal and infant biomarkers of exposure, future studies would be able to minimize postnatal exposure misclassification by using internal biomarkers of exposure such as urine, hair, or nails. Furthermore, urinary arsenic biomarkers would provide an opportunity to examine whether arsenic methylation capacity influences individual susceptibility to infectious disease risk.

Other limitations to our study included reliance on caregiver’s recalling and self-reporting symptoms in their children. Although a parent may observe symptoms more readily, mild diarrheal symptoms may go unnoticed in children 4–5 years old because they may be sufficiently potty trained and not notify their caregivers unless they are severely ill. In addition, both diarrheal and respiratory illnesses are more common at certain times of the year, and this seasonality was not directly accounted for in this analysis. However, enrollment and active disease follow-up was evenly distributed throughout the year (data not shown) so any seasonality in infectious diseases should be randomly distributed throughout the follow-up period.

Despite these limitations, our study had a number of strengths including repeated measures of arsenic exposure at multiple key developmental periods in a large population-based sample. We also relied on 3-day recall of symptoms which has shown to have the least amount of recall bias. Furthermore, our cohort experienced a wide range of arsenic exposure with most occurring below the current World Health Organization drinking water recommended guideline of 10 µg/L which allowed us to examine the effect of low to modest arsenic exposure on infectious disease symptoms in children. Also, we were able to adjust for breastfeeding duration. Given that arsenic does not readily pass the mammary gland, breastfeeding is an important protective behavior against arsenic exposure and is also protective against childhood infection, our main outcomes.

In conclusion, drinking water arsenic during pregnancy was associated with a modest increase in respiratory illness, but not fever, in children between ages 4 and 5 years in Bangladesh. These results add to the growing body of evidence that environmental exposure to arsenic during fetal development is associated with increased risk of infectious diseases during childhood.

Conflicts of interest statement
The authors declare that they have no conflicts of interest with regard to the content of this report.

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