Arsenic exposure, diabetes-related genes and diabetes prevalence in a general population from Spain☆

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ABSTRACT

Inorganic arsenic exposure may be associated with diabetes, but the evidence at low-moderate levels is not sufficient. Polymorphisms in diabetes-related genes have been involved in diabetes risk. We evaluated the association of inorganic arsenic exposure on diabetes in the Hortega Study, a representative sample of a general population from Valladolid, Spain. Total urine arsenic was measured in 1451 adults. Urine arsenic speciation was available in 295 randomly selected participants. To account for the confounding introduced by non-toxic seafood arsenicals, we designed a multiple imputation model to predict the missing arsenobetaine levels. The prevalence of diabetes was 8.3%. The geometric mean of total arsenic was 66.0 μg/g. The adjusted odds ratios (95% confidence interval) for diabetes comparing the highest with the lowest tertile of total arsenic were 1.76 (1.01, 3.09) and 2.14 (1.47, 3.11) before and after arsenobetaine adjustment, respectively. Polymorphisms in several genes including *IL8RA, TXN, NR3C2, COX5A* and *GCLC* showed suggestive differential associations of urine total arsenic with diabetes. The findings support the role of arsenic on diabetes and the importance of controlling for seafood arsenicals in populations with high seafood intake. Suggestive arsenic-gene interactions require confirmation in larger studies.

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1. Brief summary of the main result of the work

In a general population from Spain with low-moderate inorganic arsenic exposure, increased arsenic exposure, assessed as urine arsenic concentrations, was associated with higher diabetes prevalence. The reported association was stronger after adjustment for urine arsenobetaine reflecting the importance of accounting for seafood consumption in populations with high seafood intake.

Carriers of specific genotypes may have increased susceptibility to arsenic-related diabetes, although larger studies are needed to confirm these suggestive findings.

2. Introduction

Epidemiological studies support that people with higher inorganic arsenic exposure levels are more likely to have diabetes (Navas-Acien et al., 2006) but the evidence at low to moderate exposure levels is not sufficient. Moreover, most studies have been conducted in Asia and the Americas, with very few studies on arsenic and diabetes being conducted in Europe. Epidemiological studies in diverse populations are needed given the elevated burden of diabetes, a world-wide rapidly growing disease (World Health Organization, 2016).

The sum of urine concentrations of inorganic arsenic (arsenite [AsIII] plus arsenate [AsV]) and its methylated species (monomethylarsonate [MMA] and dimethylarsinate [DMA]) is an established biomarker of inorganic arsenic exposure in populations with low seafood intake (Navas-Acien et al., 2011). It is well known that inorganic arsenic (iAs) is highly toxic to humans (World Health Organization, 2001) and is associated with a wide range of adverse health effects, including cancer (Bates et al., 1992), cardiovascular disease (Moon et al., 2012) and others (D'Ippoliti et al., 2015). People are exposed to inorganic arsenic mainly through drinking contaminated water (National Research Council, 1999) and through food intake (Wei et al., 2014). Organic arsenic species, such as arsenobetaine (Asb), arsenolipids and arsenosugars, are mostly found in saltwater finfish and shellfish and are considered not harmful to human health (Mozaffarian and Rimm, 2006). Among the seafood arsenicals, arsenobetaine is the most common compound, which is rapidly cleared from the bloodstream and excreted unchanged in the urine (Molin et al., 2014), contributing to the total urinary arsenic concentrations. Arsenosugars and arsenolipids are metabolized in the body resulting in multiple metabolites in the urine, including DMA, and contributing to the total amount of inorganic arsenic species. As a result, in the presence of seafood intake, it is necessary to account for the potential residual confounding of organic arsenic in the interpretation of urinary arsenic concentrations as a biomarker of inorganic arsenic exposure (Navas-Acien et al., 2011).

The objective of this study was to evaluate the association of inorganic arsenic exposure, assessed as total urinary arsenic concentrations, with the prevalence of type 2 diabetes in a population from Spain with relatively high seafood consumption. This research was complicated by the presence of missing completely at random urine arsenobetaine concentrations for the ~80% of the study sample. We thus developed and implemented a multiple imputation model based in Markov Chain Monte Carlo (MCMC) methods using Gibbs sampling to account for the potential residual confounding by organic arsenicals in the whole study population. Given the well-established role of genetic variation on diabetes traits (Mahajan et al., 2014) and the paucity of gene-environmental interaction studies, we also evaluated the interaction of arsenic and diabetes-related candidate polymorphisms as a potential determinant of diabetes.

3. Methods

3.1. Study population

The present study was conducted among adult participants of the Hortega Study, who were beneficiaries of the public health system assigned to the Rio Hortega University Hospital's catchment area (Valladolid, northwestern Spain). The Hortega Study uses a complex sampling design to obtain a representative sample of the general population. The sampling design and methodology have been previously described (Mena Martin et al., 2003). In 2001—2003, baseline information on socio-demographic, behavioral, dietary and other health-related factors were collected from in-person interviews, examinations, and review of health records (see e-Appendix 1). After signing an informed consent form, urine and blood samples were collected and stored at $-80\,°C$. A total of 1502 individuals had sufficient urine and plasma samples available for metal determinations. After excluding 21 participants with missing total arsenic concentrations, 2 missing urine creatinine, 10 missing seafood intake, and 18 missing other relevant covariates, 1451 participants were included in the present study.

3.2. Arsenic measurements and arsenobetaine imputation

Urine arsenic determinations were conducted by the Laboratory of Analytical and Bioanalytical Chemistry of Huelva University (Spain) in 2013. Total urine arsenic and arsenic species concentrations were measured by inductively coupled-plasma mass spectrometry (ICPMS) on an Agilent 7500CEx ICPMS (Agilent Technologies, Santa Clara, California) and by anion exchange liquid chromatography coupled to ICPMS with octapole reaction cell, respectively, following a standardized protocol (see e-Appendix 2). Urine arsenic speciation, including AsIII, AsV, MMA, DMA, and Asb concentrations, occurred only in a random subsample of 295 individuals, leaving 1156 (79.7%) of the 1451 participants with missing completely at random urine arsenic species concentrations. Asb and DMA levels below the limit of detection (12 and 1 undetectable values, respectively) were imputed by the limit of detection divided by the square root of 2 (Hornung RW and Reed LD, 1990). Total plasma arsenic levels were measured in the whole study population in 2010 by atomic absorption spectrometry with graphite furnace at Cerba International Laboratories Ltd. 42.6% of total plasma arsenic concentrations were under the limit of detection. See limits of detection in e-Appendix 2.

We used the arsenic speciation information available in the random subsample to generate 5 complete datasets (for the 1451 participants) in which the Asb values missing completely at random where imputed as the 30th, 40th, 50th, 60th and 70th percentiles of each subject-specific posterior distribution obtained from a MCMC by Gibbs sampling nested linear model (Tellez-Plaza et al., 2010), implemented with WinBUGS software (Lunn et al., 2000) (see e-Appendix 3). Total plasma arsenic and DMA where strongly correlated with Asb (spearman correlation coefficients = 0.65 and 0.54, respectively). Thus, we incorporated the correlation of total plasma arsenic, DMA and Asb conditionally in our imputation model. We did not considered AsIII, AsV and MMA as potential Asb predictors. As a result, the MCMC arsenobetaine imputation model consisted of a tri-variate model with nested equations for Asb, DMA and total plasma arsenic, each based on strong predictors selected by a backward stepwise process starting from total urine concentrations and a list of socio-demographic and established arsenic exposure determinants (age, gender, smoking status, cotinine, body mass index, seafood, rice and chicken consumption) (e-Appendix 3, e-Table 1). For Asb and DMA, the predictive equations were standard linear models, whereas for total plasma arsenic the predictive equation was a tobit linear model for truncated data. The WinBUGS code of the imputation model is provided in e-Appendix 3. We conducted a *post-hoc* validation process to measure the predictive error of the MCMC imputation model using data from the Aragon Workers Health Study, an independent dataset with data on total urine arsenic, urine arsenic species and total plasma arsenic (e-Appendix 4 and e-Table 2).