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Arsenic exposure and hepatitis E virus infection during pregnancy

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ABSTRACT

Background: Arsenic has immunomodulatory properties and may have the potential to alter susceptibility to infection in humans.
Objectives: We aimed to assess the relation of arsenic exposure during pregnancy with immune function and hepatitis E virus (HEV) infection, defined as seroconversion during pregnancy and postpartum.
Methods: We assessed IgG seroconversion to HEV between 1st and 3rd trimester (TM) and 3 months postpartum (PP) among 1100 pregnancies in a multiple micronutrient supplementation trial in rural Bangladesh. Forty women seroconverted to HEV and were matched with 40 non-seroconverting women (controls) by age, parity and intervention. We assessed urinary inorganic arsenic plus methylated species (∑As) (μg/L) at 1st and 3rd TM and plasma cytokines (pg/mL) at 1st and 3rd TM and 3 months PP.
Results: HEV seroconverters' urinary ∑As was elevated throughout pregnancy. Non-seroconverters' urinary ∑As was similar to HEV seroconverters at 1st TM but declined at 3rd TM. The adjusted odds ratio (95% confidence interval) of HEV seroconversion was 2.17 (1.07, 4.39) per interquartile range (IQR) increase in average-pregnancy urinary ∑As. Increased urinary ∑As was associated with increased concentrations of IL-2 during the 1st and 3rd TM and 3 months PP among HEV seroconverters but not non-seroconverters.
Conclusions: The relation of urinary arsenic during pregnancy with incident HEV seroconversion and with IL-2 levels among HEV-seroconverting pregnant women suggests arsenic exposure during pregnancy may enhance susceptibility to HEV infection.

1. Background

Arsenic represents a major threat to global health, with millions of people exposed through contaminated food and water, particularly in areas of the world where arsenic is naturally occurring in the environment (IARC, 2012). In Bangladesh, an estimated 45 million people are exposed to arsenic concentrations in drinking water greater than the World Health Organization (WHO) guideline value (10 μg/L) and 20 million people are exposed to concentrations greater than the Bangladeshi national standard (50 μg/L) (Flanagan et al., 2012). A recognized toxicant (IARC, 2012) and carcinogen (IARC, 2012), arsenic has been related to increased risk of cardiovascular, lung, kidney, bladder, skin, prostate, and liver disease (IARC, 2012; Liu and Waalkes, 2008; Wu et al., 2014). Arsenic has also been linked to increased systemic inflammation via oxidative stress (Ahmed et al., 2011) and chronic metabolic disorders including diabetes (Navas-Acien et al., 2008). Appreciation of the immunotoxic effects of arsenic and its potential to alter immune function is emerging (Dangleben et al., 2013). Laboratory studies reveal that arsenic inhibits antigen-presentation of macrophages (Banerjee et al., 2009) and T cell proliferation (Soto-Pena and Vega, 2008), decreases CD4+ T cell numbers in the spleen (Sikorski et al., 1989), and suppresses contact

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hypersensitivity responses (Patterson et al., 2004). The ability of arsenic to alter susceptibility to infection by affecting immune response is becoming increasingly recognized (Bailey et al., 2013; Farzan et al., 2013b; Kozul et al., 2009; Rager et al., 2014).

Pregnancy represents a unique period of susceptibility to infection (Kourtis et al., 2014) in part because of a shift toward an anti-inflammatory immune response (Kraus et al., 2012) due to changes in estrogen and progesterone levels (Shelly et al., 2012). No studies, to our knowledge, have investigated the dynamics of specific objectively measured infections among pregnant women exposed to arsenic. One study among 140 pregnant women in Matlab, Bangladesh, showed that increasing urinary arsenic concentrations were associated with increasing frequency of self-reported days of fever and diarrhea during pregnancy (Raqib et al., 2009). Though this study lacked objective measures of specific infections, its findings suggest a possible involvement of arsenic exposure in susceptibility to infection during pregnancy. Most attention has focused on the relation of in-utero arsenic exposure with the fetal immune repertoire (Nadeau et al., 2014) and infectious outcomes during early childhood (Farzan et al., 2013a, 2013b; Rahman et al., 2011). Studies have also shown that in-utero and childhood arsenic exposure can alter vaccine immune responses in children(Ahmed et al., 2014; Saha et al., 2013). This emerging literature reflects a growing understanding of the immunomodulatory properties of arsenic; however, whether (and how) this affects susceptibility to specific infections in humans is not well understood.

Hepatitis E virus (HEV) is a non-enveloped positive-strand RNA virus, transmitted primarily via the fecal-oral route (*e.g.*, contaminated surface water). HEV infection can be measured objectively via assessment of anti-HEV IgG seroconversion (Innis et al., 2002). Among individuals who seroconvert, HEV infection can be either asymptomatic or cause disease (hepatitis E) which is usually self-limited. However, some individuals with hepatitis E experience severe disease, known as acute fulminant hepatitis. HEV is a leading global cause of acute viral hepatitis (Mahtab et al., 2009; Rein et al., 2012) and causes significant morbidity and mortality during pregnancy (Labrique et al., 2012), particularly in South Asia where seasonal floods and poor sanitation have led to widespread contamination of surface water supplies with HEV and large epidemics of HEV infection (Gurley et al., 2014; Labrique et al., 2010). For reasons that are unclear, the case-fatality rate is markedly elevated (10–40%) in some pregnant women infected with HEV in South Asia, compared to the general population (3%) (Hamid et al., 1996; Tsega et al., 1992).

Although the liver is a known target of arsenic carcinogenesis (Liu and Waalkes, 2008) and emerging data support arsenic immunotoxicity (Stone, 1969), whether arsenic exposure during pregnancy can enhance susceptibility to objectively measured HEV infection during pregnancy has not been investigated. The aims of this nested case-control study were to assess arsenic exposure during pregnancy and evaluate its association with incident HEV seroconversion and changes in cytokine concentrations.

2. Methods

2.1. Study population and assessment of HEV seroconversion

Women in this nested case-control study were participants in a cluster-randomized, controlled trial of antenatal multiple micronutrients (15 vitamins and minerals including iron-folic acid) at a dietary reference intake (RDA) versus iron-folic acid alone in Gaibandha, Bangladesh to assess the impact of antenatal supplementation on infant mortality and adverse birth outcomes (West et al., 2013, 2014). Of the 44,567 pregnancies enrolled into the trial, 1526 women from a limited geographic area ($\sim$450 km^2) contributed a blood sample at 1st and 3rd trimester (TM) and 3 months postpartum (PP) as part of a more intensive biospecimen sub-study. The samples from the first available 1100 women were assessed for incident IgG seroconversion to HEV at the 1st and 3rd TM and 3 months PP using a well-characterized National Institutes of Health reference enzyme immunoassay (EIA) (Tsarev et al., 1993). The sensitivity of this anti-HEV IgG EIA is 96% and the specificity is 98% (Engle et al., 2015; Mast et al., 1998). Among the 1100 women, 39 women seroconverted between the 3rd TM and 3 months PP and 1 woman seroconverted to HEV between the 1st and 3rd TM. These 40 women (cases) were matched with 40 non-seroconverting women (controls) by age, parity, and intervention group. All procedures were approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board (IRB 00000570) and the Bangladesh Medical Research Council (BMRC/ERC/2007-2010 935) and the trial was registered at Clinical Trials.gov (NCT00860470).

2.2. Urine arsenic analysis

Total arsenic and arsenic species concentrations were measured in spot urine samples of the 40 HEV seroconverting (cases) and 40 non-seroconverting (controls) women at 1st and 3rd TM. The analyses were conducted at the Trace Element Laboratory of the Institute of Chemistry-Analytical Chemistry, University of Graz, Austria. All samples were analyzed blinded to HEV seroconversion status. The analytical methods used to perform arsenic and arsenic species analysis and the associated quality control criteria have been described in detail (Scheer et al., 2012). In brief, total arsenic concentrations in urine were measured using inductively coupled plasma mass spectrometry (ICPMS) (Agilent 7700x ICPMS, Agilent Technologies, Waldbronn, Germany). Arsenic species were measured by high performance liquid chromatography (HPLC)-ICPMS (Agilent 1100 HPLC and Agilent 7700x ICPMS). Urinary arsenic analysis variables were adjusted for urine dilution using specific gravity. The method limits of detection were 0.1 μg As/L for all the species. A total of 4% of participants were $<$LOD for arsenobetaine, 7.5% $<$ LOD for inorganic arsenic, and 0% $<$ LOD for all other arsenic species. In previous population-based studies, the inter-assay coefficients of variation for inorganic arsenic, methylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine plus other arsenic cations in the in-house reference urine were 6.0%, 6.5%, 5.9% and 6.5%, respectively (Scheer et al., 2012). The median (interquartile range) of arsenobetaine and other arsenic cations was 1.0 (0.6, 1.7) μg As/L confirming that seafood intake was relatively low in the population. We used the sum of inorganic and methylated arsenic species ($\sum$As) in urine as the biomarker of inorganic arsenic exposure. To assess arsenic metabolism, we calculated the relative proportion of inorganic and methylated arsenic metabolites in urine to their sum: % inorganic arsenic, % MMA and % DMA (Vahter,2000).

2.3. Cytokine analysis

Plasma cytokine measurements have been described in detail elsewhere (Kmush et al., In press). In brief, cytokine levels of all HEV seroconverting and non-seroconverting women were determined by the electrochemiluminescence-based Meso Scale Discovery (MSD) immunoassay in the format of the Human Th1/Th2 10-Plex Ultra-Sensitive Kit for IFN-γ, IL-10, IL-12, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, and TNF-α according to manufacturer protocols (Meso Scale Discovery, Gaithersburg, MD). Plates were read using the SECTOR Imager 2400 and data acquired using Discovery Workbench 3.0 software (Meso Scale Discovery, Gaithersburg, MD). For all cytokine concentrations below the lower limit of

detection (LOD), one-half the LOD was imputed. The limit of detection for cytokines was as follows: IFN-γ=0.3 pg/mL, IL-10=1.2 pg/mL, IL-12=0.7 pg/mL, IL-13=3.3 pg/mL, IL-1β=0.5 pg/mL, IL-2=0.4 pg/mL, IL-4=1.1 pg/mL, IL-5=0.1 pg/mL, IL-8=0.4 pg/mL, and TNF-α=0.2 pg/mL. We did not include in the analyses the cytokines with greater than 20% of samples below the LOD at one or more of the pregnancy time points (*i.e.*, IL-1β, IL-4, IL-13).

2.4. Demographic and nutritional factors

Micronutrient measurements have been described in detail elsewhere (West et al., 2014). As part of participation in the trial of antenatal multiple micronutrients (West et al., 2014), women provided information during their 1st TM about sociodemographic (age, parity, gestational age at enrollment, living standards index) and nutritional (food intake) factors. Trained anthropometrists measured women's weight, body mass index [BMI], and middle-upper arm circumference [MUAC]. Information about the season of enrollment (summer, rainy, winter) was also collected.

2.5. Statistical analysis

For demographic, nutritional and environmental factors we examined differences using the Wilcoxon rank sum test for continuous variables (mean [standard deviation]) and the proportion test for categorical and binary variables. Urinary arsenic, arsenic species and plasma cytokine data were ln-transformed before analysis. Differences in median (interquartile-range [IQR]) urinary arsenic and arsenic species concentrations by HEV seroconversion status were assessed by Fisher's exact test of the equality of medians, comparing HEV seroconverters to non-seroconverters. We also examined differences in urinary arsenic and arsenic species concentrations across pregnancy time points, comparing 3rd to1st TM using Fisher's exact test of the equality of medians. To visualize median trends across 1st and 3rd TM of pregnancy, we plotted the median of urine ∑As concentration (μg/L) for each woman and the overall group median trend, by HEV seroconversion status. Due to the non-independence of repeated measurements within person, we also examined longitudinal associations of changes in mean ∑As concentration (μg/L) across pregnancy time points by fitting generalized linear mixed regression models.

Odds ratios and 95% confidence intervals of the urinary arsenic-HEV seroconversion association were estimated using conditional logistic regression models. Models were adjusted for potential determinants of immune response to infection that could also be related to arsenic exposure or metabolism – living standards index, folate, vitamin D, and zinc. We used the Fisher's exact test of the equality of medians to assess differences in median (IQR) plasma cytokine concentrations by HEV seroconversion status and also between adjoining pregnancy time points (1st TM, 3rd TM, 3 months PP) within each seroconversion group (i.e., comparing 1st to 3rd TM; comparing 3rd TM to 3 months PP). We used generalized linear mixed regression models to estimate the changes in plasma cytokine concentrations at 1st and 3rd TM and 3 months PP – included as dependent variable in model – for an IQR-unit change in urinary ∑As at 1st and 3rd TM and the average of 1st and 3rd TM ∑As (as a measure of average pregnancy arsenic exposure) – included as independent variables in separate models – by HEV seroconversion status. Multiplicative interaction coding between ∑As and HEV seroconversion status variables was used in generalized linear mixed regression models to examine potential effect measure modification by HEV seroconversion by comparing a model with interaction terms to a model without using a likelihood ratio test. All analyses were completed using Stata version 11 (StataCorp LP, College Station, Texas, USA).

3. Results

3.1. Participant characteristics by HEV seroconversion status

HEV seroconverting (cases) and non-seroconverting (controls) women were similar by the matching factors (age, parity, and intervention group) as well as other sociodemographic, nutritional, anthropometric and pregnancy-related factors (Table 1). There was also no difference by season of enrollment over the 4-year period between groups

3.2. Urinary arsenic levels during pregnancy and HEV seroconversion

First TM median and IQR ∑As concentrations in urine were similar among HEV seroconverters (median 65.6 μg/L; IQR 40.8, 132.8 μg/L) and non-seroconverters (61.6 μg/L; 37.0, 82.2 μg/L) ($p=1.0$; Table 2). Urinary concentrations of ∑As remained high in the 3rd TM among HEV seroconverting women (64.7 μg/L; 26.2, 105.9 μg/L), but declined in the 3rd TM among non-seroconverting women (35.9 μg/L; 25.6, 77.4 μg/L) ($p=0.002$; Table 2; Fig. 1). During the 1st TM of pregnancy, non-seroconverting HEV women had higher % DMA (median 80.3%; IQR=75.6, 82.9%) in urine compared to seroconverting women (76.7%; 74.3, 80.5%) ($p=0.003$; Table 2).

Increasing urinary arsenic concentration during the 1st TM of pregnancy was associated with increasing odds of incident HEV seroconversion. The adjusted odds ratio (95% confidence interval [CI]) of incident HEV seroconversion was 2.06 (1.07, 3.97) for every IQR-unit increase in 1st TM urinary ∑As concentration (Table 3). A similar association was observed for the average of 1st and 3rd TM urinary ∑As concentration (odds ratio 2.17, 95% CI 1.07, 4.39) (Table 3). Arsenic methylation profiles were non-statistically significantly associated with incident HEV seroconversion. The adjusted odds ratio (95% CI) per IQR-unit increase in average 1st and

Table 1
Participant characteristics at 1st trimester of pregnancy by HEV seroconversion status.

	HEV seroconverters	Non-seroconverter	p^a
N	40	40	
Age (years)	22.1 (4.7)	22.1 (4.6)	1
Parity	0.9 (0.91)	0.9 (0.92)	1
Gestational age at enrollment (weeks)	10.1 (3.3)	11.5 (5.1)	0.23
Weight (kg)	43.0 (8.2)	44.0 (6.5)	0.52
Height (cm)	148.9 (4.8)	148.7 (4.9)	0.94
BMI (kg/m^2)	19.3 (3.4)	19.9 (2.5)	0.17
BMI < 18.5 (%)	43	25	0.10
MUAC (cm)	23.4 (3.0)	24.3 (3.4)	0.21
MUAC ≤ 22.5 cm (%)	35	23	0.22
Plasma			
Folate (nmol/L)	21.1 (8.0)	20.5 (9.7)	0.68
Vitamin D (nmol/L)	39.8 (6.0)	41.0 (11.3)	0.57
Zinc (μmol/L)	11.5 (2.0)	12.5 (2.3)	0.04
Living standards index	0.04 (1.05)	0.09 (1.13)	0.93
Season (%)			
Summer	25	28	0.80
Rainy	55	48	0.50
Winter	20	25	0.59

Note. Data are mean (standard deviation) or %. Participants were matched on age, parity and nutritional intervention. BMI=body mass index. MUAC=middle-upper arm circumference. Summer consists of January to May; Rainy of June to August; and Winter of September to December.

[a] p derived from Wilcoxon rank-sum test for continuous variables, proportion test for categorical and binary variables.

Table 2
Median (interquartile range – IQR) arsenic concentrations (μg/L) and percent arsenic metabolites during pregnancy by HEV seroconversion status.

	HEV seroconverter	p^a	Non-seroconverter	p^a	p^b
Arsenobetaine (μg/L)					
1st TM	1.2 (0.8, 1.8)		1.5 (1.0, 2.5)		0.26
3rd TM	0.6 (0.4, 1.1)	0.01	0.7 (0.5, 1.1)	< 0.001	0.82
∑As (μg/L)					
1st TM	65.6 (40.8, 132.8)		61.6 (37.0, 82.2)		1.00
3rd TM	64.7 (26.2, 105.9)	1.00	35.9 (25.6, 77.4)	0.002	0.03
% DMA					
1st TM	76.7 (74.3, 80.5)		80.3 (75.6, 82.9)		0.003
3rd TM	84.3 (80.4, 87.3)	< 0.001	84.8 (78.6, 88.1)	0.003	0.50
% MMA					
1st TM	7.6 (5.8, 9.6)		6.7 (4.7, 8.7)		0.50
3rd TM	4.8 (4.1, 7.0)	0.01	5.0 (3.7, 6.3)	0.03	0.82
% As(V)+As(III)					
1st TM	14.7 (12, 18)		13.1 (11, 17)		0.12
3rd TM	10.2 (7.9, 13)	0.001	9.9 (7.4, 13)	0.07	0.37
Total As (μg/L)					
1st TM	75.8 (41.3, 136.0)		63.6 (44.6, 85.9)		0.50
3rd TM	66.4 (27.9, 119.2)	0.82	37.7 (27.4, 79.5)	0.007	0.03

Note. TM=trimester. PP=postpartum. ∑As=inorganic arsenic plus methylated species. As=arsenic. DMA=dimethylarsinic acid. MMA=methylarsonic acid.

[a] *p* derived from Fisher's exact test of the equality of medians comparing 1st and 3rdTM.

[b] *p* derived from Fisher's exact test of the equality of medians comparing HEV seroconverters to non-seroconverters.

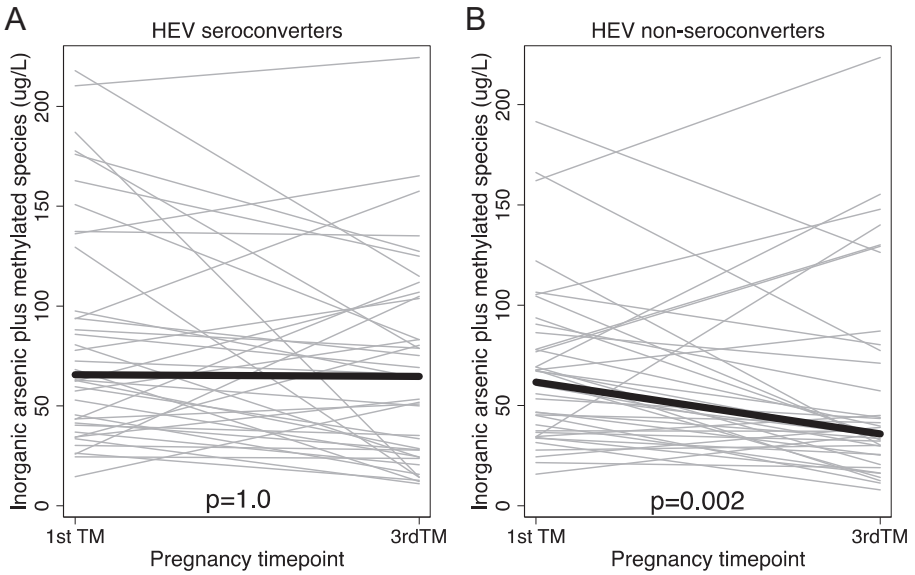

Fig. 1. Trends of inorganic arsenic plus methylated species (∑As) concentration (μg/L) in urine during the 1st and 3rd trimester (TM) of pregnancy among: A. HEV seroconverting ($n=40$) and B. non-seroconverting ($n=39$) women. Gray lines depict individual trajectories for each pregnant woman and the black line denotes the median trajectory within each group. *Note.* The *p*-value shown compares 1st and 3rd TM urinary ∑As derived from Fisher's exact test of the equality of medians. The beta (standard error), *p*-value from a linear mixed model of the temporal association between trimester (3rd vs 1st) and ln-transformed ∑As concentration (μg/L) was −0.27 (0.13), $p<0.04$ among HEV seroconverters and −0.34 (0.11), $p<0.003$ among non-seroconverters.

3rd TM % MMA was 1.77 (0.84, 3.74) (Table 3). The corresponding odds ratio for % DMA was 0.69 (0.38, 1.25) (Table 3).

3.3. IL-2 and urinary arsenic levels by HEV seroconversion status

The median plasma concentration of IL-2 was 1.73 (IQR 1.22, 2.61), 1.78 (IQR 0.90, 3.28) and 2.71 (IQR 1.65, 3.95) pg/mL at 1st TM, 3rd TM and 3 months PP, respectively among HEV-seroconverting women and was 1.69 (IQR 0.83, 2.58), 1.75 (IQR 1.05, 2.68) and 1.94 (IQR 1.51, 2.66) pg/mL at 1st TM, 3rd TM and 3 months PP, respectively among non-seroconverting women. HEV seroconverting women had higher levels of IL-2 compared to non-seroconverting women at 3 months PP ($p=0.04$). Among HEV seroconverting women, an IQR-unit increase in 1st TM ∑As was associated with a 0.46 pg/mL (95% CI 0.23, 0.68; $p<0.0001$) increase in plasma IL-2 concentration across the 1st TM, 3rd TM, and

Table 3
Odds ratio (95% confidence interval) of incident HEV seroconversion during pregnancy per interquartile-range change in urinary ΣAs concentration (μg/L) and in arsenic methylation profiles (%MMA and %DMA).

	OR	95% CI	*p*	aOR[a]	95% CI	*p*
IQR-unit change in ΣAs (μg/L)						
1st TM	1.52	0.92, 2.51	0.10	2.06	1.07, 3.97	0.03
3rd TM	1.42	0.85, 2.36	0.18	1.52	0.88, 2.64	0.14
Average (1st and 3rd TM)	1.53	0.90, 2.60	0.03	2.17	1.07, 4.39	0.03
IQR-unit change in % MMA						
1st TM	2.07	0.89, 4.82	0.09	1.93	0.81, 4.59	0.14
3rd TM	1.62	0.93, 2.83	0.09	1.53	0.86, 2.72	0.15
Average (1st and 3rd TM)	1.79	0.90, 3.59	0.10	1.77	0.84, 3.74	0.13
IQR-unit change in % DMA						
1st TM	0.63	0.37, 1.08	0.09	0.54	0.27, 1.06	0.07
3rd TM	0.63	0.36, 1.08	0.09	0.55	0.29, 1.06	0.07
Average (1st and 3rd TM)	0.74	0.43, 1.26	0.26	0.69	0.38, 1.25	0.22

Note. OR=odds ratio. CI=confidence interval. IQR=interquartile range. ∑As=inorganic arsenic plus methylated species. TM, trimester. DMA=dimethylarsinic acid. MMA= methylarsonic acid.

[a] Adjusted for living standards index and plasma concentration of folate (nmol/L), vitamin D (nmol/L) and zinc (μmol/L).

3 months PP (Table 4). The corresponding increase in plasma IL-2 concentration across the 3rd TM and 3 months PP for an IQR-unit increase in mean 1st and 3rd trimester pregnancy ∑As was 0.35 pg/mL (95% CI 0.12, 0.58; $p<0.003$) among HEV seroconverting women (Table 4). Associations of urinary ∑As with IL-2 among non-seroconverting pregnant women were around the null (Table 4). We observed evidence of effect measure modification (heterogeneity) of ∑As-IL-2 associations by HEV seroconversion status for 1st TM ∑As (χ^2 (1 df) 5.19, $p<0.02$) and average ∑As (χ^2 (1 df) 3.25, $p<0.07$) (Table 4). Although other cytokines varied significantly over time within HEV seroconverters (IFN-γ, TNF-α, and IL-5) and non-seroconverters (IL-8 and IL-5) and by HEV seroconversion status (IL-8, IL-10, IL-12) (Supplemental Data File Table S1), none showed an association with urinary arsenic concentrations within strata of HEV seroconversion status as observed for IL-2 (data not shown).

4. Discussion

Results of this study suggest that elevated urinary arsenic levels during pregnancy may be related to HEV seroconversion. In the 1st TM, prior to HEV seroconversion, urinary ∑As levels were similar between women who later went on to seroconvert to HEV and those who did not. By the 3rd TM, however, urinary ∑As levels decreased significantly among non-seroconverters, while urinary ∑As levels did not decline among HEV seroconverters. Higher urinary arsenic levels during early pregnancy and on average across pregnancy were associated with incident HEV seroconversion, particularly after adjustment for socioeconomic factors and nutritional biomarkers. Although the sample size was relatively small, strengths of the study include the prospective design, high quality exposure and outcome assessment, adjustment for relevant confounders and the evaluation of immune markers.

The biological basis of enhanced susceptibility to HEV seroconversion among women with high urinary inorganic arsenic levels during pregnancy is supported by knowledge of arsenic hepato- and immunotoxicity. The liver is a primary target site of arsenic metabolism, which involves biomethylation through one-carbon metabolism into monomethylated and dimethylated arsenic species (Vahter, 2009). As one-carbon metabolism increases during the course of pregnancy, the methylation of inorganic arsenic into dimethylated arsenic species typically increases, (Gardner et al., 2011) resulting in increased proportion of DMA in urine. (Vahter, 2009) DMA is considered a less toxic metabolite than MMA and we found that HEV seroconversion was associated with higher % MMA and lower % DMA in urine, especially during the 1st TM of pregnancy, although the model estimates were imprecise, likely due to the small sample size. The association of higher % MMA and lower % DMA with increased risk of disease has been shown for cardiovascular disease and cancer in other studies (IARC, 2012; Wu et al., 2014). These results could identify an adverse profile of arsenic-related metabolism that is associated with increased risk of HEV seroconversion among pregnant women.

Arsenic is known to alter key functions of the innate and adaptive immune system. Arsenic has been shown to disturb innate immune responses, including the inhibition of macrophage function (Banerjee et al., 2009). Such disturbance of innate immune responses is believed to be one potential contributor to the increased frequency of symptoms of respiratory tract infections, diarrhea, and fever among arsenic-exposed pregnant women (Kile et al., 2014; Raqib et al., 2009). During pregnancy the immune response is associated with a shift to an anti-inflammatory or Th2-type response (Chaouat et al., 1997). While beneficial for the

Table 4
Generalized linear mixed regression models of the association of temporal changes in plasma IL-2 concentrations (pg/mL) with an interquartile range-unit change in urinary inorganic arsenic plus methylated species (∑As) concentration (μg/L) during pregnancy by HEV seroconversion status.

	HEV seroconverter Interleukin 2 (IL-2)			Non-seroconverter Interleukin 2 (IL-2)			
	N	Beta (95% CI)[a]	*p*[a]	N	Beta (95% CI)[a]	*p*[a]	*p*-interaction[b]
IL-2 (1st TM, 3rd TM, 3 months PP)							
∑As (1st TM)	120	0.46 (0.23, 0.68)	<0.0001	120	−0.03 (−0.39, 0.34)	0.88	0.02
IL-2 (3rd TM & 3 months PP)							
∑As (3rd TM)	80	0.18 (−0.03, 0.39)	0.09	78	0.04 (−0.21, 0.29)	0.78	0.38
∑As (Average)[c]	80	0.35 (0.12, 0.58)	0.003	78	0.01 (−0.29, 0.31)	0.96	0.07

Note. TM=trimester. PP=postpartum. For IL-2 concentrations below the limit of detection, 1/2 the limit was imputed. CI=confidence interval. ∑As=inorganic arsenic plus methylated species.

[a] Beta coefficient, 95% CI, and *p* derived from generalized linear mixed regression models with pregnant women's repeated IL-2 (pg/mL) measurements as dependent variable.

[b] *p*-value derived from a 1 df likelihood ratio test of interaction between ∑As and seroconversion status in a generalized linear mixed model.

[c] Average of 1st and 3rd TM urinary ∑As.

outcome of pregnancy, reduced inflammatory processes may enhance women's susceptibility to certain infections that require Th1-type immunity for clearance. Th1- and Th2-type responses transcriptionally down-regulate each other – a strong Th2-type response should reduce a Th1-type response and vice-versa (Lee et al., 2006; Spilianakis and Flavell, 2007). Research suggests that arsenic exposure may exacerbate anti-inflammatory immune responses during pregnancy (Cho et al., 2012), which could further enhance susceptibility to infection, particularly viral infections that require Th1-type immune responses for successful clearance. It is possible that arsenic exposure during pregnancy may alter Th1-type immune responses resulting in susceptibility to seroconversion and viral infection at a lower challenge dose of virus than in the absence of arsenic exposure. A compromised immune response, increasing morbidity, and higher pulmonary viral titers were observed following a sub-lethal influenza virus challenge dose in mice exposed to arsenic relative to arsenic unexposed mice (Kozul et al., 2009), but human population-based studies of arsenic immunotoxicity and infection among pregnant women are lacking. We could not investigate whether arsenic-induced immune alteration is related to symptomatic HEV disease because pregnant women in our study did not experience symptomatic HEV disease (as reflected by anti-HEV IgG seroconversion without reports of acute viral hepatitis symptoms).

Arsenic is known to impair adaptive immunity. It is recognized that arsenic delays or inhibits adaptive T-cell immune response. One mechanism that has been described in detail is the delay of T cell proliferation (Soto-Pena and Vega, 2008), and promotion of T cell anergy, senescence or tolerance (Soto-Pena et al., 2006) after arsenic exposure. Impairment of T cell proliferation has been shown to have adverse effects when an infectious agent (such as HEV) is encountered because a delayed or ineffective T-cell response (Galicia et al., 2003), combined with ineffective innate immune response, could enhance susceptibility to infection. Among the cytokines examined in this study, IL-2, an indicator of immune activation, were higher in HEV seroconverters and its levels showed a significant positive association with urinary arsenic concentration only among pregnant women who seroconverted to HEV, potentially indicating there was activation of the immune response by arsenic. IL-2 plays a critical role in initiating the adaptive immune response and T cell proliferation. Laboratory studies suggest that after inducing an initial delay in IL-2 production, arsenic may subsequently induce a “ramping-up” of IL-2 production to achieve an effective adaptive immune response (Soto-Pena et al., 2006; Soto-Pena and Vega, 2008). This is supportive of a possible role of arsenic exposure in the dynamics of HEV seroconversion, and future studies involving functional measures of immune response (e.g., immune cell populations, immunoglobulin isotypes) could improve understanding of this exposure–response relationship.

Limitations of the study include a small sample size and a lack of assessment of arsenic in drinking water and food. However, we measured urine arsenic, which is a well-established biomarker that integrates all sources of arsenic exposure (including drinking water and food). The observed differences in urinary arsenic levels were unlikely due to changes to an alternative water supply because sources of drinking water in the study area remained constant, nor was it likely due to differences in hydration status since all analyses were adjusted for urine dilution via specific gravity. Future studies should measure arsenic and pathogens in water and food and assess urinary arsenic levels during the postpartum period to address these limitations.

Despite the small sample size, important strengths of the study include use of a prospective design, nested within a large cohort, objective measures of arsenic exposure in urine, objective assessment of outcome (incident seroconversion) for a pathogen that causes a significant burden of morbidity and mortality in pregnancy (HEV), measurement of pro- and anti-inflammatory cytokines in plasma, the inclusion of objectively measured micronutrients as confounders, and the availability of repeated measurements during pregnancy and postpartum. These strengths represent an improvement on previous studies of arsenic exposure and infection during pregnancy because most involved classification of exposures or outcomes via participant self-reports of symptoms (Kile et al., 2014; Raqib et al., 2009). Anti-HEV IgG seroconversion constitutes incident HEV infection; however, pregnant women who seroconverted to HEV in our study did not report symptoms of acute HEV disease – *e.g.*, none reported icterus, fever, clay-colored stools during the follow-up period. Our findings may therefore provide important insights into the dynamics of arsenic exposure with early sentinel immune responses and asymptomatic infection and could inform novel hypotheses for future research of symptomatic HEV infection during pregnancy in population-based and clinical settings.

The possibility of reverse causation should be considered. Pregnant women who seroconverted to HEV may have experienced a cascade of events related to viral seroconversion that could have led to reduced metabolism of arsenic in the liver and thus resulted in higher unmethylated arsenic, which has a longer half-life than methylated arsenic species, and then higher urinary $\sum$As levels. To address this question, future studies should study the relation of arsenic exposure among pregnant women with symptomatic HEV disease because this may reflect dynamics of more severe metabolic stress on the liver than among asymptomatic HEV infections observed in our study. The small sample size precluded a more extensive analysis of confounding and/or effect measure modification. Micronutrients included in our analysis were those known to influence one-carbon metabolism and arsenic metabolism (folate) (Gamble et al., 2006) and those hypothesized to be associated with immune response (zinc, vitamin D) (Bartley,2010; Mahalanabis et al., 2004). However, given that all women in this nested case-control study received antenatal multiple micronutrients at a daily requirement level or the same level of iron-folic acid alone as part of an intervention trial, future studies should be conducted among women not receiving micronutrient supplements in order to assess the potential impact of micronutrient deficiency status on arsenic exposure, infection, and immune response.

5. Conclusions

Our findings contribute to understanding arsenic immunotoxic effects during pregnancy and postpartum. In Bangladesh, a large proportion of the population relies on water from tubewells that are contaminated with arsenic (Karagas, 2010). In Bangladesh and other areas where genotype 1 HEV is endemic, transmission occurs via the fecal-oral route principally via fecally-contaminated surface water. During pregnancy, it may be important to consider the context of exposure to arsenic and HEV (as well as other pathogens related to water, hygiene, and sanitation conditions). In rural Bangladesh, near the location of the present study, HEV seroprevalence estimates range between 20% and 23% (Labrique et al., 2010) and have been observed as high as 40–80% in other areas of Bangladesh (Mahtab et al., 2009). With such high HEV endemicity and co-occurring arsenic exposure, if the associations we observed are causal, the population at risk could be large. A more complete understanding of the effects of arsenic on immune alterations and other infectious diseases during pregnancy could inform interventions to reduce morbidity and mortality where exposure to pathogenic and immunotoxic agents co-exists.

Competing interests

All authors report no competing or conflict of interest.

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Previous presentation

Portions of this work were presented as abstract #1736 on October 11, 2014 at the annual meeting of the Infectious Diseases Society of America (IDSA) IDWeek in Philadelphia, PA.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.07.004.

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