Arsenic Exposure and Risk of Spontaneous Abortion, Stillbirth, and Infant Mortality

Anisur Rahman,[a,b] *Lars-Åke Persson,*[b] *Barbro Nermell,*[c] *Shams El Arifeen,*[a] *Eva-Charlotte Ekström,*[b] *Allan H. Smith,*[d] *and Marie Vahter*[c]

Background: Millions of people worldwide are drinking water with elevated arsenic concentrations. Epidemiologic studies, mainly cross-sectional in design, have suggested that arsenic in drinking water may affect pregnancy outcome and infant health. We assessed the association of arsenic exposure with adverse pregnancy outcomes and infant mortality in a prospective cohort study of pregnant women.

Methods: A population-based, prospective cohort study of 2924 pregnant women was carried out during 2002–2004 in Matlab, Bangladesh. Spontaneous abortion was evaluated in relation to urinary arsenic concentrations at gestational week 8. Stillbirth and infant mortality were evaluated in relation to the average of urinary arsenic concentrations measured at gestational weeks 8 and 30.

Results: The odds ratio of spontaneous abortion was 1.4 (95% confidence interval [CI] = 0.96–2.2) among women with urine arsenic concentrations in the fifth quintile (249–1253 μg/L; median = 382 μg/L), compared with women in the first quintile (<33 μg/L). There was no clear evidence of increased rates of stillbirth. The rate of infant mortality increased with increasing arsenic exposure: the hazard ratio was 5.0 (95% CI = 1.4–18) in the fifth quintile of maternal urinary arsenic concentrations (268–2019 μg/L; median = 390 μg/L), compared with the first quintile (<38 μg/L).

Conclusions: We found evidence of increased risk of infant mortality with increasing arsenic exposure during pregnancy, with less evidence of associations with spontaneous abortion or stillbirth risk.

(*Epidemiology* 2010;21: 797–804)

Arsenic is widely distributed in the environment, and people are exposed mainly through well water and, to a lesser extent, through the food chain.[1,2] Many millions of people drink water with inorganic arsenic concentrations exceeding the World Health Organization guideline value of 10 μg/L.[2–4] Arsenic is an established carcinogen and is also associated with a wide range of other chronic illnesses, such as diabetes, hypertension, and vascular diseases.[2,5]

Arsenic is known to readily cross the placental barrier.[6] Animal studies have found arsenic to be embryotoxic and teratogenic at high doses,[2,7,8] but results are difficult to extrapolate to humans because of major differences in kinetics and susceptibility.[2,9] Also, a number of epidemiologic studies suggest that arsenic exposure via drinking water may affect early human development, but findings are mixed.[10,11] Several studies have reported an association of arsenic exposure with spontaneous abortion and stillbirth, with about 2 to 3 times higher risks among women with high arsenic concentrations in their drinking water (>50 μg/L).[12–16] Elevated neonatal and postneonatal mortality was observed during a period of high water arsenic concentration (800 μg/L) in Chile compared with an area having essentially no arsenic in the drinking water.[17] A recent retrospective cohort study in Bangladesh found a weak association between arsenic exposure via drinking water and fetal loss, and a more clear association with infant mortality.[18] These inconsistent findings may be related to the fact that several of the studies used retrospective assessment of outcomes, which can be subject to recall bias. Also, exposure assessments were based mainly on measurements of arsenic concentrations in water, with no assessment of water consumption during pregnancy, and not all studies adjusted adequately for confounding factors.

The aim of the present study was to evaluate the associations of individual arsenic exposure (assessed by arsenic concentrations in urine during pregnancy) with spontaneous abortion, stillbirth, and infant mortality in a prospective

Submitted 16 February 2009; accepted 21 June 2010.

From the [a]International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; [b]International Maternal and Child Health, Department of Women's and Children's Health, University Hospital, Uppsala University, Uppsala, Sweden; [c]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; and [d]School of Public Health, University of California, Berkeley, CA.

Supported by United Nations Children's Fund (UNICEF), Swedish International Development Cooperation Agency (SIDA), UK Medical Research Council, Swedish Research Council, Department for International Development (DfID), International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Global Health Research Fund-Japan, Child Health and Nutrition Research Initiative (CHNRI), Uppsala University and United States Agency for International Development (USAID) (for The MINIMat project). This arsenic-related substudy was further supported by Swedish International Development Agency (Sida), World Health Organization, United States Agency for International Development (USAID), Swedish Research Council, and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. Matlab HDSS is primarily supported by the Department for International Development (DfID).

Correspondence: Anisur Rahman, International Maternal and Child Health, Department of Women's and Children's Health, Uppsala University Hospital, SE-75185 Uppsala, Sweden. E-mail: anisur.rahman@kbh.uu.se.

ISSN: 1044-3983/10/2106-0797
DOI: 10.1097/EDE.0b013e3181f56a0d

cohort study of pregnant women with a wide range of arsenic exposure.

METHODS

Study Area

The study was carried out in the subdistrict of Matlab, Bangladesh, located 53 km southeast of the capital, Dhaka. The International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) has been running a health and demographic surveillance system since 1966 in a population of about 220,000 in 142 villages of Matlab. The present study was conducted in half of the surveillance system area, where ICDDR,B provides health care to women of reproductive age and children less than 5 years of age. This area is divided into 4 administrative blocks, each with a population of about 25,000–27,000. Each block has a subcenter clinic, where paramedical staff provide maternal and child care, including delivery services 24 hours a day. The clinics are supported by a hospital located at the Matlab Center. Data on vital statistics and selected child and maternal morbidity events are collected in monthly household visits by community health research workers.

Study Design and Participants

This prospective population-based cohort study was nested within a food and micronutrient supplementation trial—Maternal and Infant Nutrition Interventions, Matlab Study. The analysis was based on a cohort of pregnancies identified by the regular monthly home visits of the community health research workers. Urine-based pregnancy testing (ACON, United States) was offered to all women who reported that their menstrual period was at least 2 weeks overdue. Women with positive pregnancy tests were invited to provide the remaining urine sample for analysis of arsenic concentration. The urine samples were collected around gestational week 8 (median = 8 weeks; mean = 9 [standard deviation {SD} = 3]). Between February 2002 and January 2003, 2924 women donated urine samples for arsenic analysis in early gestation (Figure). All women with a positive pregnancy test in early gestation were invited to visit their health center for evaluation of eligibility to enroll in the Maternal and Infant Nutrition Interventions, Matlab study. A woman was enrolled if the following eligibility criteria were met: the fetus was viable, with gestational age less than 14 weeks by ultrasound examination; the woman had no severe illness; and she consented to participate. An additional urine sample was collected in the late gestational period, around gestational week 30 (median = 30.5 weeks; mean = 30 [SD = 2] weeks). In total, 1725 women had urine arsenic concentrations measured in both early and late gestation (Figure).

Ethical Considerations

We were not able to inform women about the level of arsenic concentrations in their urine samples during pregnancy, as analyses were performed abroad and results were not available until after the end of pregnancy. However, a parallel study analyzed water arsenic concentrations in all tube-wells in the study area; based on these results, pumps were painted red if the water arsenic concentrations exceeded the national drinking water standard of 50 μg/L, whereas the others were painted green.[19] Pregnant women, in particular, were advised to take water from the green tube-wells. In addition, several mitigation options (such as pond sand filter, rain-water harvesting, and various household filters) were provided to the arsenic-exposed families.[19] The pregnancy cohort study was approved by the ethical review committee of ICDDR,B and the Regional Ethical Committee at the Karolinska Institutet, Stockholm.

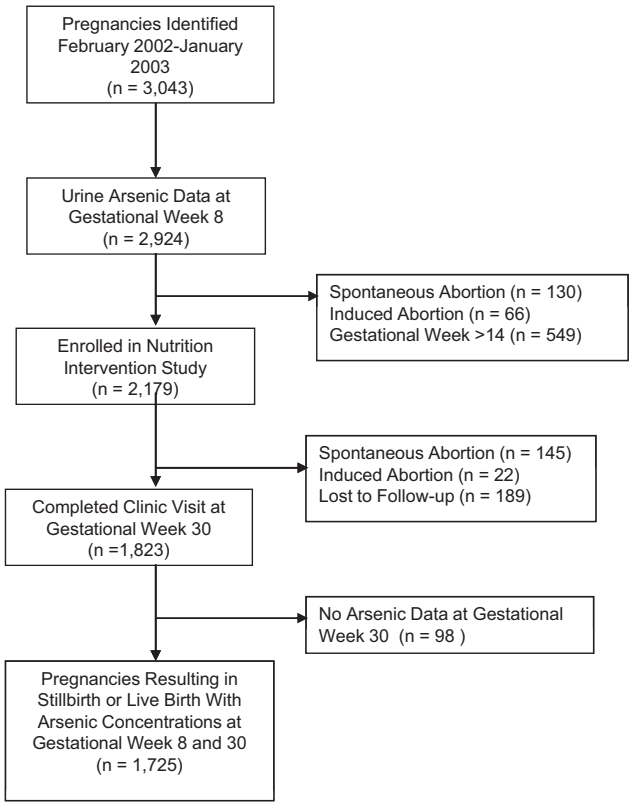

FIGURE. Study participation of the 2002–2003 Pregnancy Cohort in Matlab, Bangladesh.

Exposure Assessment

Exposure was assessed by measuring the concentration of inorganic arsenic and its metabolites in urine, which provides a measure of the ongoing exposure to inorganic arsenic from all sources. The sum of inorganic arsenic and the methylated metabolites was measured by hydride generation atomic absorption spectroscopy.[20] To compensate for variation in the dilution of the urine (caused by variation in fluid intake, time of sampling, temperature, and physical activity), we adjusted the concentrations by specific gravity (to the

average of 1.012 g/mL). Our group has previously reported the advantage of using specific gravity for adjustment over the commonly used creatinine adjustment in this study population.[21] Many of the studied women were malnourished, with very low urinary concentrations of creatinine at gestational week 8 (total range = 0.10–1.6 g/L; mean = 0.57 g/L),[22] and so adjustment by creatinine would lead to variation in urine arsenic concentrations depending on nutritional status. Also, urinary creatinine has been found to be associated with urinary arsenic, which further disqualifies creatinine adjustment.[21]

Details of exposure assessment have been described elsewhere.[20] The collected urine samples were transferred to arsenic-free 24-mL plastic bottles, immediately chilled with cooling blocks or refrigerator, and transported to Matlab hospital laboratory on the same day, where samples were stored frozen at −70°C. All samples were analyzed for arsenic concentrations at the Institute of Environmental Medicine, Karolinska Institutet, Sweden.

Pregnancy Outcomes

After diagnosis of pregnancy by urine pregnancy test during monthly household visits by health workers, women were advised to attend the subcenter clinic for an ultrasound examination to verify the pregnancy and its gestational age. Outcome information was then collected prospectively by a team especially recruited and trained. Pregnant women were personally interviewed each month to identify pregnancy outcomes (spontaneous abortion, induced abortion, stillbirth, live birth) and survival in infancy after the birth of the child. Spontaneous abortion was defined as unintended loss of the fetus in the first 28 weeks of gestation, as determined by the last reported menstrual period. Induced abortion was defined as intended loss of fetus in the first 28 weeks of gestation. Stillbirth was defined as birth of a dead fetus after 28 weeks of gestation. Live birth was defined as birth of a fetus with any sign of viability. Infant death was defined as the death of a live-born baby before 12 months of age.

Information on the cause of death was collected through the "verbal autopsy" method. Using a modified structured questionnaire developed by the World Health Organization,[23] interviews were conducted with the caretakers or relatives who had lived with the infant in the same household during the terminal stages of illness and death of the infant. A physician reviewed each verbal autopsy sheet and filled out death certificates based on the list of 3-character categories of the International Classification of Diseases version 10 (ICD10) codes, with notes of the points in favor of their diagnoses. We did not collect information on the types of stillbirth.

Covariates

Detailed information on women's age, gravidity, parity, education, and household assets were collected from the surveillance system databases and from interviews with the cohort participants. Gravidity was defined as number of pregnancies including the present one, and parity as the number of live or dead children before the current pregnancy. Educational status was assessed as number of years completed at school. Economic status was assessed by generating scores through principal-components analysis based on household assets, housing structure, land occupation, and income. These scores were then indexed into quintiles, where 1 represents the poorest and 5 the richest.[24] Last menstrual period date was determined by recall during the pregnancy-identification interview at the monthly household visit. Gestational age at pregnancy outcome was measured by subtracting the last menstrual period date from date of pregnancy outcome and was expressed in weeks. Season of birth was categorized as premonsoon (January–May), monsoon (June–September), and postmonsoon (October–December). In addition, information on the geographical location of the women's residence was obtained.

Women's weight and height were measured during the visit to health facilities at study enrollment (usually at gestational week 9). Weight was measured by electronic scales (SECA, Hamburg, Germany) with a precision of 100 g, and height was measured with locally made wooden scales with a precision of 0.1 cm. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Statistical Analysis

Logistic regression was used to analyze the association between arsenic exposure and spontaneous abortion and stillbirth. A time-to-event approach using Cox proportional hazards models was employed to determine the risk of infant death. We estimated odds ratios (ORs) or hazards ratios (HRs) with 95% confidence intervals (CIs) for each outcome in relation to urine arsenic concentrations.

Spontaneous abortion risk was evaluated in relation to urinary arsenic concentration in early gestation with the lowest quintile of arsenic exposure used as the reference category. We also analyzed associations by gestational age: <8, 8–11, 12–15, 16–19, and ≥ 20 weeks of gestation. In this analysis we compared women who had less than the median and at least the median values of urinary arsenic concentrations at gestational week 8.

Stillbirth and infant mortality were assessed in relation to the average of 2 urinary arsenic concentrations measured at early and late gestation. To evaluate whether variation in arsenic exposure from early to late gestation had any effect on stillbirth and infant mortality, we stratified the exposure as low (<50 μg/L) in both periods, low at gestational week 8 and high (≥ 50 μg/L) at gestational week 30, high at gestational week 8 and low at gestational week 30, and high in both periods.

Analyses of infant mortality were performed with all cases of infant death, as well as excluding those deaths that

were related to intrapartum care (birth asphyxia) or accidents. Birth time was set at time = 0 for infant survival, and follow-up lasted for 365 days.

Associations of covariates with outcomes and exposure were evaluated by Wald test, χ^2 test or a nonparametric test. Potential confounders, associated with exposure and outcome at $P < 0.20$ significance level, were identified, and those found to change the effect estimation by 5% or more were included in the multivariate model. We also included sociodemographic and biologically relevant covariates, associated with outcomes at $P < 0.10$ level, for adjustment in the final multivariate model, to check the robustness of the observed findings.

RESULTS

The 2924 pregnancies with data on arsenic in urine in early pregnancy resulted in 275 (9.4%) spontaneous abortions, 88 (3.0%) induced abortions, 52 (1.8%) stillbirths (late fetal loss), 2509 (85.8%) live births, and 98 infant deaths (39/1000 live births). The mean gestational week for spontaneous abortions was 13 (SD = 6); for induced abortion, 11 (SD = 5); for stillbirth, 37 (SD = 5); and for live birth, 39 (SD = 3) weeks. Table 1 presents background characteristics of the women as were associations of these characteristics with arsenic exposure and with spontaneous abortion. The mean age of women at the time of pregnancy outcome was 26.5 (SD = 6) years. The mean years attending school was 5 (SD = 4), and about one-third of the women were illiterate. None of them smoked or used alcohol. The median urinary arsenic concentration in gestational week 8 was 80 μg/L (range = 1–1253 μg/L). The mean concentration was 154 μg/L (SD = 176).

Women with urine arsenic concentrations in the fifth quintile in early gestation had 44% increased risk of spontaneous abortion (OR = 1.44 [95% CI = 0.96–2.15]), in comparison with women who had arsenic concentrations in the first quintile (Table 2). In the stratified analyses of events occurring in gestational weeks <8 (n = 31), 8–11 (n = 98), 12–15 (n = 89), 16–19 (n = 37), and ≥20 (n = 20), the ORs of spontaneous abortion risk among women with higher exposure levels (≥80 μg/L in early gestation) were 0.73 (95% CI = 0.35–1.49), 1.19 (0.79–1.78), 1.63 (1.06–2.52), 0.86 (0.45–1.65), and 0.54 (0.22–1.37), respectively, in comparison with women exposed to lower urinary arsenic concentrations (less than median value).

Of the 1725 pregnancies for which urine arsenic concentrations at both gestational weeks 8 and 30 were available, 32 (1.8%) resulted in stillbirth and 44 (2.6%) in infant death. The mean gestational week was 38 (SD = 3) for stillbirths and 39 (SD = 2) for live births. The mean age of infant deaths was 34 (SD = 66) days, with a median of 3 days. The median urinary arsenic concentrations at gestational week 30 was 82 μg/L, the mean was 171 μg/L (SD = 217), and the range was 2–3384 μg/L; the concentrations were significantly correlated with those in gestational week 8 (Spearman $r = 0.61$). For the average of weeks 8 and 30, the median urinary arsenic concentration was 94 μg/L and the mean was 163 μg/L

TABLE 1. Background Characteristics and Their Association With Urine Arsenic Concentrations and With Spontaneous Abortions in the 2002–2003 Cohort in Matlab, Bangladesh

Characteristics	No. (%)	Urine Arsenic in Gestational Week 8 Median (μg/L)	Spontaneous Abortion OR (95% CI)
Age (years)			
<20	491 (18)	89	1.31 (0.88–1.96)
20–24[a]	791 (28)	76	1.00
25–29	726 (26)	81	1.12 (0.77–1.62)
30–39	727 (26)	79	1.89 (1.34–2.65)
≥40	49 (2)	68	4.48 (2.25–8.90)
Gravidity			
1[a]	848 (30)	77	1.00
2	690 (25)	76	1.04 (0.74–1.47)
3	520 (19)	88	0.88 (0.59–1.30)
≥4	726 (26)	81	1.43 (1.04–1.98)
Education (years)			
0	702 (25)	85	1.25 (0.92–1.70)
1–5	881 (32)	88	1.15 (0.86–1.55)
>5[a]	1201 (43)	72	1.00
Asset index			
1 (poorest)	611 (22)	94	1.42 (0.96–2.10)
2	593 (21)	97	0.85 (0.55–1.31)
3	521 (19)	82	1.33 (0.88–2.00)
4	536 (19)	69	1.24 (0.82–1.87)
5 (richest)[a]	523 (19)	58	1.00
Season			
Premonsoon	1104 (40)	81	1.61 (1.17–2.23)
Monsoon	869 (31)	70	1.51 (1.07–2.12)
Postmonsoon[a]	811 (29)	94	1.00

[a]Reference category.

TABLE 2. Association of Arsenic Exposure (Quintiles in Early Pregnancy) With Spontaneous Abortion in the 2002–2003 Pregnancy Cohort in Matlab, Bangladesh[a]

Arsenic Concentrations (μg/L)		No. Pregnancies	Spontaneous Abortions	
Interval	Median		No.	OR (95% CI)
<33[b]	23	553	45	1.00
33–57	42	558	57	1.28 (0.85–1.93)
58–121	80	567	63	1.41 (0.94–2.11)
122–248	177	549	47	1.06 (0.69–1.62)
249–1253	382	557	63	1.44 (0.96–2.15)

[a]No significant confounding factor found; therefore, crude estimate is presented.
[b]Reference category.

(SD = 173) (range = 5–2020 μg/L). Although the cohort average exposure was similar in early and late pregnancy, there was a marked intraindividual variation in urine arsenic concentrations during pregnancy. Urinary arsenic concentrations decreased by at least 50 μg/L between gestational weeks 8 and 30 for 198 women, whereas for 255 women the concentrations increased by at least 50 μg/L.

Table 3 presents associations of background factors with average arsenic concentration, and with stillbirth and infant mortality. The highest odds ratio for stillbirth (3.4 [0.92–13]) was observed for women with average urinary arsenic in the range 134–267 μg/L (fourth quintile), in comparison with women who had arsenic concentrations below 38 μg/L (first quintile) (Table 4).

Dose-response associations were observed for infant mortality with arsenic exposure (quintiles of average arsenic concentrations in urine, P for linear trend = 0.02). In comparison with women in the first quintile of urinary arsenic, the

TABLE 3. Background Characteristics and Their Association With Average Urine Arsenic Concentrations (Mean of Gestational Weeks 8 and 30) and With Stillbirth and Infant Mortality in the 2002–2003 Cohort in Matlab, Bangladesh

Characteristics	Pregnancies No. (%)	Arsenic Concentrations No. (%)	Stillbirth OR (95% CI)	Infant Mortality OR (95% CI)
Age (years)				
<20	285 (17)	109	0.75 (0.23–2.45)	1.70 (0.63–4.59)
20–24	501 (29)	97	1.18 (0.48–2.87)	1.71 (0.71–4.11)
25–29[a]	482 (28)	93	1.00	1.00
≥30	457 (26)	87	0.94 (0.36–2.45)	1.87 (0.78–4.50)
Parity				
0	580 (34)	92	0.65 (0.28–1.52)	1.67 (0.72–3.92)
1[a]	456 (26)	92	1.00	1.00
2–3	549 (32)	102	0.62 (0.26–1.48)	1.66 (0.70–3.92)
≥4	140 (8)	92	0.27 (0.03–2.06)	1.20 (0.31–4.59)
Education (years)				
0	420 (24)	112	0.80 (0.33–1.96)	1.99 (0.97–4.07)
1–5	531 (31)	102	0.82 (0.36–1.86)	1.26 (0.60–2.68)
>5[a]	773 (45)	86	1.00	1.00
Asset index				
1 (poorest)	368 (21)	118	1.16 (0.35–3.84)	3.19 (1.16–8.81)
2	364 (21)	111	1.37 (0.43–4.37)	2.40 (0.84–6.88)
3	323 (19)	101	1.55 (0.49–4.93)	1.56 (0.49–4.98)
4	315 (18)	87	1.59 (0.50–5.06)	0.91 (0.24–3.41)
5 (richest)[a]	355 (21)	75		1.00
Height (m)				
<1.50	870 (50)	98	1.90 (0.91–3.96)	0.99 (0.55–1.81)
≥1.50[a]	855 (50)	93	1.00	1.00
BMI (kg/m^2)				
<18.5	510 (30)	112	0.82 (0.17–3.84)	1.28 (0.29–5.73)
18.5–24	1118 (65)	92	0.87 (0.20–3.77)	1.17 (0.27–4.97)
≥25[a]	93 (5)	79	1.00	1.00
Gestational age in weeks				
<37	233 (14)	103	5.23 (2.57–10.68)	2.61 (1.32–5.15)
≥37[a]	1492 (87)	94	1.00	1.00
Seasons				
Premonsoon[a]	678 (39)	85	1.00	1.00
Monsoon	560 (33)	87	0.60 (0.24–1.50)	0.59 (0.28–1.23)
Postmonsoon	487 (28)	124	1.10 (0.49–2.43)	0.69 (0.33–1.44)
Women's residence				
Block A[a]	481 (28)	92	1.00	1.00
Block B	506 (29)	88	0.39 (0.14–1.12)	1.71 (0.78–3.74)
Block C	398 (23)	176	1.11 (0.48–2.54)	0.72 (0.26–2.09)
Block D	340 (20)	58	0.46 (0.15–1.45)	1.41 (0.58–3.42)

[a]Reference category.

TABLE 4. Association Between Average Arsenic Exposure (Mean of Gestational Weeks 8 and 30) and Stillbirth in the 2002–2003 Pregnancy Cohort in Matlab, Bangladesh

Arsenic Concentrations μg/L		No. Pregnancies	No. Stillbirths	OR (95% CI)	Adjusted OR (95% CI)[a]
Quintile	Median				
<38[b]	30	341	3	1.00	1.00
39–67	50	348	6	1.98 (0.49–7.97)	2.06 (0.51–8.38)
68–133	94	346	7	2.33 (0.60–9.07)	2.35 (0.60–9.23)
134–267	189	345	10	3.36 (0.92–12.33)	3.41 (0.92–12.63)
268–2019	390	345	6	1.99 (0.49–8.04)	2.02 (0.50–8.24)

[a]Adjusted for asset index and gestational age.
[b]Reference category.

TABLE 5. Association Between Average Arsenic Exposure (Mean of Gestational Weeks 8 and 30) and Infant Death (Excluding Birth Asphyxia and Accident) in the 2002–2003 Pregnancy Cohort in Matlab, Bangladesh

Arsenic Concentrations (μg/L)		No. Infants	No. Infant Deaths	HR (95% CI)	Adjusted HR (95% CI)[a]
Quintile	Median				
<38[b]	30	338	3	1.00	1.00
39–67	50	342	6	1.98 (0.50–7.93)	1.78 (0.44–7.16)
68–133	94	339	6	2.01 (0.50–8.02)	1.83 (0.45–7.35)
134–267	189	335	7	2.35 (0.61–9.11)	2.29 (0.58–9.05)
268–2019	390	339	14	4.69 (1.35–16.34)	5.01 (1.41–17.84)

[a]Adjusted for asset index, gestational age, season, and location of women's residence. Test for linear trend $P < 0.005$.
[b]Reference category.

HR was 1.5 (95% CI = 0.49–4.6), 1.7 (0.57–5.2), 1.5 (0.46–4.7), and 3.4 (1.2–9.4) for women in the second, third, fourth, and fifth quintiles, respectively (adjusted for asset index, gestational age, season, and location of women's residence).

Of the 44 infant deaths, 15 were due to infections, 13 were the result of premature birth and/or impaired growth, 4 were due to maternal and/or obstetric complications, 2 were caused by congenital defects, and 2 had unspecified causes. In addition, 8 cases were related to birth asphyxia and accidents. When analyses excluded the 8 deaths due to birth asphyxia and accidents, the HR for infant mortality was about 5 times higher (HR = 5.0 [1.4–18]) for women with average urinary arsenic concentrations at the fifth quintile (268–2019 μg/L) in comparison with women had average arsenic concentrations below 38 μg/L (first quintile). A dose-response trend was observed for infant mortality (P value for linear trend = 0.005) (Table 5).

We did not observe meaningful changes in the associations of arsenic with stillbirth and infant death when we stratified by exposure pattern, ie, low in both periods, low at 8 weeks and high at 30 weeks, high at 8 weeks and low at 30 weeks, and high in both periods (data not shown).

DISCUSSION

In this prospective cohort study of pregnancies with a wide range of exposure to arsenic, we observed a clear increase in infant mortality with increasing prenatal arsenic concentrations in mother's urine. However, there was little evidence of increased risks of spontaneous abortions or stillbirths; some odds ratios for these outcomes were elevated, but the confidence intervals were wide, and there was no clear evidence of dose-response relationships.

The strengths of this study include the prospective cohort study design with a relatively large sample size, pregnancies identified by urine test early in gestation, and individual exposure assessment based on urinary arsenic concentrations (which reflect exposure to inorganic arsenic from both water and food). Exposure assessment was conducted in both early and late pregnancy. The prospectively collected outcome data are a particular strength of the study design; most previous studies on arsenic and pregnancy outcomes have had to rely on recall of pregnancy outcomes. Adjustment was made for relevant covariates, including socioeconomic measures, seasonality, and nutritional status. Measurement errors were minimized by careful handling of

urine samples from the field to the laboratory, intensive training of health workers, and a quality-assurance team checking a subsample of outcome and covariate data.

We found little evidence of an association between arsenic exposure and risk of either spontaneous abortion or stillbirth. Although spontaneous abortions were identified more completely than in previous arsenic studies,[12,15,16,18] the rate was still low in comparison with studies that meticulously identified early pregnancies.[25] Pregnancy identification based on reported missing of menstrual periods might exclude women who were not willing to disclose pregnancy at such an early stage and who subsequently had an abortion before the next visit. With pregnancy tests at 9 weeks of gestation, on the average, very early pregnancy losses are obviously missed. Many such early abortions are not identified by the women herself and, thus, are not likely to be reported in interviews later in time. Perhaps arsenic concentrations measured in the first trimester were not satisfactorily representing exposure in the second trimester. For stillbirth, the misclassification bias from failure to identify fresh stillbirths, arising purely from lack of quality intrapartum care, might also lead to dilution of the odds ratios.

We observed clear evidence of an association between arsenic exposure and infant mortality. Although the possibility of residual confounding cannot be ruled out completely, the observed dose-response gradient for infant mortality is supportive of a causal relationship. Arsenic exposure of the infants themselves was not measured directly in this study. Infants who are exclusively breast-fed have low arsenic exposure, as very little arsenic is transferred to breast milk.[26] In the present study, approximately 70% of the deceased infants died within the first month, during which time about 80% of the infants were exclusively breast-fed.[27] Therefore, it is likely that the increased risk of dying in infancy was related to prenatal arsenic exposure. However, we did not measure other elements in drinking water, eg, manganese, which has been associated with infant mortality in Bangladesh.[28]

The mechanisms for arsenic-induced fetal and infant mortality are not known. Arsenic may cause spontaneous abortions by defective implantation and zygote development, and also by aneuploidy. Meiotic anomalies, including spindle disruption and chromosomal misalignment leading to defective preimplantation development of zygotes, has been reported in arsenic-treated mice.[29] Arsenic may also cause spontaneous abortion by aberrant placental vasculogenesis and placental insufficiency, as shown in animals.[30] Several studies have reported that arsenic causes oxidative stress and perturbation of oxidative defense,[9,31] which may be associated with a wide range of reproductive problems through defective placentation and even pre-eclampsia in later pregnancy.[32] Arsenic may also alter immune function either by direct effects or by impaired fetal growth. Studies in Bangladesh, Chile, and Taiwan have previously reported a possible association of arsenic exposure with low birth weight.[33–35] In experimental studies, arsenic has been found to be an endocrine disruptor,[36–38] and therefore, may modulate the effects of steroid receptors responsible for fetal programming on growth and immune function. Further studies are needed to evaluate whether arsenic affects human immune system and morbidity.

Epidemiologic studies of the effects of arsenic exposure through drinking water on reproductive outcomes and child health are scarce, and to our knowledge, no previous study has assessed individual exposure during pregnancy using a biologic marker of exposure. Therefore, it is difficult to compare the exposure range in the present study with that of earlier studies. Use of water arsenic concentration as exposure measures does not reflect exposure from multiple drinking water sources and also through food sources. There are increasing numbers of studies showing a rather high arsenic exposure by means of food in Bangladesh, probably corresponding to urinary arsenic concentrations of about 20–50 μg/L.[20,39]

An ecological study in Hungary indicated an increased occurrence of spontaneous abortions in relation to higher arsenic exposure through drinking water[13]; however, the full report was not published. Two cross-sectional studies in Bangladesh, with retrospective measurement of outcome data, showed 2- to 3-fold increases in spontaneous abortions and stillbirths in women exposed to arsenic through tube-well water, with concentrations more than the local drinking water standard ($\geq$50 μg/L).[12,15] In contrast, a study that used outcome data collected by a food supplementation program reported an association of arsenic exposure ($>$50 μg/L) with malformation (OR = 1.005 per μg/L water concentration [95% CI = 1.001–1.010]) but not with other negative pregnancy outcomes.[40] A cross-sectional study in West Bengal, India, found an association of high arsenic exposure during pregnancy ($>$200 μg/L) with stillbirths (OR = 6.1 [95% CI = 1.5–24]), but not with spontaneous abortions (1.0 [0.38–2.7]).[16] In a recent ecological study in Bangladesh, an increased risk of stillbirth (1.8 [1.1–2.9]) was reported among women with arsenic content in tube-well water above the reference level ($>$10 μg/L).[14] The present study did not find clear increases in poor pregnancy outcomes. In our earlier study of a historical cohort within the Matlab surveillance system that assessed exposure by arsenic concentrations in tube-well water, we found associations of high arsenic concentrations ($>$50 μg/L) with fetal loss (relative risk (RR) = 1.14 [95% CI = 1.04–1.25]) and infant death (1.17 [1.03–1.32]).[18] Furthermore, the dose-dependent association of arsenic exposure with infant mortality in the present study is in the same direction as the dose-response observed in the earlier retrospective study.[18]

In conclusion, we have found new evidence of a strong association of maternal arsenic exposure during pregnancy with infant mortality. The findings were robust even after adjustment for sociodemographic and biologically important covariates. However, unlike some previous studies, we found little evidence for increased risks of spontaneous abortion or stillbirth.

REFERENCES

1. Huq SM, Joardar JC, Parvin S, Correll R, Naidu R. Arsenic contamination in food-chain: transfer of arsenic into food materials through groundwater irrigation. *J Health Popul Nutr*. 2006;24:305–316.
2. NRC. *Arsenic in Drinking Water: 2001 Update.* Washington, DC: National Academy Press; 2001.
3. Chowdhury UK, Biswas BK, Chowdhury TR, et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect*. 2000;108:393–397.
4. Smith AH, Lingas EO, Rahman M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ*. 2000;78:1093–1103.
5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr Eval Carcinog Risks Hum*. 2004;84:1–477.
6. 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.
7. Hill DS, Wlodarczyk BJ, Finnell RH. Reproductive consequences of oral arsenate exposure during pregnancy in a mouse model. *Birth Defects Res B Dev Reprod Toxicol*. 2008;83:40–47.
8. Wang A, Holladay SD, Wolf DC, Ahmed SA, Robertson JL. Reproductive and developmental toxicity of arsenic in rodents: a review. *Int J Toxicol*. 2006;25:319–331.
9. Vahter M. Health effects of early life exposure to arsenic. *Basic Clin Pharmacol Toxicol*. 2008;102:204–211.
10. Smith AH, Steinmaus CM. Health effects of arsenic and chromium in drinking water: recent human findings. *Annu Rev Public Health*. 2009; 30:107–122.
11. Vahter M. Effects of arsenic on maternal and fetal health. *Annu Rev Nutr*. 2009;29:381–399.
12. Ahmad SA, Sayed MH, Barua S, et al. Arsenic in drinking water and pregnancy outcomes. *Environ Health Perspect*. 2001;109:629–631.
13. Borzsonyi M, Bereczky A, Rudnai P, Csanady M, Horvath A. Epidemiological studies on human subjects exposed to arsenic in drinking water in southeast Hungary. *Arch Toxicol*. 1992;66:77–78.
14. Cherry N, Shaikh K, McDonald C, Chowdhury Z. Stillbirth in rural Bangladesh: arsenic exposure and other etiological factors: a report from Gonoshasthaya Kendra. *Bull World Health Organ*. 2008;86:172–177.
15. Milton AH, Smith W, Rahman B, et al. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology*. 2005;16: 82–86.
16. von Ehrenstein OS, Guha Mazumder DN, Hira-Smith M, et al. Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. *Am J Epidemiol*. 2006;163:662–669.
17. Hopenhayn-Rich C, Browning SR, Hertz-Picciotto I, Ferreccio C, Peralta C, Gibb H. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environ Health Perspect*. 2000;108:667–673.
18. Rahman A, Vahter M, Ekstrom EC, et al. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. *Am J Epidemiol*. 2007;165:1389–1396.
19. Rahman M, Vahter M, Wahed MA, et al. Prevalence of arsenic exposure and skin lesions. A population based survey in Matlab, Bangladesh. *J Epidemiol Community Health*. 2006;60:242–248.
20. Vahter ME, Li L, Nermell B, et al. Arsenic exposure in pregnancy: a population-based study in Matlab, Bangladesh. *J Health Popul Nutr*. 2006;24:236–245.
21. Nermell B, Lindberg AL, Rahman M, et al. Urinary arsenic concentration adjustment factors and malnutrition. *Environ Res*. 2008;106:212–218.
22. Li L, Ekstrom EC, Goessler W, et al. Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. *Environ Health Perspect*. 2008;116:315–321.
23. Baqui AH, Black RE, Arifeen SE, Hill K, Mitra SN, al Sabir A. Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. *Bull World Health Organ.* 1998;76:161–171.
24. Gwatkin DR, Rutstein S, Johnson K, Pande R, Wagstaff A. Socio-economic differences in health nutrition and population in Bangladesh. Washington, DC: The World Bank; 2000. Available at: http://siteresources.worldbank.org/INTPAH/Resources/Publications/Country-Reports/bangladesh.pdf.
25. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med*. 1988;319:189–194.
26. Fangstrom B, Moore S, Nermell B, et al. Breast-feeding protects against arsenic exposure in Bangladeshi infants. *Environ Health Perspect*. 2008;116:963–969.
27. Saha KK, Frongillo EA, Alam DS, Arifeen SE, Persson LA, Rasmussen KM. Appropriate infant feeding practices result in better growth of infants and young children in rural Bangladesh. *Am J Clin Nutr*. 2008;87:1852–1859.
28. 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.
29. Navarro PA, Liu L, Keefe DL. In vivo effects of arsenite on meiosis, preimplantation development, and apoptosis in the mouse. *Biol Reprod.* 2004;70:980–985.
30. He W, Greenwell RJ, Brooks DM, Calderon-Garciduenas L, Beall HD, Coffin JD. Arsenic exposure in pregnant mice disrupts placental vasculogenesis and causes spontaneous abortion. *Toxicol Sci*. 2007;99:244–253.
31. Wang TC, Jan KY, Wang AS, Gurr JR. Trivalent arsenicals induce lipid peroxidation, protein carbonylation, and oxidative DNA damage in human urothelial cells. *Mutat Res*. 2007;615:75–86.
32. Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig*. 2004;11:342–352.
33. Hopenhayn C, Ferreccio C, Browning SR, et al. Arsenic exposure from drinking water and birth weight. *Epidemiology*. 2003;14:593–602.
34. Rahman A, Vahter M, Smith AH, et al. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am J Epidemiol*. 2009;169:304–312.
35. Yang CY, Chang CC, Tsai SS, Chuang HY, Ho CK, Wu TN. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. *Environ Res*. 2003;91:29–34.
36. Bodwell JE, Kingsley LA, Hamilton JW. Arsenic at very low concentrations alters glucocorticoid receptor (GR)-mediated gene activation but not GR-mediated gene repression: complex dose-response effects are closely correlated with levels of activated GR and require a functional GR DNA binding domain. *Chem Res Toxicol*. 2004;17:1064–1076.
37. Davey JC, Bodwell JE, Gosse JA, Hamilton JW. Arsenic as an endocrine disruptor: effects of arsenic on estrogen receptor-mediated gene expression in vivo and in cell culture. *Toxicol Sci*. 2007;98:75–86.
38. Kaltreider RC, Davis AM, Lariviere JP, Hamilton JW. Arsenic alters the function of the glucocorticoid receptor as a transcription factor. *Environ Health Perspect*. 2001;109:245–251.
39. Lindberg AL, Ekstrom EC, Nermell B, et al. Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. *Environ Res*. 2008;106:110–120.
40. Kwok RK, Kaufmann RB, Jakariya M. Arsenic in drinking-water and reproductive health outcomes: a study of participants in the Bangladesh Integrated Nutrition Programme. *J Health Popul Nutr*. 2006;24:190–205.

