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Renal function is associated with indicators of arsenic methylation capacity in Bangladeshi adults

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ABSTRACT

Background: Arsenic (As) methylation capacity in epidemiologic studies is typically indicated by the proportions of inorganic As (%InAs), monomethylarsonic acid (%MMA), and dimethylarsinic acid (%DMA) in urine as a fraction of total urinary As. The relationship between renal function and indicators of As methylation capacity has not been thoroughly investigated.

Objectives: Our two aims were to examine (1) associations between estimated glomerular filtration rate (eGFR) and %As metabolites in blood and urine, and (2) whether renal function modifies the relationship of blood %As metabolites with respective urinary %As metabolites.

Methods: In a cross-sectional study of 375 As-exposed Bangladeshi adults, we measured blood and urinary As metabolites, and calculated eGFR from plasma cystatin C.

Results: In covariate-adjusted linear models, a 1 ml/min/1.73 m^2 increase in eGFR was associated with a 0.39% increase in urinary %InAs ($p < 0.0001$) and a mean decrease in urinary %DMA of 0.07 ($p = 0.0005$). In the 292 participants with measurable blood As metabolites, the associations of eGFR with increased blood %InAs and decreased blood %DMA did not reach statistical significance. eGFR was not associated with urinary or blood %MMA in covariate-adjusted models. For a given increase in blood %InAs, the increase in urinary %InAs was smaller in those with reduced eGFR, compared to those with normal eGFR ($p = 0.06$); this effect modification was not observed for %MMA or %DMA.

Conclusions: Urinary excretion of InAs may be impaired in individuals with reduced renal function. Alternatively, increased As methylation capacity (as indicated by decreased urinary %InAs) may be detrimental to renal function.

1. Introduction

Exposure to inorganic arsenic (As) in drinking water is a public health problem estimated to impact over 140 million people globally (World Health Organization, 2008). Arsenic exposure has been associated with skin, lung, and bladder cancers, cardiovascular disease, nonmalignant respiratory illness, and neurologic deficits (National Research Council, 2013). Arsenic metabolism is thought to play an important role in the toxicity of As (Hughes, 2002).

The metabolism of inorganic As (InAs) occurs in a series of oxidative methylation and reduction reactions which promote As excretion in urine. Arsenite (As^{III}) undergoes oxidative methylation to form monomethylarsonic acid (MMA^{V}). MMA^{V} is reduced to MMA^{III}, which then undergoes oxidative methylation to form dimethylarsinic acid (DMA^{V}) (Challenger, 1951). The methylation reactions are catalyzed by arsenic methyltransferase (AS3MT) (Lin et al., 2002). The toxicity of As metabolites varies by methylation and valence state, with MMA^{III} being most toxic, and DMA^{V} being least toxic (Styblo et al., 2002).

Methylation of As is crucial for As elimination through urine; AS3MT knockout mice retain greater amounts of As in their tissues (primarily InAs) and excrete less As in urine than wild-type mice (Drobna et al., 2009; Hughes et al., 2010). The reduced ability of

Abbreviations: As, arsenic; AS3MT, arsenic methyltransferase; bAs, blood arsenic; CKD, chronic kidney disease; DMA, dimethylarsinic acid; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; InAs, inorganic arsenic; MMA, monomethylarsonic acid; SG, specific gravity; uAs, urinary arsenic

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the AS3MT knockout mice to excrete As is likely due to impaired synthesis of DMA, which is rapidly excreted in urine (Buchet et al., 1981). DMA is the most prevalent As species in human urine (approximately 60–80% of total urine As on average), while MMA (10–20%) and InAs (10–30%) comprise a much smaller proportion of total urine As (Vahter, 2000).

The proportions of InAs, MMA, and DMA in urine are often used as indicators of As methylation capacity; these proportions, particularly the percentage of MMA in urine (u%MMA), have been associated with risk for various diseases including atherosclerosis, bladder cancer, lung cancer, skin cancer, and skin lesions (Chen et al., 2013; Steinmaus et al., 2010). An important yet overlooked factor in using these indicators is the potential relationship between renal function and the urinary excretion of As metabolites. Other than studies on the reabsorption of $InAs^V$ in dogs (Ginsburg and Lotspeich, 1963; Tsukamoto et al., 1983) and the effect of nephrectomy on $InAs^V$ methylation in rabbits (De Kimpe et al., 1999), very little is known regarding the renal handling of As species and how this might be influenced by reductions in glomerular filtration rate (GFR) or by proximal tubule injury. The dearth of knowledge regarding renal filtration, secretion, and reabsorption of As species, and the influence of these factors on urine composition of As metabolites, was highlighted in a review by Carter et al. (2003), yet research in this area is still severely lacking. In a previous report of two cross-sectional samples of As-exposed Bangladeshi adults, we observed that the estimated GFR (eGFR) was associated with increased %InAs in urine (u%InAs) in both samples, and decreased u%DMA in one of the samples, while there was no association of eGFR with u%MMA (Peters et al., 2014). We hypothesized that these associations could be due to renal function influencing the urinary excretion of As metabolites, or that As metabolites are differentially detrimental to renal function.

The current analysis builds upon a rich dataset from the Folate and Oxidative Stress (FOX) study (Hall et al., 2013), a cross-sectional study designed to examine the dose–response relationship between As exposure and biomarkers of oxidative stress. Two advantages of the FOX study are: (1) by design, participants had a wide range of As exposure, and (2) As metabolites were measured in both blood and urine. In this study, we examined the association of eGFR with As metabolite proportions in both blood and urine, and tested whether eGFR modifies the relationship between blood and urinary As metabolite proportions. The latter analysis may elucidate whether reduced renal function impedes the excretion of some As species from blood to urine.

2. Materials and methods

2.1. Study population and design

The cross-sectional Folate and Oxidative stress (FOX) study included 379 adults from Araihazar, Bangladesh who were selected based on the As concentration of their wells [Group A: 0–10 μg/L ($n=76$), Group B: 10–100 μg/L ($n=104$), Group C: 100–200 μg/L ($n=86$), Group D: 200–300 μg/L ($n=67$), and Group E: > 300 μg/L ($n=45$)]. The study has been described in detail previously (Hall et al., 2013). Briefly, we recruited individuals between the ages of 30 and 65 who had been drinking from their current well for at least 3 months. Exclusion criteria were as follows: (1) women who were pregnant, (2) individuals taking nutritional supplements, and (3) individuals having known diabetes, cardiovascular disease, renal disease, chronic obstructive pulmonary disease, or cancer. For the current analysis, participants missing data on water As, plasma folate, specific gravity, or BMI were excluded, leaving a final sample size of $N=375$. Blood As (bAs) metabolites were only measured in participants with total bAs ≥ 5 μg/L, because one or more As metabolites would likely fall below detection limits in those with total bAs < 5 μg/L, leaving a subset of $N=292$ for analyses with bAs metabolites.

Oral informed consent was obtained by our Bangladeshi field staff physicians, who read an approved assent form to the study participants. This study was approved by the Bangladesh Medical Research Council and the institutional review board of Columbia University Medical Center.

2.2. Water arsenic

Water samples were analyzed for total As by high-resolution inductively coupled plasma mass spectrometry, as previously described (Cheng et al., 2004). The detection limit of the method is < 0.2 μg/L. A standard with an As concentration of 51 μg/L was run multiple times in each batch. The intra- and inter-assay coefficients of variation (CVs) for this standard were 6.01% and 3.76%, respectively.

2.3. Total blood arsenic

As described previously (Hall et al., 2006), total blood As concentrations were measured using a Perkin-Elmer Elan DRC II ICP-MS equipped with an AS 93+ autosampler. The intra- and inter-assay CVs were 2.1% and 4.9%, respectively.

2.4. Blood and urine arsenic metabolites

The As metabolites [arsenite (A^{III}), arsenate (As^V), monomethylarsonous acid plus monomethylarsonic acid (MMA^{III+V}), and dimethylarsinous acid plus dimethylarsinic acid (DMA^{III+V})] were measured in blood and urine by coupling HPLC to dynamic reaction cell inductively coupled plasma MS (Reuter et al., 2003). The percentages of $InAs^{III+V}$ (%InAs), MMA^{III+V} (%MMA), and DMA^{III+V} (%DMA) were calculated using the sum of the inorganic and methylated metabolites as the denominator. The intra-assay CVs for urinary As^{III}, As^V, MMA, and DMA were 3.6%, 4.5%, 1.5%, and 0.6%, respectively; those for blood were 0.9%, 11.5%, 3.6%, and 2.6%, respectively. The inter-assay CVs for urinary metabolites were 9.7%, 10.6%, 3.5%, and 2.8%, respectively, whereas those for blood were 3.7%, 23.2%, 2.9%, and 3.5%, respectively. The total urinary As (uAs) and blood As (bAs) variables that are used in our analyses were calculated as the sum of As^{III}, As^V, MMA, and DMA in urine and blood, respectively. Specific gravity (SG) was measured by refractometer, and total uAs was adjusted for SG using the following formula: [uAs*(overall mean SG–1)/(measured SG–1)] (Cone et al., 2009).

2.5. Plasma cystatin C and eGFR

GFR is typically estimated from plasma creatinine and/or cystatin C using population-based equations; we calculated eGFR from plasma cystatin C using the 2012 CKD-EPI Cystatin C equation (Inker et al., 2012). Plasma cystatin C was measured by ELISA according to the manufacturer's protocol (R&D Systems Human Cystatin C Duoset Catalog# DY1196). We used a 6 point standard curve with a high standard of 3000 pg/ml. Samples were diluted 1:2000 in PBS with 10% fetal bovine serum (Sigma Aldrich F6178). Recovery of the IFCC certified reference material for serum cystatin C (ERM-DA 471/IFCC) was 104%. The intra- and inter- assay CVs were 7% and 10%, respectively.

2.6. Plasma nutrients

Plasma folate and B12 were measured by radio-protein binding assay (SimulTRAC-S, MP Biomedicals). The intra- and inter-assay