Published in final edited form as:

Sci Total Environ. 2012 September 1; 433C: 362–370. doi:10.1016/j.scitotenv.2012.06.085.

The role of drinking water sources, consumption of vegetables and seafood in relation to blood arsenic concentrations of Jamaican children with and without Autism Spectrum Disorders

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Abstract

Arsenic is a toxic metal with harmful effects on human health, particularly on cognitive function. Autism Spectrum Disorders (ASDs) are lifelong neurodevelopmental and behavioral disorders manifesting in infancy or early childhood. We used data from 130 children between 2-8 years (65 pairs of ASD cases with age- and sex-matched control), to compare the mean total blood arsenic concentrations in children with and without ASDs in Kingston, Jamaica. Based on univariable analysis, we observed a significant difference between ASD cases and controls (4.03µg/L for cases vs. 4.48µg/L for controls, $P < 0.01$). In the final multivariable General Linear Model (GLM), after controlling for car ownership, maternal age, parental education levels, source of

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drinking water, consumption of “yam, sweet potato, or dasheen”, “carrot or pumpkin”, “callaloo, broccoli, or pak choi”, cabbage, avocado, and the frequency of seafood consumption per week, we did not find a significant association between blood arsenic concentrations and ASD status (4.36µg/L for cases vs. 4.65µg/L for controls, $P = 0.23$). Likewise, in a separate final multivariable GLM, we found that source of drinking water, eating avocado, and eating “callaloo, broccoli, or pak choi” were significantly associated with higher blood arsenic concentrations (all three $P < 0.05$). Based on our findings, we recommend assessment of arsenic levels in water, fruits, and vegetables, as well as increased awareness among the Jamaican population regarding potential risks for various exposures to arsenic.

Keywords

Arsenic; Autism Spectrum Disorders; Fruits; Vegetables; Drinking water; Cooking water; Seafood; Jamaica

1. Introduction

Arsenic (As) is a toxic, sulfhydryl-reactive metal shown to have harmful effects on the human nervous system, even at low levels of exposure (ATSDR, 2007b; USEPA, 2002). Arsenic is classified broadly into organic and inorganic forms. Inorganic arsenic, the most toxic form of arsenic, is found mainly in soil and rocks (ATSDR, 2007b). Since the inorganic arsenic in soil has a high water-solubility fraction, it is more readily mobile and bioavailable within the environment (Beesley et al., 2010; Vahidnia et al., 2007). Inorganic arsenic is considered carcinogenic (De Gieter et al., 2002) and affects neurodevelopment (ATSDR, 2007b). Although organic arsenic (arsenobetaine and arsenocholine) is much less toxic than inorganic arsenic (Abernathy et al., 2003), some studies have shown that it can also have adverse effects on neurodevelopment (ATSDR, 2007b). Exposure to arsenic could occur during prenatal, perinatal, and postnatal developmental periods. For example, studies have shown that arsenic can easily cross the placenta and find its way to fetal tissues (ATSDR, 2007b). For the postnatal period, it has been reported that higher levels of arsenic are associated with reduced cognitive function in children including visual-spatial abilities (Rosado et al., 2007), intelligence quotient (IQ) (Wang et al., 2007; Wasserman et al., 2004), verbal learning and memory, verbal IQ (Wright et al., 2006), decreased long-term memory, language skills, attention, and comprehension (Calderon et al., 2001).

Sources of human exposure to inorganic arsenic can include, but are not limited to: 1) arsenic trioxide, used in pesticides, defoliants (Murunga and Zawada, 2007), and wood preservatives (e.g., exterior decks and playgrounds) (ATSDR, 2010), is also released into the air through industrial combustion processes; 2) gallium arsenide, used in discrete microwave devices, lasers, and semiconductor devices; 3) arsine gas (the most toxic arsenical), used in commercial microelectronics and sometimes accidentally released during mining; 4) arsenite (As^{III}), released into ground water from mineral ore, which contaminates wells used for drinking water (WHO, 2001); and 5) arsenate (As^{V}), released from erosion of natural land sources and industrial contamination of soil that is carried via rain runoff into surface water used as a drinking water source (ATSDR, 2007b). People living in arsenic-contaminated soil areas, who consume vegetables grown in the same area, can become exposed to inorganic arsenic through these arsenic-contaminated crops (Laizu, 2007). Although humans are regularly exposed to arsenic through consumption of seafood, such as bottom feeding fish or seaweed (ATSDR, 2007b), only 10-15% of arsenic in edible parts of seafood is inorganic (ATSDR, 2007b). However, inorganic arsenic can be bioaccumulated in the human body through high frequency of seafood consumption for a long period of time (Vieira et al., 2011).

People living in many developing and some developed countries suffer from high arsenic exposure (Mandal and Suzuki, 2002; Sun et al., 2006; WHO, 2010a) and are at risk for chronic arsenic exposure through the aforementioned sources. High arsenic levels have been detected in surface water, groundwater (Vahidnia et al., 2007), and vegetables (Laizu, 2007) in Bangladesh, Taiwan (Wu et al., 2001), and Vietnam (Hanh et al., 2011). Similarly, high arsenic levels found in seafood, soil, and water in some parts of China (Chen et al., 2011; Sun et al., 2006; Wang et al., 2007), the gold mining industry in Nicaragua (Wickre et al., 2004), and in seafood consumed by people living in some fishing communities in Canada (Elliott et al., 2009) and Belgium (Baeyens et al., 2009) were reported to result in chronic arsenic exposure.

Autism Spectrum Disorders (ASDs) are complex neurodevelopmental disorders that manifest in early childhood (Genuis, 2009; Volkmar and Chawarska, 2008). ASDs affect language development, communication, imagination, and social interactions. Repetitive, stereotyped behaviors are characteristic features of ASDs (Rapin, 1997). The etiology of ASDs is believed to be multifactorial (Gardener et al., 2011). Some genetic factors have been associated with ASDs (Kumar and Christian, 2009). However, many researchers believe that ASDs are in part caused by environmental factors, either directly (Landrigan, 2010) or through interaction with some genes (Hallmayer et al., 2011).

Several studies have investigated the possible association between exposure to arsenic and ASDs, but their findings are conflicting (Fido and Al-Saad, 2005; Kern et al., 2007; Obrenovich et al., 2011). For example, a case-control study of 45 children (1-6 years) with an ASD and 45 non-affected age-, gender-, and race/ethnicity-matched children from Dallas, Texas reported lower levels of arsenic in the hair of children with an ASD (mean hair As of 0.06 $\mu\text{g/g}$ in ASD cases vs. 0.09 $\mu\text{g/g}$ in controls; $P < 0.05$) (Kern et al., 2007). In contrast, another study from Cleveland, Ohio reported higher levels of arsenic in the hair of children with an ASD compared to controls ($P < 0.0001$) (Obrenovich et al., 2011). On the other hand, another study reported no significant difference in levels of arsenic in the hair among 40 age-matched boys (4-8 years of age) with an ASD compared to a group of typically developing (TD) boys (0.13 $\mu\text{g/g}$ in both cases and controls) (Fido and Al-Saad, 2005).

As an island nation, Jamaica has very specific sources of exposure to environmental contaminants, including arsenic, such as water and agricultural soil that might affect levels of arsenic in fruits, vegetables, and seafood (Howe et al., 2005; Lalor, 1996). We investigated whether there is an association between blood arsenic concentrations and ASDs in children. Our study allows for investigation of the importance of the levels and routes of exposures to arsenic (e.g., frequency of seafood consumption). Thus, we identified factors associated with total blood arsenic concentrations among children in Kingston, Jamaica.

2. Materials and Methods

2.1. General description

The Jamaican Autism study is a NIH-supported age- and sex-matched case-control study that began enrollment (children age 2-8 years) in December 2009, investigating whether environmental exposures to several heavy metals, including arsenic, have a role in the onset of an ASD. Information regarding the recruitment and assessment of ASD cases and controls have been described previously (Rahbar et al., 2012a; Rahbar et al., 2012b). In short, children listed in the University of the West Indies' (UWI) Jamaica Autism Database, who were previously identified as being at risk for an ASD based on Diagnostic Statistical Manual of Mental Disorders (DSM-IV-TR) criteria (American Psychiatric Association, 2000) and the Childhood Autism Rating Scale (CARS) (Schopler et al., 1980) were invited

to participate for reassessment of their ASD status. The Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2002) and the Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al., 2003b) were administered by a trained clinician to these children and their parents/guardians, respectively, to confirm the diagnosis of an ASD for the purposes of this research. The inclusion criteria for all children in the study were that each child must be born in Jamaica and be between 2-8 years of age at the time of enrollment. For ascertainment of ASD status, we used standard algorithms developed for scoring ADOS (Lord et al., 2000) and ADI-R and established cut-off points (Lord et al., 1997). Each ASD case was confirmed based on both ADI-R and all three domains in ADOS. For each case, an age- and sex-matched control was identified from schools and well-child clinics. The criteria for matching required that the age of control children be within six months of their matched cases. The Lifetime form of the Social Communication Questionnaire (SCQ) (Rutter et al., 2003a) was administered to the parents/guardians of control children to rule out symptoms of ASDs. We set the criteria for including children in the control groups as having a SCQ score of 0-6. This cut-off point of 6 is one standard deviation above the mean SCQ score of TD school children (Mulligan et al., 2009).

We also administered a pre-tested questionnaire to the parents/guardians of both cases and controls to collect demographic and socioeconomic (SES) information (e.g., ownership of a car by the family), parental education levels, and potential exposure to arsenic through water sources and food by asking how often the child ate certain food items within a week, with a particular focus on the types and frequency of fruits, vegetables, and seafood. These food frequency data represent current typical consumption of food items by children. The types of fruits and vegetables, based on their characteristics and species, were classified into the following categories: 1) two classes of root vegetables [class 1A = (yam, sweet potato, or dasheen), class 1B = (carrot, or pumpkin)]; 2) three classes of leafy vegetables [class 2A = (lettuce), class 2B = (callaloo, broccoli, or pak choi), class 2C = (cabbage)]; 3) legumes (string beans); and 4) three different fruits (tomatoes, ackee, or avocado). The types of seafood we considered included salt water fish, fresh water fish (pond fish, tilapia), sardine or mackerel (canned fish), tuna (canned fish), salt fish (pickled mackerel), shellfish (lobsters, crabs), and shrimp.

At the end of each interview, the project coordinator at the UWI, who has received phlebotomy training, collected about 2 mL of venous whole blood from each child into plastic tubes containing EDTA which were prescreened for mercury, cadmium, and lead. We also collected hair samples (only from children who had hair that was at least 3 inches long). The blood samples were frozen and stored at -20 °C until they were transported to the Michigan Department of Community Health (MDCH) Trace Metals Lab at ambient temperature on ice packs for trace metal analyses, including arsenic. All participating parents provided written informed consent. This study was approved by the Institutional Review Boards of the University of Texas Health Science Center at Houston (UTHealth) and the UWI, Mona campus, in Kingston, Jamaica. The data presented herein represent analysis of 65 1:1 matched case-control pairs (130 children) from the Kingston area that were interviewed from December 2009 to September 2010.

2.2. Assessment of Arsenic Exposure

In this study, participants' exposure to arsenic was assessed by total blood arsenic concentration. Both organic and inorganic arsenic are absorbed in blood, but organic arsenic has a short half-life in blood. Inorganic arsenic can be measured through urine, blood, hair, and nails (Caussy, 2005; Subcommittee on Arsenic in Drinking Water, 1999). Most studies in the literature recommend assessment of arsenic in urine (adjusted for creatinine in urine) as a more reliable method for measuring arsenic exposure (ATSDR, 2007b). Depending on the duration of exposure, arsenic levels could be assessed from other biological samples, like

blood, hair, or fingernails. Blood arsenic concentrations are useful when there is a continuous exposure, whereas urine arsenic concentrations represent more recent exposure (several days) and arsenic levels in hair or fingernails reflect exposure from the past several months (Wu et al., 2001). The World Health Organization (WHO) has confirmed that for areas with a high level of arsenic exposure or chronic (continuous) arsenic exposure, blood arsenic concentration is a reliable method for measuring chronic arsenic exposure (WHO, 2001). For example, in Bangladesh, it has been shown that total blood arsenic concentrations correlated well with creatinine-adjusted urine arsenic concentrations ($r = 0.85$) (Hall et al., 2006). Since Jamaican soils have high arsenic levels (Lalor, 1996), and Jamaicans consume large amounts of seafood and vegetables (Howe et al., 2005), we believe the Jamaican community has had continuous exposure to arsenic; hence, the blood arsenic concentrations should be considered reliable. In this study, whole venous blood samples were assayed for total arsenic by the Trace Metals Lab at MDCH, which is certified by the Centers for Disease Control and Prevention (CDC) for analysis of trace metals. All samples were diluted and analyzed on a PerkinElmer Elan DRC II inductively-coupled plasma mass spectrometer (PerkinElmer, Waltham, MA).

2.3. Statistical Analysis

Descriptive analyses were conducted to compare demographic and SES characteristics of ASD cases and controls. Since the distribution of total blood arsenic concentrations was skewed, we transformed the data using the natural logarithm ($\ln$) to normalize the distribution of the measurements. The means of the $\ln$ transformed blood arsenic concentrations were then transformed to their original scale (i.e., $\mu\text{g/L}$) by applying an exponential function, herein called geometric means (i.e., $\text{Exp.}[\text{Mean}(\ln\text{As})] = \text{geometric mean}$), for ease of interpretation of study results. In addition, since this is a sex- and age-matched case-control study, we used General Linear Models (GLM) with random effects to compare the cases and controls with respect to total blood arsenic concentrations. Moreover, we controlled for the effect of matching by including 64 dummy variables ($65-1=64$, one pair serving as a referent category) to represent the 65 matched pairs as described in our previously published work (Rahbar et al., 2012a; Rahbar et al., 2012b). Associations between the categorical exposure variables and case status were assessed using Conditional Logistic Regression (CLR). Using GLMs, we also assessed the association between blood arsenic concentrations and various exposures of interest, including frequency of eating various kinds of seafood or fish (e.g., mackerel), root vegetables (e.g., yam), leafy vegetables (e.g., broccoli), and fruits (e.g., avocado). In order to minimize any potential effects of multicollinearity due to a high correlation between maternal and paternal education levels, we created a binary variable indicating whether both parents had education up to high school or at least one of the parents obtained education beyond high school. We used multivariable GLMs to identify factors associated with total blood arsenic concentrations in Jamaican children. Finally, we fitted two final multivariable GLMs to assess the relationship between exposure to environmental arsenic and ASDs. In the process of developing the first final multivariable model, we investigated the role of potential confounders related to SES (e.g., car ownership) and socio-demographic variables. In developing the second final multivariable GLM, we examined the role of potential confounders associated with various sources of arsenic exposure including sources of water for drinking and cooking, and child's consumption of vegetables (e.g., roots or leafy), fruits (e.g., avocado), and frequency of seafood consumption, while keeping the SES and socio-demographic covariates in the model. The identification of potential confounders was based on both *a priori* and empirical considerations. First, important factors associated with the ASD status (e.g., maternal age, parental education, SES), from our previous work (Rahbar et al., 2012a) and the literature (Hertz-Picciotto et al., 2010a; Lando and Zhang, 2011), were considered. Second, the covariates which were associated with both blood arsenic concentration and the ASD status

($P < 0.25$) were considered as potential confounders. A covariate was considered to be a confounder if the regression coefficient of the ASD status varied by nearly 10% or more when the covariate was present or absent from the model (Tong and Lu, 2001). All statistical analyses were conducted at a 5% level of significance using SAS 9.2 (SAS Institute Inc., 2008).

2.4. Sample Size and Power

As shown in our previously published work (Rahbar et al., 2012a), based on the available 130 children (65 1:1 matched pairs), we have at least 80% power to detect moderate effect sizes of greater than or equal to 0.35 [i.e., $0.35 \times \text{standard deviation (SD)}$ of blood arsenic concentrations] between ASD cases and controls at a 5% level of significance. As such, we have at least 80% power to detect minimum differences of $0.35 \times \text{SD}$ of total blood arsenic concentrations between children grouped into two categories of exposure (e.g., those who ate avocado vs. those who did not eat avocado), assuming an even split of children between the exposed and unexposed categories. We acknowledge that for exposure variables with significant departure from a 50-50 split of children between the exposed and unexposed categories, a larger effect size than 0.35 will be detected with 80% power at a 5% level of significance.

3. Results

In our sample, 86.2% of the cases and controls were male, which is fairly consistent with the usual gender ratio seen in the ASD population. The mean age of cases and controls was about 65 months. The cases and controls were 96.9% and 98.5% Afro-Caribbean, respectively. Paternal education was significantly higher in the case group compared to controls (49.2% of fathers in the case group had education beyond high school compared to 8.2% in the control group, $P < 0.01$). Similarly, a significantly higher level of education was obtained by mothers in the case group compared to the control group (47.7% in the case group had education beyond high school vs. 19.0% in the control group, $P < 0.01$). In terms of SES, the percentage of families who owned a car was 69.2% in the case families and 33.8% in the control families ($P < 0.01$). Demographic and SES characteristics of the cases and matched controls are reported in Table 1.

In the univariable analysis, we observed significantly lower geometric mean blood arsenic concentrations in ASD cases ($4.03 \mu\text{g/L}$ for cases vs. $4.48 \mu\text{g/L}$ for controls, $P < 0.01$). As part of our effort to identify and control for potential confounding variables, we found that parental education levels were significantly higher in the case group compared to the control group, [Matched Odds Ratio (MOR) = 5.60, 95% CI (2.16, 14.50), $P < 0.01$]. A similar comparison revealed that a significantly lower proportion of ASD cases reported eating sardine or mackerel fish [MOR = 0.22, 95% CI (0.08, 0.66), $P = 0.01$], and identical results were observed for salt fish (pickled mackerel). As shown in Table 2, parents of ASD cases reported significantly lower consumption of some fruits and vegetables compared to children in the control group. For example, parents of ASD cases reported a lower consumption of "avocado" [MOR = 0.17, 95% CI (0.07, 0.30), $P < 0.01$].

A comparison of geometric mean blood arsenic concentrations between children who had different levels and types of exposures revealed the following findings. In the univariable analysis, the geometric mean blood arsenic concentration of children who had piped water as their source of drinking water was significantly lower than that of children who received water from other sources ($4.20 \mu\text{g/L}$ vs. $5.49 \mu\text{g/L}$, $P = 0.02$). Similar results were observed for children whose families used piped water for cooking compared to those who reported using other sources ($4.20 \mu\text{g/L}$ vs. $5.77 \mu\text{g/L}$, $P = 0.01$). A significantly higher geometric mean blood arsenic concentration was observed for children who ate avocado compared to

those who did not eat avocado (4.49 μ g/L vs. 4.04 μ g/L, $P = 0.04$), and the geometric mean blood arsenic concentration of children who ate root vegetables class 1A (yam, sweet potato, or dasheen) was significantly higher than that of children who did not eat these vegetables (4.35 μ g/L vs. 3.85 μ g/L, $P = 0.04$). The geometric mean blood arsenic concentration of children who had a higher consumption of seafood (ate more than 6 meals per week containing seafood) was significantly higher than that of children who ate a lower amount of seafood (4.60 μ g/L vs. 4.11 μ g/L, $P = 0.02$). In the final multivariable model that was developed to identify factors associated with blood arsenic concentrations, only three variables maintained their statistical significance: source of drinking water, eating avocado, and eating leafy vegetables class 2B (callaloo, broccoli, or pak choi). It is important to note that while eating “callaloo, broccoli, or pak choi” was marginally significant ($P = 0.09$) in the univariable analysis, when adjusted in the multivariable model, we observed a significantly higher adjusted geometric mean blood arsenic concentration (4.81 μ g/L vs. 4.30 μ g/L, $P < 0.05$) for children who ate these leafy vegetables compared to children who did not. Comparisons of geometric mean blood arsenic concentrations (unadjusted and adjusted) for other exposure variables are displayed in Table 3.

Finally, in order to explain the higher geometric mean blood concentration in the control group, we fitted two additional multivariable models. In a multivariable model that only controlled for car ownership, maternal age, and parental education levels, we observed a significantly higher adjusted geometric mean blood arsenic concentration for controls (4.01 μ g/L vs. 4.44 μ g/L, $P = 0.01$). Subsequently, in our final multivariable model, we compared the geometric mean blood arsenic concentrations between ASD cases and controls, while controlling for car ownership, maternal age, parental education levels, source of drinking water, consumption of root vegetables [class 1A = (yam, sweet potato, or dasheen) and class 1B = (carrot or pumpkin)], two classes of leafy vegetables [class 2B = (callaloo, broccoli, or pak choi) and class 2C = (cabbage)], avocado, and the frequency of seafood consumption per week. Based on this final model, there is no significant difference between the adjusted geometric mean blood arsenic concentration for ASD cases and controls, (4.36 μ g/L for cases and 4.65 μ g/L for controls, $P = 0.23$).

4. Discussion

4.1. Blood arsenic concentrations and ASD

Our results do not support an association between postnatal total blood arsenic measured in Jamaican children 2-8 years of age and ASD case status. The univariable analysis showed a significantly higher geometric mean blood arsenic concentration ($P < 0.01$) in controls, and this difference was still significant ($P < 0.01$) when adjusted for SES and socio-demographic variables (car ownership, maternal age, and parental education levels). However, this difference was attributed to the differences between ASD cases and controls in terms of their eating habits and other sources of arsenic exposure. Specifically, when we adjusted for potential confounding by car ownership, maternal age, parental education levels, source of drinking water, consumption of “yam, sweet potato, or dasheen”, “carrot or pumpkin”, “callaloo, broccoli, or pak choi”, “cabbage”, “avocado”, and frequency of seafood consumption, there is no evidence ($P = 0.23$) to indicate an association between ASD status in children and elevated total blood arsenic concentrations. Our univariable (unadjusted) results are consistent with Kern *et al.* (2007), who reported lower means for arsenic in the hair of children with an ASD than that of controls (Kern *et al.*, 2007). However, these findings are in contrast with those of Obrenovich *et al.* (2011), who reported higher levels of arsenic in the hair of children with an ASD than in controls (Obrenovich *et al.*, 2011), and those reported by Fido and Al-Saad (2005), who indicated no significant difference between levels of arsenic in the hair of children with an ASD and controls (Fido and Al-Saad, 2005). It is important to note that none of the other studies controlled for potential confounding

effects due to differences in the diets of children with and without an ASD. A comparison of the results in our univariable and multivariable models suggest a significant confounding effect by diet. It is well established that children with an ASD have a higher incidence of gastrointestinal (GI) problems (70% vs. 28%) (Valicenti-McDermott et al., 2006), a higher incidence of constipation (33.9% vs. 17.6%) (Horvath and Perman, 2002), and feeding issues which may result in food selectivity for children with an ASD (24.5% vs. 16.1%) compared to TD children (Ibrahim et al., 2009). Consistent with our findings, Schreck *et al.* (2006) and Cermak *et al.* (2010) reported lower levels of consumption of fruits and vegetables by children with an ASD compared to TD children (Cermak et al., 2010; Schreck and Williams, 2006). Our findings also indicate that the consumption of some vegetables and fruits is associated with higher levels of blood arsenic concentrations, which suggests that the above factors could play a major role in confounding of the association between blood arsenic concentrations and ASDs. In order to obtain unbiased results when assessing associations between blood arsenic concentrations and ASDs, we believe it is important to identify potential confounding variables and control for them in the multivariable analysis. To our knowledge, we are the first to report on the importance of the role of diet as a major confounder when investigating an association between blood arsenic concentrations and ASDs in children. However, the Childhood Autism Risk from Genetics and Environment (CHARGE) study has emphasized the importance of controlling for a variety of factors, including children's fish consumption, when assessing the association between blood mercury concentrations and ASD status (Hertz-Picciotto et al., 2010b). We acknowledge that the blood arsenic concentrations in this study represent arsenic exposure only during the postnatal period, and we did not collect information regarding arsenic exposures during the perinatal and prenatal periods. Furthermore, the postnatal exposure to arsenic through diet may not necessarily represent arsenic exposure through diet during a time that may be causally related to ASDs.

4.2. Blood arsenic concentrations in Jamaican children

We observed that children living in Jamaica have higher total blood arsenic concentrations than children in some other countries, depending on the sources and levels of exposure to arsenic. For example, the arithmetic mean blood arsenic concentration of the control group (4.55 µg/L) in our study was about 4.5 times the blood arsenic concentration of < 1 µg/L that is considered baseline for unexposed individuals in the US by the ATSDR (ATSDR, 2007a). However, it was much lower than that of participants in the Health Effects of Arsenic Longitudinal Study (HEALS) who reside in Arai-hazar, Bangladesh (4.55 µg/L vs. 10.8 µg/L) (Hall et al., 2006). Our study suggests a baseline geometric mean blood arsenic concentration of 4.48 µg/L for TD children (2-8 years) in Kingston, Jamaica.

4.3. Vegetable and fruit consumptions and blood arsenic concentrations

To our knowledge, there are no previously published reports regarding arsenic levels in blood, urine, or hair of Jamaican children, but there are published reports indicating that high levels of arsenic were found in the soil of central Jamaica (Howe et al., 2005; Lalor, 1996) as well as in the drinking water (Lalor et al., 1999). The mean inorganic arsenic levels in some areas of the Jamaican soil could be 2.5 times that of Canadian soil (Howe et al., 2005). However, in Kingston, Jamaica, the mean arsenic level in the soil is 1.3 times that of Canadian soil. Levels of arsenic in agricultural soil could affect fruits, vegetables, and other foods (Lalor et al., 1999). For example, it has been reported that ginger has the highest level of arsenic in Jamaica, but generally the concentrations of arsenic were low in root and crop vegetables (Howe et al., 2005). In contrast, root and tuber vegetables and leafy vegetables have been reported to have high inorganic arsenic levels in other countries, such as Bangladesh (Laizu, 2007). Arsenical pesticides are shown to be a source of arsenic exposure in agricultural products. Although the production of arsenical pesticides in the US was

banned in the 1990s (Lopez et al., 2011), it was not completely phased out until the end of 2009 (USEPA, 2009). In some countries arsenical pesticides are used widely (Reigart, 1999). In Jamaica, the Pesticides Control Authority (PCA) of the Ministry of Health supervises importation and usage of pesticides. PCA banned some arsenical pesticides in 1999 (Pesticides Control Authority, 1999; Pesticides Control Authority, 2010). Although earlier reports from Jamaica reported low levels of arsenic in vegetables (Howe et al., 2005), the bioaccumulation of arsenic in the human body is an important issue for people living in areas with continuous exposure to arsenic. Our findings from our multivariable analysis indicate that Jamaican children who ate avocado had higher postnatal geometric mean blood arsenic concentrations (4.78 μ g/L vs. 4.33 μ g/L, $P=0.04$). Similar results were found for those who consumed “callaloo, broccoli, or pak choi” (4.81 μ g/L vs. 4.30 μ g/L, $P<0.05$). Since it has been shown that a higher frequency of fruit and vegetable consumption has positive effects on cognitive development in children (Gale et al., 2009), additional research focused on risk assessment for consumption of fruits and vegetables in Jamaican children is recommended.

4.4. Seafood consumption and blood arsenic concentrations

Residents of island communities usually consume a higher amount of seafood than residents who live in other settings. Seafood consumption is an important source of exposure to arsenic for humans, particularly young children (Borak and Hosgood, 2007; USEPA, 1997). Our findings, based on univariable analysis, indicate that children with a higher frequency of seafood consumption had a significantly higher total geometric mean blood arsenic concentration than those who reported a lower frequency of seafood consumption (4.60 μ g/L vs. 4.11 μ g/L, $P=0.02$). This finding is consistent with studies that reported mean total blood arsenic concentrations of 5-10 μ g/L for areas with high seafood consumption (Subcommittee on Arsenic in Drinking Water, 1999). However, when we controlled for the effects of avocado and “callaloo, broccoli, or pak choi” in our multivariable GLM, the effect of seafood consumption on blood arsenic concentration was no longer significant. Therefore, seafood consumption was removed from the final model that assessed factors associated with blood arsenic concentrations. We recognize that this does not mean that seafood consumption does not have any role in blood arsenic concentrations, but in the presence of other factors in the model, seafood does not explain any additional variance in the blood arsenic concentrations beyond what has already been explained by other variables in the model.

Greater consumption of seafood in coastal areas and fishing communities has been a concern. This has been of particular interest to high-risk populations as inorganic arsenic exposure in pregnant women has been associated with increased incidence of adverse birth outcomes (Milton et al., 2005). In contrast, Oken *et al.* (2005) reported better infant cognition among mothers with higher fish consumption during pregnancy (Oken et al., 2005). Several studies reported benefits of seafood consumption, including the positive effects of omega-3 fatty acids on cognitive function (Oken et al., 2005; Vinot et al., 2011). We recommend further research focused on assessment of risks and benefits of consumption of seafood in Jamaica.

4.5. Blood arsenic concentrations and sources of water for drinking and cooking

Water, particularly groundwater, is an important source of inorganic arsenic exposure (Subcommittee on Arsenic in Drinking Water, 1999). Highly contaminated soil resulting from activities such as mining or volcanic bedrock can contribute to increased levels of arsenic found in drinking and cooking water sources (Concha et al., 1998; Sarker, 2011). Our findings, based on univariable analyses, indicate that children who drank piped water had significantly lower geometric mean blood arsenic concentrations than those who did not use

pipled water for drinking (4.20 μ g/L vs. 5.49 μ g/L, $P = 0.02$) and cooking (4.20 μ g/L vs. 5.77 μ g/L, $P = 0.01$). The source of water used for cooking could play a role in blood arsenic concentrations particularly in relation to the preparation of food that involves certain vegetables and seafood. However, it is almost impossible to separate the effects of cooking water source from those of vegetables and seafood. Another difficulty in the assessment of the role of cooking water sources in determining blood arsenic concentrations is its high correlation with sources of water for drinking. In order to avoid multicollinearity (Rahbar et al., 2012b) due to a high correlation between sources of drinking and cooking water, we only kept source of drinking water in our final GLM that assessed factors associated with blood arsenic concentrations. We recognize that this does not mean that source of cooking water does not have any role in blood arsenic concentrations.

Other studies reported a significant association between drinking water and elevated blood arsenic concentrations. For example, Vahter *et al.* (1995) reported that women in northwest Argentina, which has elevated levels of arsenic in the drinking water (contaminated by volcanic bedrock), had a high arithmetic mean total blood arsenic concentration (8.0 μ g/L, Range 2.7 μ g/L - 18.0 μ g/L) (Vahter et al., 1995). In the same area, Concha *et al.* (1998) reported a high arithmetic mean total blood arsenic concentration in children (9.1 μ g/L, Range 6.0 μ g/L - 15.0 μ g/L). Rosado *et al.* (2007) reported that children who lived in an arsenic-contaminated area had a high mean total urine arsenic concentration (58.1 μ g/L) and reduced cognitive ability (Rosado et al., 2007).

Our findings from the final multivariable analysis, that assessed factors associated with blood arsenic concentrations, indicate that Jamaican children who live in the Kingston area who did not use pipled water for drinking and ate avocado and “callaloo, broccoli, or pak choi” had significantly higher blood arsenic concentrations. The Jamaican Government has been collaborating with the WHO and the Pan American Health Organization (PAHO) on a program (Country cooperation strategy, 2010-2015) to improve food and water safety (PAHO, 2010; WHO, 2010b). We believe that Jamaicans could benefit from the experience of countries such as Bangladesh (Hoque et al., 2000) with respect to increasing awareness among the Jamaican population regarding potential risks for various exposures to arsenic.

4.6. Limitations

We acknowledge several limitations in this study. First, since the control children for this study were selected to match the ASD cases by sex and age from the Kingston area, they may not represent a random sample from the population of all children in Jamaica. Additionally, our controls belonged to a lower SES group than our cases. Therefore, the findings reported in this study may not be generalizable to populations other than that in which the samples were selected. Moreover, even though the frequency of seafood and vegetable consumption is measured through a previously utilized and culturally appropriate food questionnaire in Jamaica, we cannot completely rule out the potential for recall bias. On the other hand, our data cannot provide reliable information regarding the timing of the arsenic exposure as the blood arsenic concentrations collected in this study are more likely to represent only recent exposure, which may not be a susceptible period for developing ASDs. While we did not find differences between ASD case and control groups with respect to levels of arsenic exposure, it is possible that there will be differences in individual susceptibility to arsenic As Palmer (2010) highlighted, without testing for potential gene-environment interactions, these findings should not be interpreted as an indication of the safety of exposure to arsenic for all (Palmer, 2010). We recognize this issue and acknowledge that a formal test for gene-environment interactions will require a much larger sample size of ASD cases and controls than the 65 matched pairs that we analyzed in this study. We also acknowledge our inability to investigate the role of other dietary factors, such as eating rice in relation to blood arsenic in Jamaica. Since rice production takes place

in four parishes in Jamaica (St. Catherine, St. Elizabeth, Clarendon and Westmoreland), we collected data regarding consumption of rice by children. However, since 99.2% of the children reported eating rice, our ability to analyze the relationship between rice consumption and blood arsenic in Jamaican children was limited. Finally, due to limited resources, in this study we assessed only total blood arsenic concentrations. However, we acknowledge that assessment of inorganic and organic urine arsenic concentrations would allow a better assessment for risk of exposure to inorganic arsenic in Jamaican children.

5. Conclusions

In this study, we demonstrated that children living in Kingston, Jamaica, have higher total blood arsenic concentrations compared to children with lower exposures to arsenic in the US. In addition, we showed that eating “callaloo, broccoli, or pak choi”, avocado, and living in a family that did not use piped water for drinking are significantly associated with having elevated total blood arsenic concentrations. Furthermore, after adjusting for potential confounding variables in a multivariable GLM that included car ownership, maternal age, parental education levels, source of drinking water, consumption of root vegetables (class 1A and class 1B), leafy vegetables (class 2A and class 2B), avocado, and the frequency of seafood consumption per week, we did not find a significant association between ASDs and total blood arsenic concentrations. Further research, including risk-benefit analysis studies, is needed to assess optimal levels for consumption of fruits and vegetables in Jamaica.

Acknowledgments

This research is co-funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institutes of Health Fogarty International Center (NIH-FIC) by a grant [R21HD057808] awarded to the University of Texas Health Science Center at Houston (UTHealth). We also acknowledge the support provided by the Biostatistics/ Epidemiology/ Research Design (BERD) component of the Center for Clinical and Translational Sciences (CCTS) for this project. CCTS is mainly funded by the NIH Centers for Translational Science Award (NIH CTSA) grant (UL1 RR024148), awarded to the University of Texas Health Science Center at Houston in 2006 by the National Center for Research Resources (NCRR). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD or the NIH-FIC or the NCRR. Finally, we acknowledge contributions by colleagues in the Trace Metals Lab at MDCH for analyzing and storing the blood samples for arsenic concentrations.

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Highlights

We investigated the role of exposure to arsenic on autism in Jamaican children

We identified factors associated with blood arsenic levels in Jamaican children

We did not find an association between autism and blood arsenic in Jamaican children

We found that eating some vegetables and avocado were associated with blood arsenic

We found that source of drinking and cooking water were associated with blood arsenic

Table 1

Demographic and socioeconomic characteristics of children and their parents by ASD case status

Variables	Categories	Case (n=65) N (%)	Control (n=65) N (%)	P-value
Age of child^a (months)	Age < 48	10 (15.4)	9 (13.8)	0.85
	48 age < 72	34 (52.3)	34 (52.3)	
	Age 72	21 (32.3)	22 (33.9)	
Maternal education (at child's birth)	Up to high school	34 (52.3)	51 (81.0)	< 0.01
	Beyond high school	31 (47.7)	12 (19.0)	
Paternal education (at child's birth)	Up to high school	32 (50.8)	56 (91.8)	< 0.01
	Beyond high school	31 (49.2)	5 (8.2)	
Assets owned	TV	65 (100.0)	62 (95.4)	NR
	Refrigerator	65 (100.0)	54 (83.1)	NR
	Freezer	8 (12.3)	12 (18.8)	0.35
	Living room set	57 (87.7)	31 (47.7)	< 0.01
	Washing machine	54 (83.1)	33 (50.8)	< 0.01
	Cars or other vehicles	45 (69.2)	22 (33.8)	< 0.01
	Cable/Satellite connection	46 (70.8)	22 (33.8)	< 0.01

NR: Not reported due to unstable estimates caused by no observations in at least one cell

Up to high school education include: attended Primary/Jr. Secondary, and Secondary/High/Technical schools

Beyond high school education include: attended Vocational, Tertiary College, or University

Maternal education was missing for 2 controls

Paternal education was missing for 2 cases and 4 controls

Assets owned (freezer) was missing for 1 control family

^aAge of the controls is within 6 months of their matched cases

Table 2
Association between potential confounding variables and ASD case status using Conditional Logistic Regression (65 pairs)

Variables	Categories	Case N (%)	Control N (%)	Matched OR (MOR)	95% CI for MOR	P-value
Child's sex	Male	56 (86.2)	56 (86.2)	1.00	(0.06, 15.99)	1.00
Child's age	More than 4 years	55 (84.6)	56 (86.2)	NR	NR	NR
Paternal age (at child's birth)	More than 35 years	33 (52.4)	18 (29.5)	3.00	(1.28, 7.06)	0.01
Maternal age (at child's birth)	More than 35 years	17 (26.2)	7 (11.3)	3.00	(1.09, 8.25)	0.03
Parental education levels (at child's birth)	At least one of the parents had education beyond high school	42 (66.7)	14 (23.7)	5.60	(2.16, 14.50)	< 0.01
Source of drinking water	Piped water	61 (93.6)	63 (98.4)	0.25	(0.03, 2.24)	0.22
Source of water for cooking	Piped water	62 (95.4)	63 (98.4)	0.33	(0.04, 3.21)	0.34
Root vegetables (class 1)	A. Yam, sweet potato, or dasheen	48 (73.8)	55 (85.9)	0.43	(0.17, 1.12)	0.08
	B. Carrot or pumpkin	54 (83.1)	63 (98.4)	0.10	(0.01, 0.78)	0.03
Leafy vegetables (class 2)	A. Lettuce	26 (40.0)	38 (59.4)	0.52	(0.27, 1.00)	0.05
	B. Callaloo, broccoli, or pak choi	45 (69.2)	60 (93.8)	0.20	(0.07, 0.58)	< 0.01
Fruits and vegetables consumption	C. Cabbage	40 (61.5)	60 (93.8)	0.16	(0.06, 0.46)	< 0.01
Legumes	String beans	20 (30.8)	25 (39.1)	0.68	(0.34, 1.38)	0.30
	Tomatoes	37 (56.9)	52 (81.3)	0.27	(0.11, 0.67)	0.01
Fruits	Ackee	33 (50.8)	58 (90.6)	0.04	(0.01, 0.28)	< 0.01
	Avocado	18 (27.7)	43 (67.2)	0.17	(0.07, 0.30)	< 0.01
High seafood consumption (more than 6 meals per week)		13 (20.0)	26 (40.0)	0.44	(0.21, 0.91)	< 0.07
Frequency of seafood meals consumed weekly		NA	NA	1.28	(1.11, 1.49)	< 0.01
Seafood consumption	Ate salt water fish	49 (75.4)	57 (87.7)	0.38	(0.14, 1.08)	0.07
	Ate fresh water fish (pond fish, tilapia)	29 (44.6)	26 (40.0)	1.23	(0.59, 2.56)	0.58
	Ate sardine, mackerel (canned fish)	45 (69.2)	59 (90.8)	0.22	(0.08, 0.66)	0.01
	Ate tuna (canned fish)	18 (27.7)	27 (41.5)	0.53	(0.24, 1.13)	0.10
	Ate salt fish (pickled mackerel)	45 (69.2)	59 (90.8)	0.22	(0.08, 0.66)	0.01
	Ate shellfish (lobsters, crabs)	1 (1.5)	6 (9.2)	0.17	(0.02, 1.38)	0.10
	Ate shrimp	7 (10.8)	16 (24.6)	0.40	(0.16, 1.03)	0.06

NR: Not reported due to unstable estimates caused by a limited number of observation in at least one of the cells
Paternal age was missing for 1 case and 4 controls

Maternal age was missing for 3 controls

Parental education levels was missing for 2 cases and 5 controls

Source of drinking water was missing for 1 control

Source of water for cooking was missing for 1 control

For all variables related to fruits and vegetables consumption information was missing for 1 control

NA: not applicable, because frequency of seafood meals consumed weekly is analyzed as a continuous variable

Table 3
Factors associated with total blood arsenic concentration using General Linear Model (65 Pairs)

Exposure variables	Category	Univariable					Multivariable		
		Yes		No		P-value	Yes Adjusted Mean As	No Adjusted Mean As	Adjusted P-value
		Mean As (µg/L)	N	Mean As (µg/L)	N				
Child's sex	Male	4.09	112	5.36	18	0.13	-	-	-
Child's age	More than 4 years	4.00	111	6.01	19	0.11	-	-	-
Paternal age (at child's birth)	More than 35 years	4.23	51	4.25	74	0.93	-	-	-
Maternal age (at child's birth)	More than 35 years	4.18	24	4.27	103	0.72	-	-	-
Parental education levels (at child's birth)	At least one of the parents had education beyond high school	4.21	54	4.27	69	0.78	-	-	-
Source of drinking water	Piped water	4.20	124	5.49	5	0.02	4.06	5.09	0.04
Source of water for cooking	Piped water	4.20	125	5.77	4	0.01	-	-	-
Fruits and vegetables consumption	Root vegetables (class 1)	4.35	103	3.85	26	0.04	-	-	-
		4.31	117	3.72	12	0.07	-	-	-
	Leafy vegetables (class 2)	4.26	64	4.23	65	0.83	-	-	-
		4.32	105	3.92	24	0.09	4.81	4.30	< 0.05
		4.31	100	4.02	29	0.20	-	-	-
	Legumes	4.36	45	4.18	84	0.37	-	-	-
	Fruits	4.25	89	4.24	40	0.96	-	-	-
		4.28	91	4.16	38	0.64	-	-	-
		4.49	61	4.04	68	0.04	4.78	4.33	0.04
	Avocado								

Exposure variables	Category	Univariable				Multivariable			
		Yes		No		P-value	Yes		Adjusted P-value
		Mean As (µg/L)	N	Mean As (µg/L)	N		Adjusted Mean As	Adjusted Mean As	
Seafood consumption	High seafood consumption (more than 6 meals per week)	4.60	39	4.11	91	0.02	-	-	-
	Frequency of seafood meals consumed weekly	NA	-	NA	-	0.06	-	-	-
	Ate salt water fish	4.28	106	4.13	24	0.59	-	-	-
	Ate fresh water fish (pond fish, tilapia)	4.19	55	4.29	75	0.63	-	-	-
	Ate sardine, mackerel (canned fish)	4.34	104	3.91	26	0.08	-	-	-
	Ate tuna (canned fish)	4.38	45	4.18	85	0.35	-	-	-
	Ate salt fish (pickled mackerel)	4.33	104	3.94	26	0.11	-	-	-
	Ate shellfish (lobsters, crabs)	4.46	7	4.24	123	0.61	-	-	-
	Ate shrimp	4.08	23	4.29	107	0.40	-	-	-

NA: not applicable, because frequency of seafood meals consumed weekly is analyzed as a continuous variable

The "Yes" column includes subjects who met the category specified in front of each exposure variable

The "No" column includes subjects who did not meet the category specified in front of each exposure variable

Paternal age was missing for 1 case and 4 controls

Maternal age was missing for 3 controls

Parental education levels was missing for 2 cases and 5 controls

Source of drinking water was missing for 1 control

Source of water for cooking was missing for 1 control

Mean As indicates the geometric mean = Exp. [Mean (ln As)]

Adjusted P-value for "Callaloo, broccoli, or pak choi" is 0.487 but reported as $P < 0.05$