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Thyroid hormones and neurobehavioral functions among adolescents chronically exposed to groundwater with geogenic arsenic in Bangladesh

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Abstract

Groundwater, the major source of drinking water in Bengal Delta Plain, is contaminated with geogenic arsenic (As) enrichment affecting millions of people. Children exposed to tubewell water containing As may be associated with thyroid dysfunction, which in turn may impact neurodevelopmental outcomes. However, data to support such relationship is sparse. The purpose of this study was to examine if chronic water As (WAs) from Holocene alluvial aquifers in this

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Disclosure Summary

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region was associated with serum thyroid hormone (TH) and if TH biomarkers were related to neurobehavioral (NB) performance in a group of adolescents. A sample of 32 healthy adolescents were randomly drawn from a child cohort in the Health Effects of Arsenic Longitudinal Study (HEALS) in Araihaaz, Bangladesh. Half of these participants were consistently exposed to low WAs (<10 µg/L) and the remaining half had high WAs exposure (10 µg/L) since birth. Measurements included serum total triiodothyronine (tT₃), free thyroxine (fT₄), thyrotropin (TSH) and thyroperoxidase antibodies (TPOAb); concurrent WAs and urinary arsenic (UAs); and adolescents' NB performance. WAs and UAs were positively and significantly correlated with TPOAb but were not correlated with TSH, tT₃ and fT₄. After accounting for covariates, both WAs and UAs demonstrated positive but non-significant relationships with TSH and TPOAb and negative but non-significant relationships with tT₃ and fT₄. TPOAb was significantly associated with reduced NB performance indicated by positive associations with latencies in simple reaction time (b=82.58; p<0.001) and symbol digit (b=276.85; p=0.005) tests. TSH was significantly and negatively associated with match-to-sample correct count (b=-0.95; p=0.05). Overall, we did not observe significant associations between arsenic exposure and TH biomarkers although the relationships were in the expected directions. We observed TH biomarkers to be related to reduced NB performance as hypothesized. Our study indicated a possible mechanism of As-induced neurotoxicity, which requires further investigations for confirmatory findings.

Keywords

arsenic in aquifer; drinking water arsenic; mechanism of neurotoxicity; thyroid biomarkers

1. Introduction

Geogenic arsenic (As) occurs in elevated concentrations in the Holocene alluvial aquifers of rural Bangladesh resulting in chronic As exposure in millions of people who heavily rely on water in aquifers for both drinking and cooking. As concentration in drinking water in Bengal Basin often exceeds the World Health Organization (WHO) and US-EPA safety standard of 10 µg/L (Biswas et al., 2012; Halder et al., 2012; von Bromssen et al., 2007). In addition, several other toxic elements such as manganese (Mn), chromium (Cr), and lead (Pb) have been found in groundwater in Bengal Basin (Frisbie et al., 2009; Halder et al., 2013; Rahman et al., 2013). However, considering a large 150 million As-affected global population and more frequent detection of unsafe level of As in well water relative to other metals and metalloids As remains the most important public health challenge in different parts of the world (Shankar et al., 2014).

It is anticipated that more than 3 million wells in Bangladesh have As above this threshold value (Raessler, 2018). In addition, rice and other crops, fruits, juices and poor air quality can lead to higher arsenic exposure (Halder et al., 2012; Wilson et al., 2012). Arsenic (As) is associated with increased risk for several cancers in addition to non-carcinogenic health effects in the respiratory, cardiovascular and nervous systems in adults (Chen et al., 2011; Chen et al., 2007; Hughes et al., 2011; Parvez et al., 2010). Both experimental and epidemiological studies have reported associations of chronic water arsenic (WAs) exposure with several neurodevelopmental functions such as intelligence, learning, memory and motor

function in early life (Rodriguez-Barranco et al., 2013; Tyler and Allan, 2014). However, the underlying mechanisms of As-induced neurotoxicity have yet to be elucidated.

Thyroid hormone (TH) is important in the development and maturation of the central nervous system (CNS) from the pre-natal period to the middle of childhood (Rovet, 2014; Zoeller and Rovet, 2004). A very small change in maternal TH – even within the population reference range – may be associated with up to a 10% decrease in child intelligence (i.e. loss of approximately 5-10 IQ points) (Bath et al., 2013; Haddow et al., 1999; Henrichs et al., 2010; Li et al., 2010). On the other hand, As may have a direct effect on the thyroid function through the inhibition of enzymes involved in TH synthesis and disruption of TH receptor-mediated gene regulation (Davey et al., 2007; Davey et al., 2008). Epidemiological studies including the NHANES study have reported positive association of As exposure with thyroid stimulating hormone (TSH) and negative associations of As with free thyroxine (fT₄) and total triiodothyronine (tT₃) (Meltzer et al., 2002; Molin et al., 2017); (Meeker et al., 2009); (Ciarrocca et al., 2012). Arsenic also triggers autoimmune thyroid disease resulting in elevated levels of biomarkers such as thyroid peroxidase antibody (TPOAb) (Brent, 2010; Langer et al., 2007). Thus, it is reasonable to hypothesize that As may exert a neurotoxic effect during child development by interfering with TH biomarkers such as fT₄, tT₃, TSH and TPOAb.

We conducted a pilot study in a group of As-exposed adolescent children for gathering preliminary data and laying the foundation for a future study with a larger sample size, which will eventually test whether TH mediates As-induced neurotoxicity. Mothers of these children are participants of an ongoing Health Effects of Arsenic Longitudinal Study (HEALS) in Araihaazar, Bangladesh, which started in 2000 (Wasserman et al., 2007; Wasserman et al., 2004). These adolescent children (born after 2000) and their mothers have been consuming tubewell water since birth as evident in the literature (Wasserman et al., 2007; Wasserman et al., 2004). Longitudinal As exposure data for these adolescents was collected from HEALS database. Based on the data, it was possible to identify a subset of children who had either consistently high (i.e. above the WHO WAs standard of 10 µg/L) or low (i.e. below the WHO WAs standard) lifetime WAs exposure. We considered these adolescents as our potential study participants. During adolescence, several important components of the brain such as the frontal and limbic regions demonstrate stable performance in cognitive functions at adolescence (de Graaf-Peters and Hadders-Algra, 2006; Spear, 2013). Specific key areas of the brain that support working memory do not become functional until early adolescence as well (Sander et al., 2012). For these reasons, any measured neurodevelopmental outcome during this critical window is most likely to reflect the functional outcome during the remainder of the life course.

At the time of recruitment for the present study, all of our adolescent participants were between 15 to 17 years of age living in Araihaazar, Bangladesh in the HEALS study area. We hypothesized that consistent WAs exposure from birth to childhood predicts the levels of four serum TH biomarkers through negative associations with total triiodothyronine (T₃) and free thyroxine (T₄), and positive associations with thyroid stimulating hormone (TSH), and antibodies to thyroperoxidase (TPOAb). Also, we tested the hypothesis that concurrent TH biomarker levels are associated with reduced neurobehavioral (NB) performance measured

by a computer test battery, the Behavioral Assessment and Research System (BARS) (Rohlman et al., 2001; Rohlman et al., 2007a; Rohlman et al., 2003). Collectively, our long-term goal was to generate preliminary evidence regarding possible mediation effects of TH on the association between geogenic As exposure and NB outcomes, which may eventually lay the foundation for a larger study.

2. Materials and Methods

2.1. Study area and participants

The population in Araihaazar, Bangladesh is exposed to a wide range of WAs with the majority of the HEALS participants consuming low-to-moderate doses (0-150 µg/L) of WAs (Figure 1). In the first three years of the study, the HEALS recruited 11,746 adults between the age of 18 and 75 years including 726 women who had children born between 2000 and 2002. At the time of our study conducted in 2017, these HEALS children were between 15 to 17 years of age. These children were enrolled in a prospective child development study when recruitment for our pilot study began. Past studies among children in the HEALS demonstrated strong correlations ($r=0.75$) between mother's urinary arsenic (UAs) and children's UAs, which were adjusted for urinary creatinine providing evidence that both mothers and children largely consume water from the same wells (Wasserman et al., 2007; Wasserman et al., 2004). This is because every mother brings water for all family members from a specific well, the only source of drinking water for the household. At the time of recruitment, WAs and UAs data were available for these mothers covering at least five critical stages of childhood: birth, age 3, 6, 9, and 12, which helped us identify the children who were consistently exposed to WAs either above or below 10 µg/L throughout childhood. We found 304 healthy adolescent children with consistent exposure to low WAs, i.e. $WAs < 10 \mu\text{g/L}$ and 252 children with consistent exposure to high WAs ($WAs \geq 10 \mu\text{g/L}$) since birth. At the time of recruitment, all participants of this longitudinal child development study were between 15 to 17 years of age. We randomly selected 16 healthy adolescents from the high WAs group and equal number of participants from the low WAs exposure group to conduct this pilot study using a cross-sectional design. All initially selected participants went through physical examinations by a HEALS study physician and none of them was identified with any symptom of subclinical hypothyroidism. Arsenic guideline value of 10 µg/L of WAs recommended by the World Health Organization (WHO) and US Environmental Protection Agency (EPA) was chosen instead of the Bangladesh standard (i.e. 50 µg/L) to make the findings of the study relevant to other As-affected populations across the globe. To accommodate necessary field and laboratory costs for this pilot project, a small number of sample ($n=32$) was chosen. Children with disability, known iodine deficiency, diagnosed thyroid disease or other chronic diseases were excluded. The recruitment of adolescent participants took place between May to July of 2017. This study was approved by the Columbia University Medical Center Institutional Review Board (IRB), Indiana University IRB, and the Bangladesh Medical Research Council IRB. Parental informed consent and child assent were obtained once the mother agreed to allow her child to participate in the study.

2.2. Procedures

Appointments were made for mother and child to visit the HEALS field clinic in Araihaazar, Bangladesh to complete the NB assessment. The ongoing child development study (Wasserman et al., 2018) had earlier collected concurrent urine and serum samples that were used for analyses of biomarker of exposure (i.e. urinary arsenic) and serum TH (total T₃, free T₄, TSH and TPOAb) respectively. Adolescents received a small age-appropriate gift in appreciation for participation.

2.3. Exposure assessment

Information about As concentrations in well water used by the mothers and their children was collected from HEALS WAs exposure database to verify lifetime consistent high or low WAs exposure in adolescent participants. Additionally, concurrent well WAs concentration for each mother and child was also obtained from this database. Laboratory analysis procedure for arsenic in drinking water has been described elsewhere (Cheng et al., 2004; Van Geen et al., 2005). Concurrent UAs concentration was measured by graphite furnace atomic absorption spectrophotometry (GFAA), using a Perkin-Elmer Analyst 800 system as described (Nixon et al., 1991). All the samples were above the detection limit of 2 µg/L. Urinary creatinine (UCr) concentrations were analyzed by a colorimetric method based on Jaffe's reaction (Heinegard and GunnarTiderstrom, 1973).

2.4. Serum thyroid hormone assessment

Measurement of total triiodothyronine (T₃), free thyroxine (T₄), and thyroid stimulating hormone (TSH) are generally regarded as the most comprehensive diagnostic tests for assessing thyroid function. ELISA was used to quantify total T₃ (Abnova, Walnut, CA), free T₄ and TSH (Eagle Biosciences, Nashua, NH) in serum samples collected from the adolescent participants analyzed according to manufacturer's instructions. Thyroperoxidase antibody (TPOAb) levels are often used to detect autoimmune thyroid diseases. Serum levels of TPOAb were quantified using a commercially available ELISA kit (Eagle Biosciences, Nashua, NH) according to manufacturer's instructions. Unknown values were calculated from standard curves generated using a 4-parameter logistic regression. The precision of the analyte measurements was assessed using quality control serum (Lyphochek Immunoassay Plus Control, levels 1-3, and Liquechek Specialty Immunoassay Control, levels LTA 1-3; Bio-Rad, Irvine, CA). All samples were analyzed in a single 96 well plate for each biomarker, so there was no need to normalize across plates.

2.5. Neurobehavioral assessment in adolescents

Neurobehavioral performance of the participants was evaluated through a computerized test battery, the Behavioral Assessment and Research System (BARS). The BARS has been used to detect subtle effects of neurotoxic chemicals in young age groups. Cultural adaptation and research use of the BARS have been reported in rural and low-income populations in developing countries including Ecuador, Egypt, Costa Rica, and Thailand (Ismail et al., 2014; Khan et al., 2019; Lu et al., 2009; Rohitrattana et al., 2014; Rohlman et al., 2014). The BARS was found to be culturally adaptable in rural Bangladesh with moderate to high test-retest reliability (data not shown). Testing in BARS does not require prior experience in

computer use. Any participant can be tested if the person can count from 1 through 9, because the special response unit used for all BARS tests contains nine buttons (i.e. keys) that are labeled with one of these nine single digit numbers. The nine-button response unit fits over the laptop computer keyboard. Participants are instructed to use one or more of the nine keys while participating in the tests. The computer-based tests measure attention, response speed, coordination, and memory by recording time and accuracy of the participant's responses. The test battery included five tests, Simple Reaction Time (SRT) to measure response speed, Match to Sample (MTS) to measure visual memory, Finger Tapping Test (FTT) to measure motor performance and procedural learning, Continuous Performance Test (CPT) to measure sustained attention, and Symbol Digit Test (SDT) to measure information processing speed. Initial selection of the tests in the battery was based on those demonstrating deficits in studies of children and adolescents conducted in different parts of the world (Abdel Rasoul et al., 2008; Butler-Dawson et al., 2016; Ismail et al., 2014; Rohlman et al., 2005; Rohlman et al., 2007b). Each test had built in instructions (i.e. computer demonstration and practice test) allowing the testing staff to explain in local language (Bangla) how each test should be performed. Once the tests were completed, the results were calculated, aggregated, and stored automatically in computers, with periodic transfer of data to Indiana University Bloomington server by HEALS informatics team in Bangladesh.

2.6. Sociodemographic characteristics

During the child development study, sociodemographic characteristics were collected from the parents of the adolescents during the home visit, including maternal age and education. Television ownership is an indicator of higher socioeconomic status of a family in rural Bangladesh. Therefore, information about television in house was also collected during this visit. Information about the number of years of schooling completed and current school enrollment were collected from the adolescent participant during BARS NB testing. At the same time, head circumference, height, weight, and blood pressure of the participants were measured by trained field staff using a measuring tape, a digital scale, and a sphygmomanometer respectively.

2.7. Data analysis

We compared demographic characteristics between the high and low WAs exposure groups using descriptive data presented as frequency (%) or means $\pm$ standard deviations. Initial bivariate analyses were conducted to compare differences in proportions using χ^2 test and mean differences using independent t-tests. Statistical significance was defined as $p < 0.05$ and all hypothesis tests were 2-sided. Spearman correlation coefficients were calculated between As exposure variables (WAs and UAs adjusted for creatinine) and the four serum thyroid biomarkers. We constructed multivariate linear regression models to examine the association of As exposure with TH biomarkers. Exposure variables such as UAs (adjusted for creatinine) and WAs, both of which had right skewed distributions were all transformed by natural logarithmic function before being included in the models to reduce the impact of extreme values and improve model fitting. Linear regression models were constructed to examine the relationships between TH biomarkers and BARS neurobehavioral outcomes. In both cases, potential confounding variables selected for initial inclusion in the cross-

sectional statistical models were those previously reported to be associated with neurocognitive effects of As in healthy children (i.e., age, sex, BMI and maternal education) or documented socioeconomic risk factors for neurocognitive effects such as home ownership and maternal occupation (Felfe and Hsin, 2012; Leist et al., 2013; Patra et al., 2016; Ronfani et al., 2015). We examined whether these covariates changed the estimated associations between exposure and outcomes; variables were retained in the model if there was any substantial change (i.e. >10%) in the association of interest. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary NC).

3. Results

3.1. Sample characteristics

Co-variables including age, BMI, blood pressure, maternal and paternal education (i.e. whether the parent went to school for formal education), presence of television in house, and house constructions did not differ by WAs exposure groups (Table 1). The educational status of all participants did not differ between the two groups as all of them were enrolled in high schools (not shown in Table 1). As expected, adolescent participants in the high WAs exposure group had significantly higher WAs and creatinine adjusted UAs ($p<0.001$). The difference between high and low WAs exposure groups in terms of TSH and TPOAb levels was statistically non-significant. The low WAs exposure group performed better in all neurobehavioral tests as indicated by lower latencies and higher counts for key BARS outcome variables in this group. For simple reaction time latency and number of taps in finger tapping test by preferred hand, the differences between the two groups were statistically significant ($p=0.05$) (Table 1).

3.2. Correlations of As exposure with serum thyroid hormones

Arsenic in drinking water (WAs) was positively and significantly correlated with creatinine-adjusted UAs (Spearman correlation coefficient $r=0.73$; $p<0.001$). We observed that WAs demonstrated negative but non-significant correlation with free T_4 ($r=-0.16$) but did not show any correlation with total T_3 . UAs, on the other hand, demonstrated non-significant negative correlations with total T_3 ($r=-0.15$) but did not show correlation with free T_4 ($r=-0.06$). Furthermore, significant positive correlations between WAs and TPOAb ($r=0.37$; $p=0.04$) and UAs and TPOAb ($r=0.43$; $p=0.004$), were found.

3.3. Associations of As exposure and demographic covariates with TH biomarkers

Associations between As exposure and four TH in serum collected from the adolescent participants after accounting for the demographic covariates age, sex and BMI are shown in Table 2. Both log-transformed WAs and UAs did not demonstrate any significant associations with the four serum TH biomarkers in the adjusted models. Among the covariates used in the models, boys had higher total T_3 and free T_4 and lower TSH and TPOAb than the girls. BMI, on the other hand, demonstrated negative relationship with total T_3 and free T_4 and positive relationship with TSH and TPOAb. Associations of age, sex (boys vs girls) and BMI with total T_3 were also statistically significant.

3.4. Associations between TH biomarkers and NB performance

For NB outcomes that measure latency (i.e. time interval between the stimulation and response in a BARS test), a positive association with As could indicate reduced neurocognitive performance (Table 3). On the other hand, a negative association of As with a NB outcome measured by counts or numbers could reflect declined performance. Among the four TH biomarkers TPOAb demonstrated most consistent and in some cases significant relationships with NB outcomes in the expected directions. Higher TPOAb was associated with significantly higher latencies (an indicator of delayed cognitive response) in the simple reaction time ($p<0.001$) and symbol digit tests ($p=0.005$). We observed a positive relationship between TPOAb and match to sample latency although it was non-significant ($p=0.10$). The other three TH biomarkers did not demonstrate any significant association with any of the NB outcomes except TSH, which demonstrated significant negative association with correct counts in match to sample test ($p=0.02$) (Table 3).

4. Discussion

It is difficult to mitigate As contamination in drinking water in Bengal delta sediments, because arsenic gets dissolved into the aquifer via a biogeochemical process due to high amount of organics in the sediments (Raessler, 2018). Therefore, potential associations between consistent groundwater As exposure in early life, biomarkers of thyroid function and brain development hinted by our pilot study data have high public health significance. Arsenic neurotoxicity was evident in our study as the low As exposure group performed better when the key outcome variables measured by the BARS test battery were compared (Table 1). Although differences in NB performance between the two exposure groups were not always significant in our study, perhaps due to the small sample size, several previous larger epidemiological studies conducted in the same study area have already documented similar neurocognitive effects of WAs in children and adolescents (Parvez et al., 2011; Wasserman et al., 2004; Wasserman et al., 2018; Wasserman et al., 2011). We wanted to understand if TH modulation could mediate the relationship between As and NB performance. Overall, we observed that the associations between WAs exposure and TH biomarkers were non-significant after accounting for covariates. However, the directions and trend of associations were consistent with our hypothesis. In an unadjusted model, we observed significant linear relationship between As exposure and TPOAb ($p=0.04$) when age, sex and BMI were not taken into account. On the other hand, significant positive associations between TPOAb and latencies (i.e. response time) in simple reaction time and symbol digit tests and significant negative association between TSH and match to sample count are suggestive of adverse effects of elevated TSH and TPOAb on specific neurobehavioral functions.

Experimental studies suggest that As has direct effect on the thyroid function through inhibition of enzymes involved in TH synthesis and inhibition of signaling at the level of receptor-mediated gene regulation. This latter effect is similar to the effect on the other nuclear hormone receptor superfamily members (Davey et al., 2007; Davey et al., 2008; Meeker et al., 2009; Palazzolo and Jansen, 2008). Evidences from several epidemiological studies are consistent with this mechanism. For instance, an Italian study found that UAs in

As exposed urban policemen was associated with increased TSH and decreased total T₃ and free T₄ when compared with rural outdoor workers (Ciarrocca et al., 2012). A pilot study on women who consumed fish contaminated with As also found negative correlations between blood As and plasma total T₃ and free T₄ (Meltzer et al., 2002) whereas in a cross-sectional study of US males there was a positive association between blood As and serum TSH after accounting for age, BMI and other covariates (Meeker et al., 2009). In an adult population in India (mean WAs exposure >202.7 ppb), significantly elevated thyroperoxidase autoantibodies were found in WAs-exposed group compared to the unexposed (Das et al., 2012). A study on rural Hispanic adults exposed to low WAs (i.e. 2-22 ppb) identified cumulative WAs as a significant predictor of hypothyroidism (based on TH levels and examination by physicians) after accounting for potential confounders (Gong et al., 2015). All these studies were conducted on adult participants. Our study is the first that examined the association between As exposure and TH in blood in an adolescent population. The specific age group has made our study unique as more TH are produced for rapid growth and sexual development during this life stage. The change in biological systems in adolescents makes them more susceptible to endocrine disrupting chemicals than the adults (McMullen et al., 2017).

There are several compelling reasons for considering adolescence age group for our study. Adolescence is an important period of brain development and maturation as both frontal and limbic regions of the brain demonstrate stable performance in cognitive functions at this development phase (de Graaf-Peters and Hadders-Algra, 2006; Spear, 2013). Although several subtle neurodevelopmental outcomes start to disappear after puberty (de Graaf-Peters and Hadders-Algra, 2006), some other functional deficits appear more significantly during brain maturation at middle or late adolescence (Hadders-Algra, 2002). Due to these reasons, neurobehavioral deficits measured during this critical window may be indicative of those during adulthood and beyond. In our study, we did not observe significant associations of total T₃, free T₄, and TSH with WAs, however, the directions of associations in the adjusted models are similar to the results of previous studies. Given that individual variations in serum TH, especially T₄ and T₃ in normal healthy subjects are relatively narrow in relation to the population variance (Andersen et al., 2002; Koulouri et al., 2013), TH levels in our sample might represent TH status at young age in the arsenic-exposed population in Bangladesh. Since all participants of the study had consistent WAs exposure, the concurrent WAs exposure also represented As exposure throughout all critical stages of child development.

Significant positive correlations between As and TPOAb in our study indicate a possible mechanism of As-induced thyroid disease, which was previously demonstrated by other environmental toxicants such as metals and polyhalogenated biphenyls (Brent, 2010; Kahn et al., 2014; Langer et al., 2007). Our data are also consistent with an adult study that demonstrated relationships between As exposure and autoimmune thyroid disease (Gong et al., 2015). Our study is the first to examine the possible effect of As on thyroid disease in adolescents by using the biomarker TPOAb. Although our study did not demonstrate significant associations between As and TPOAb after accounting for demographic covariates, perhaps due to a small sample size, the directions and magnitude of the associations still hinted at a potential effect of As in developing thyroid disease.

Sex is believed to influence TH concentrations although the precise age and sex dependent responses after exposure to hormone disruptors in the environment remain unclear. Several studies reported differential responses by the male and female participants (Clark et al., 2012; Roelfsema et al., 2009; Suzuki et al., 2012). Our data showed that adolescent boys had significantly higher total T₃ (b=0.26; p=0.03) and non-significantly higher free T₄ than the girls when As exposure is included in the models. Boys also exhibited lower TSH and TPOAb than the girls in adjusted models. Such relationships may be consistent with earlier studies that showed women to be more sensitive in TH action by expressing higher levels of TSH, and lower levels of T₃ and T₄ (Surks and Hollowell, 2007).

The findings of our study are not because of a greater proportion of adolescent participants with subclinical or overt hypothyroidism particularly in the high WAs exposure group. Only one out of 32 children had TSH above 10 µIU/ml and 27 children were within the most commonly used reference intervals of 0.4-4.0 µIU/ml (Chaker et al., 2017). Additionally, free T₄ ranged between 0.7-1.4 ng/dL is not indicative of a sample with hypothyroidism.

A body of literature suggests that As is a potent neurotoxicant. Arsenic easily crosses the placenta and is transferred to the fetus (Concha et al., 1998) and therefore As exposure appears to have strong neurocognitive effects (Tolins et al., 2014; Tyler and Allan, 2014). Among As-exposed pregnant women in Bangladesh, we have also found a significant association between maternal and cord blood As (Hall et al., 2007). We have already demonstrated adverse dose-response relationships between WAs and multiple measures of neurocognitive function including child intelligence, working memory and several other neuropsychological outcomes in the U.S. and Bangladeshi children (Parvez et al., 2011; Wasserman et al., 2014; Wasserman et al., 2007; Wasserman et al., 2004; Wasserman et al., 2011; Wasserman et al., 2016). These findings are consistent with our early studies in Bangladesh on both younger (i.e. 6-year old) and older children (10-year old) (Wasserman et al., 2007; Wasserman et al., 2004). Two of our more recent studies conducted in the same population found additional evidence in other neurobehavioral (NB) domains such as motor function and working memory in pre-adolescent children (Parvez et al., 2011; Wasserman et al., 2011). Despite consistent findings on associations between As and NB outcomes, the pathway via which As affects brain development remains unknown. Our objective was to explore if TH alteration by WAs is one of the possible pathways of As-induced neurotoxicity. For this purpose, we examined whether concurrent TH biomarkers in adolescents who have been consistently exposed to either high or low levels of WAs since birth are associated with neurocognitive functions at present time point.

The requirements for TH in the development and maturation of the central nervous system (CNS) extend from the pre-natal period to the middle of childhood (Rovet, 2014; Zoeller and Rovet, 2004). A very small change in maternal TH within reference ranges may be associated with up to a 10% decrease in IQ (i.e. approximately 5-10 IQ points) and other cognitive outcomes (Bath et al., 2013; Haddow et al., 1999; Henrichs et al., 2010; Li et al., 2010). TH is known to affect the development of the cerebral cortex, cerebellum, pons, striatum, thalamus, and basal ganglia (Gilbert et al., 2012), which control cognition, memory, learning, language development and motor function (Sanchez-Pena et al., 2010; Wang et al., 2009). Data from our study provide initial evidence that TH in adolescents may

predict their NB performance at present time, however larger studies are warranted to establish the relationship.

In addition to some environmental chemicals, several nutritional factors and chronic disease conditions in liver, kidney and cardiovascular system also limit thyroid function (Asvold et al., 2007; Hepner and Chopra, 1979; Pearce, 2012; Sarne, 2000; Schussler et al., 1978; Sun et al., 2015; Tarim, 2011; Ye et al., 2014). Deficiencies of micronutrients such as iodine, iron and selenium can have negative impacts on the thyroid system in children (Brix et al., 2011; Triggiani et al., 2009). Urinary iodine below 20 µg/L in pregnant women is correlated with lower or undetectable serum free T₄, total T₃ and higher TSH (Zimmermann, 2009; Zimmermann et al., 2008). Although we did not collect data on these covariates, future large-scale studies should include these potential effect modifiers and confounding variables to assess the As-TH relationship more accurately.

5. Potential Limitations

This was an exploratory study with a small sample size limiting the statistical power for examining exposure-outcome relationships and testing the mediation hypothesis. However, our objective was to obtain preliminary data providing hints of associations between As exposure, TH and neurodevelopmental outcomes. Our preliminary findings would lay the foundation for a larger study, which would eventually have adequate statistical power to test the potential mediation effects of TH biomarkers on As-induced neurotoxicity. Given that our sample size was small, it was therefore important to assess if the directions of association of As with TH biomarkers (positive direction with TSH and TPOAb, and negative direction with T₃ and T₄) were visible in our sample. Since directions of relationship in our preliminary data were consistent with the hypothesis our findings create the opportunity to eventually test the hypothesis regarding the TH-dependent neurotoxicity of As using a large sample size in future.

There are several methodological limitations in our study. Drinking water is the primary source of As exposure in this population and therefore, As in household well-water was used as an exposure variable in our analysis. Although seafood is not a major source of As in rural Bangladesh other dietary sources of arsenic such as rice and vegetables could contribute to total As exposure in this population (Kippler et al., 2016). The relative contribution of dietary As to overall As exposure can be more significant when the WAs concentration is below the Bangladesh As standard of 50 µg/L (Kile et al., 2007). To overcome this limitation, we have used UAs, a measure of internal exposure biomarker that takes contributions of both water and dietary As into account for the adolescent participants. Recent studies conducted in Bangladesh demonstrated that WAs remained as the primary and most important source of As in most rural households (Khan et al., 2015; Kile et al., 2007), which is also evident in our study sample due to a high and significant correlation between WAs and UAs. We have previously analyzed arsenic metabolites in HEALS participants in Araihaazar, and did not find arsenobetaine, a dietary source of As, a meaningful component of total UAs (Ahsan et al., 2007; Chen et al., 2011). The use of UAs, a biomarker of arsenic exposure also helped us minimize the limitation associated with undocumented information regarding multiple sources of water As. It was highly likely that

participants of this study might have been consuming As-containing water from school wells or other neighborhood wells.

Absence of a third category of As exposure between the WHO and Bangladesh standard (i.e. 10-50 µg/L of WAs) was another limitation. It would have been interesting to know if this specific range of moderate exposure had any influence on TH and neurobehavioral outcomes when compared with <10 µg/L of WAs. However, such category was not statistically meaningful as there were only 2 participants in this exposure range.

Lack of As speciation data is another weakness of the study. Both trivalent (As^{III}) and pentavalent (As^V) forms of iAs are present in drinking water in Bangladesh although As^{III} was identified as the dominant form in groundwater and rice samples (Halder et al., 2014; Zheng et al., 2004). In vertebrates, As^{III} and As^V undergo multiple oxidation and methylation reactions producing monomethylarsonic acid (MMA) and then dimethylarsinic acid (DMA), which are excreted via urine. MMA is considered more toxic and high MMA% and iAs% in biological samples were reported to be associated with increased risk of skin lesions, skin and bladder cancers and cardiovascular outcomes in adults (Ahsan et al., 2007; Gao et al., 2011; Huang et al., 2008; Kile et al., 2011; Pierce et al., 2013; Valenzuela et al., 2005). A small number of studies reported associations of elevated MMA% and reduced DMA% with developmental delays and poor health of school children in a dose-dependent manner (Chen et al., 2013; Hsieh et al., 2014; Hsueh et al., 2016). However, our two studies in Bangladeshi children did not find an effect of MMA% on intelligence even though associations were reported for total BAs and UAs (Wasserman et al., 2004; Wasserman et al., 2018). In future, we plan to investigate these possible effects of methylated As species on TH and neurodevelopmental outcomes.

The potential influence of other neurotoxic contaminants such as manganese (Mn) in drinking water in adolescent participants cannot be ruled out. We did not measure Mn in water or biological samples for the present study, which may be considered as a potential limitation. At the same time, it needs to be noted that our previous studies on younger HEALS children did not observe synergistic effect of simultaneous As and Mn exposures (i.e. Mn by As interactions) on multiple neurobehavioral outcomes (Wasserman et al., 2007; Wasserman et al., 2011). Therefore, any potential influence of water Mn on our findings was very unlikely.

Being a cross sectional study it hinders cause and effect inferences although we argue that the direction of the relation is exposure to outcome. It is very unlikely that the levels of TH biomarkers can influence arsenic exposure or NB outcomes can influence TH. Our future plan is to re-assess the associations using a larger sample size that would be sufficient enough to examine if TH can mediate the associations between WAs exposure and NB outcomes. Furthermore, we will continue to follow that larger cohort to examine NB performance longitudinally. Our study was conducted in families primarily with marginal economic condition living in rural Bangladesh and therefore results may be generalizable only to communities with similar rural sociodemographic characteristics.

Although the number of subjects included in this study was limited, it did allow us to perform analyses for blood levels of TH biomarkers on a single 96 well plate. As a result, there was no systematic bias in the relative levels of these analytes. That is, the absolute values are less important than the difference among subjects and this was not biased by plate differences in the assay.

Another potential limitation is the absence of data regarding iodine status among the participants. Iodine deficiency has a known impact on the thyroid system in children (Brix et al., 2011; Triggiani et al., 2009). In turn, iodine insufficiency is related to a number of neuropsychological outcomes in young age (Chen and Hetzel, 2010; Gilbert et al., 2012; Qian et al., 2005). Future studies should assess iodine status of the adolescents to examine the effect of this nutritional factor on the associations between As, TH and NB outcomes.

In vitro and *in vivo* observations indicate that As may interfere with TH action in a tissue-specific manner (Davey et al., 2007; Davey et al., 2008; Meeker et al., 2009; Palazzolo and Jansen, 2008). Because of the effect of As on TH metabolism and receptor activation, it is possible that As interferes directly with mechanisms of TH actions in target tissues even in the absence of change in these hormone biomarkers under a variety of physiologic conditions (Salvatore et al., 2014; Watanabe et al., 2006). Therefore, it is possible that serum TH may not necessarily predict TH action in tissues. Although our findings are suggestive of As-induced TH change in blood, tissue-specific disruption of TH by WAs is also possible, which may in turn lead to decline of NB functions. This alternative mechanism, however, was beyond the scope of this project.

6. Conclusion

Overall, data from our study hinted possible TH disruption in childhood by geogenic As. Significant correlations of both WAs and UAs with TPOAb, non-significant positive relationships of As and with TPOAb and TSH, and negative relationship with free T₄ in the adjusted statistical models also emphasized the need of larger epidemiological studies for conclusive evidence about these associations. Furthermore, significant associations of TSH and TPOAb with a number of NB outcomes are consistent with our hypothesis. From risk prevention point of view, this is very important, because thyroid hormones play vital roles in determining the functions of the brain and therefore, insults in early life may have adverse effects on brain development in adulthood. Our future plan is to conduct epidemiological studies with larger samples to examine As-induced neurotoxicity mediated by TH disruption in early life. This line of research may provide a model that can be extended to additional risk factors for the brain, as well as for many other disorders aggravated by thyroid disrupting chemicals. If As-induced TH disruption is elucidated as a possible mechanism of neurotoxicity, it will help develop policies or therapeutic options to minimize early life adverse effects in the developing central nervous system.

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References

- Abdel Rasoul GM, Abou Salem ME, Mechaal AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. *Neurotoxicology* 2008; 29: 833–8. [PubMed: 18662718]
- Ahsan H, Chen Y, Kibriya MG, Slavkovich V, Parvez F, Jasmine F, et al. Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1270–8. [PubMed: 17548696]
- Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab* 2002; 87: 1068–72. [PubMed: 11889165]
- Asvold BO, Bjoro T, Nilsen TI, Vatten LJ. Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population-based study. *J Clin Endocrinol Metab* 2007; 92: 841–5. [PubMed: 17200168]
- Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Lancet* 2013; 382: 331–337. [PubMed: 23706508]
- Biswas A, Nath B, Bhattacharya P, Haider D, Kundu AK, Mandal U, et al. Hydrogeochemical contrast between brown and grey sand aquifers in shallow depth of Bengal Basin: Consequences for sustainable drinking water supply. *Science of the Total Environment* 2012; 431: 402–412. [PubMed: 22706147]
- Brent GA. Environmental Exposures and Autoimmune Thyroid Disease. *Thyroid* 2010; 20: 755–761. [PubMed: 20578899]
- Brix K, Fuhrer D, Biebertmann H. Molecules important for thyroid hormone synthesis and action - known facts and future perspectives. *Thyroid Res* 2011; 4 Suppl 1: S9. [PubMed: 21835056]
- Butler-Dawson J, Galvin K, Thorne PS, Rohlman DS. Organophosphorus pesticide exposure and neurobehavioral performance in Latino children living in an orchard community. *Neurotoxicology* 2016; 53: 165–72. [PubMed: 26820522]
- Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. *Lancet* 2017; 390: 1550–1562. [PubMed: 28336049]
- Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, et al. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ* 2011; 342: d2431. [PubMed: 21546419]
- Chen Y, Hall M, Graziano JH, Slavkovich V, van Geen A, Parvez F, et al. A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 207–13. [PubMed: 17301251]
- Chen Y, Wu F, Graziano JH, Parvez F, Liu M, Paul RR, et al. Arsenic exposure from drinking water, arsenic methylation capacity, and carotid intima-media thickness in Bangladesh. *Am J Epidemiol* 2013; 178: 372–81. [PubMed: 23788675]
- Chen ZP, Hetzel BS. Cretinism revisited. *Best Pract Res Clin Endocrinol Metab* 2010; 24: 39–50. [PubMed: 20172469]
- Cheng Z, Zheng Y, Mortlock R, Van Geen A. Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry. *Anal Bioanal Chem* 2004; 379: 512–8. [PubMed: 15098084]
- Ciarrocca M, Tomei F, Caciari T, Cetica C, Andre JC, Fiaschetti M, et al. Exposure to arsenic in urban and rural areas and effects on thyroid hormones. *Inhal Toxicol* 2012; 24: 589–98. [PubMed: 22861002]

- Clark PM, Holder RL, Haque SM, Hobbs FD, Roberts LM, Franklyn JA. The relationship between serum TSH and free T4 in older people. *Postgrad Med J* 2012; 88: 668–70. [PubMed: 23097057]
- Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. Exposure to inorganic arsenic metabolites during early human development. *Toxicol Sci* 1998; 44: 185–90. [PubMed: 9742656]
- Das N, Paul S, Chatterjee D, Banerjee N, Majumder NS, Sarma N, et al. Arsenic exposure through drinking water increases the risk of liver and cardiovascular diseases in the population of West Bengal, India. *Bmc Public Health* 2012; 12. [PubMed: 22221851]
- Davey JC, Bodwell JE, Gosse JA, Hamilton JW. Arsenic as an endocrine disruptor: effects of arsenic on estrogen receptor-mediated gene expression in vivo and in cell culture. *Toxicol Sci* 2007; 98: 75–86. [PubMed: 17283378]
- Davey JC, Nomikos AP, Wungjiranirun M, Sherman JR, Ingram L, Batki C, et al. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor-and thyroid hormone receptor-mediated gene regulation and thyroid hormone-mediated amphibian tail metamorphosis. *Environ Health Perspect* 2008; 116: 165–72. [PubMed: 18288313]
- de Graaf-Peters VB, Hadders-Algra M. Ontogeny of the human central nervous system: what is happening when? *Early Hum Dev* 2006; 82: 257–66. [PubMed: 16360292]
- Felfe C, Hsin A. Maternal work conditions and child development. *Econ Educ Rev* 2012; 31: 1037–1057. [PubMed: 27642208]
- Frisbie SH, Mitchell EJ, Mastera LJ, Maynard DM, Yusuf AZ, Siddiq MY, et al. Public health strategies for western Bangladesh that address arsenic, manganese, uranium, and other toxic elements in drinking water. *Environ Health Perspect* 2009; 117: 410–6. [PubMed: 19337516]
- Gao J, Yu J, Yang L. Urinary arsenic metabolites of subjects exposed to elevated arsenic present in coal in Shaanxi Province, China. *Int J Environ Res Public Health* 2011; 8: 1991–2008. [PubMed: 21776214]
- Gilbert ME, Rovet J, Chen Z, Koibuchi N. Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. *Neurotoxicology* 2012; 33: 842–52. [PubMed: 22138353]
- Gong G, Basom J, Mattevada S, Onger F. Association of hypothyroidism with low-level arsenic exposure in rural West Texas. *Environmental Research* 2015; 138: 154–160. [PubMed: 25721242]
- Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. *Dev Med Child Neurol* 2002; 44: 561–71. [PubMed: 12206624]
- Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New England Journal of Medicine* 1999; 341: 549–555. [PubMed: 10451459]
- Halder D, Bhowmick S, Biswas A, Chatterjee D, Nriagu J, Guha Mazumder DN, et al. Risk of arsenic exposure from drinking water and dietary components: implications for risk management in rural Bengal. *Environ Sci Technol* 2013; 47: 1120–7. [PubMed: 23198808]
- Halder D, Bhowmick S, Biswas A, Mandal U, Nriagu J, Mazumdar DNG, et al. Consumption of Brown Rice: A Potential Pathway for Arsenic Exposure in Rural Bengal. *Environmental Science & Technology* 2012; 46: 4142–4148. [PubMed: 22352724]
- Halder D, Biswas A, Slejkovec Z, Chatterjee D, Nriagu J, Jacks G, et al. Arsenic species in raw and cooked rice: implications for human health in rural Bengal. *Sci Total Environ* 2014; 497–498: 200–208. [PubMed: 25129156]
- Hall M, Gamble M, Slavkovich V, Liu X, Levy D, Cheng Z, et al. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. *Environ Health Perspect* 2007; 115: 1503–9. [PubMed: 17938743]
- Heinegard D, GunnarTiderstrom. Determination of serum creatinine by a direct colorimetric method. *Clinica Chimica Acta* 1973; 43: 305–310.
- Henrichs J, Bongers-Schokking JJ, Schenk JJ, Ghassabian A, Schmidt HG, Visser TJ, et al. Maternal Thyroid Function during Early Pregnancy and Cognitive Functioning in Early Childhood: The Generation R Study. *Journal of Clinical Endocrinology & Metabolism* 2010; 95: 4227–4234. [PubMed: 20534757]

- Hepner GW, Chopra IJ. Serum thyroid hormone levels in patients with liver disease. *Arch Intern Med* 1979; 139: 1117–20. [PubMed: 485743]
- Hsieh RL, Huang YL, Shiue HS, Huang SR, Lin MI, Mu SC, et al. Arsenic methylation capacity and developmental delay in preschool children in Taiwan. *Int J Hyg Environ Health* 2014; 217: 678–86. [PubMed: 24698386]
- Hsueh YM, Chen WJ, Lee CY, Chien SN, Shiue HS, Huang SR, et al. Association of Arsenic Methylation Capacity with Developmental Delays and Health Status in Children: A Prospective Case-Control Trial. *Sci Rep* 2016; 6: 37287. [PubMed: 27853293]
- Huang YK, Huang YL, Hsueh YM, Yang MH, Wu MM, Chen SY, et al. Arsenic exposure, urinary arsenic speciation, and the incidence of urothelial carcinoma: a twelve-year follow-up study. *Cancer Causes Control* 2008; 19: 829–39. [PubMed: 18351295]
- Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic Exposure and Toxicology: A Historical Perspective. *Toxicol Sci* 2011.
- Ismail A, GA GR, Bonner MR, Hendy OM, Lasarev M, Khan K. Longitudinal assessment of exposures, symptoms and neurobehavioral performance in Egyptian adolescent pesticide applicators. 10th anniversary symposium for brain directors for the brain disorders in the developing world: Research across the life span Bethesda, MD, 2014 2014.
- Kahn LG, Liu XH, Rajovic B, Popovac D, Oberfield S, Graziano JH, et al. Blood Lead Concentration and Thyroid Function during Pregnancy: Results from the Yugoslavia Prospective Study of Environmental Lead Exposure. *Environmental Health Perspectives* 2014; 122:1134–1140. [PubMed: 24866691]
- Khan K, Strader S, Rohlman D. Evaluation of technology-based intervention to increase the use of hearing protections among the high school-going farmworkers in Iowa. Manuscript submitted for publication. 2015.
- Khan KM, Weigel MM, Yonts S, Rohlman D, Armijos R. Residential exposure to urban traffic is associated with the poorer neurobehavioral health of Ecuadorian schoolchildren. *Neurotoxicology* 2019; 73: 31–39. [PubMed: 30826345]
- Kile ML, Hoffman E, Rodrigues EG, Breton CV, Quamruzzaman Q, Rahman M, et al. A pathway-based analysis of urinary arsenic metabolites and skin lesions. *Am J Epidemiol* 2011; 173: 778–86. [PubMed: 21378128]
- Kile ML, Houseman EA, Breton CV, Smith T, Quamruzzaman Q, Rahman M, et al. Dietary arsenic exposure in bangladesh. *Environ Health Perspect* 2007; 115: 889–93. [PubMed: 17589596]
- Kippler M, Skroder H, Rahman SM, Tofail F, Vahter M. Elevated childhood exposure to arsenic despite reduced drinking water concentrations--A longitudinal cohort study in rural Bangladesh. *Environ Int* 2016; 86: 119–25. [PubMed: 26580026]
- Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. *Best Pract Res Clin Endocrinol Metab* 2013; 27: 745–62. [PubMed: 24275187]
- Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, et al. Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. *Chemosphere* 2007; 69: 118–127. [PubMed: 17537484]
- Leist AK, Glymour MM, Mackenbach JP, van Lenthe FJ, Avendano M. Time away from work predicts later cognitive function: differences by activity during leave. *Ann Epidemiol* 2013; 23: 455–62. [PubMed: 23889855]
- Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, et al. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25–30 months. *Clinical Endocrinology* 2010; 72: 825–829. [PubMed: 19878506]
- Lu C, Essig C, Root C, Rohlman DS, McDonald T, Sulzbacher S. Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. *Int J Adolesc Med Health* 2009; 21: 609–21. [PubMed: 20306773]
- McMullen J, Ghassabian A, Kohn B, Trasande L. Identifying Subpopulations Vulnerable to the Thyroid-Blocking Effects of Perchlorate and Thiocyanate. *J Clin Endocrinol Metab* 2017; 102: 2637–2645. [PubMed: 28430972]

- Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, et al. Multiple metals predict prolactin and thyrotropin (TSH) levels in men. *Environ Res* 2009; 109: 869–73. [PubMed: 19595304]
- Meltzer HM, Maage A, Ydersbond TA, Haug E, Glatte E, Holm H. Fish arsenic may influence human blood arsenic, selenium, and T4:T3 ratio. *Biol Trace Elem Res* 2002; 90: 83–98. [PubMed: 12666828]
- Molin M, Ulven SM, Dahl L, Lundebye AK, Holck M, Alexander J, et al. Arsenic in seafood is associated with increased thyroid-stimulating hormone (TSH) in healthy volunteers - A randomized controlled trial. *J Trace Elem Med Biol* 2017; 44: 1–7. [PubMed: 28965562]
- Nixon DE, Mussmann GV, Eckdahl SJ, Moyer TP. Total Arsenic in Urine - Palladium Persulfate Vs Nickel as a Matrix Modifier for Graphite-Furnace Atomic-Absorption Spectrophotometry. *Clinical Chemistry* 1991; 37: 1575–1579. [PubMed: 1893592]
- Palazzolo DL, Jansen KP. The minimal arsenic concentration required to inhibit the activity of thyroid peroxidase activity in vitro. *Biol Trace Elem Res* 2008; 126: 49–55. [PubMed: 18685814]
- Parvez F, Chen Y, Brandt-Rauf PW, Slavkovich V, Islam T, Ahmed A, et al. A prospective study of respiratory symptoms associated with chronic arsenic exposure in Bangladesh: findings from the Health Effects of Arsenic Longitudinal Study (HEALS). *Thorax* 2010; 65: 528–33. [PubMed: 20522851]
- Parvez F, Wasserman GA, Factor-Litvak P, Liu X, Slavkovich V, Siddique AB, et al. Arsenic exposure and motor function among children in Bangladesh. *Environ Health Perspect* 2011; 119: 1665–70. [PubMed: 21742576]
- Patra K, Greene MM, Patel AL, Meier P. Maternal Education Level Predicts Cognitive, Language, and Motor Outcome in Preterm Infants in the Second Year of Life. *Am J Perinatol* 2016; 33: 738–44. [PubMed: 26890439]
- Pearce EN. Update in lipid alterations in subclinical hypothyroidism. *J Clin Endocrinol Metab* 2012; 97: 326–33. [PubMed: 22205712]
- Pierce BL, Tong L, Argos M, Gao J, Farzana J, Roy S, et al. Arsenic metabolism efficiency has a causal role in arsenic toxicity: Mendelian randomization and gene-environment interaction. *Int J Epidemiol* 2013; 42: 1862–71. [PubMed: 24536095]
- Qian M, Wang D, Watkins WE, Gebiski V, Yan YQ, Li M, et al. The effects of iodine on intelligence in children: a meta-analysis of studies conducted in China. *Asia Pac J Clin Nutr* 2005; 14: 32–42. [PubMed: 15734706]
- Raessler M The Arsenic Contamination of Drinking and Groundwaters in Bangladesh: Featuring Biogeochemical Aspects and Implications on Public Health. *Arch Environ Contam Toxicol* 2018; 75: 1–7. [PubMed: 29520432]
- Rahman MM, Asaduzzaman M, Naidu R. Consumption of arsenic and other elements from vegetables and drinking water from an arsenic-contaminated area of Bangladesh. *J Hazard Mater* 2013; 262: 1056–63. [PubMed: 22939573]
- Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, Alguacil J, Gil F, Gonzalez-Alzaga B, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci Total Environ* 2013; 454–455: 562–77. [PubMed: 23570911]
- Roelfsema F, Pereira AM, Veldhuis JD, Adriaanse R, Endert E, Fliers E, et al. Thyrotropin secretion profiles are not different in men and women. *J Clin Endocrinol Metab* 2009; 94: 3964–7. [PubMed: 19773394]
- Rohittrattana J, Siri Wong W, Suittwan P, Robson MG, Strickland PO, Rohlman DS, et al. Adaptation of a neurobehavioral test battery for Thai children. *Rocz Panstw Zakl Hig* 2014; 65:205–12. [PubMed: 25247800]
- Rohlman DS, Arcury TA, Quandt SA, Lasarev M, Rothlein J, Travers R, et al. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *Neurotoxicology* 2005; 26: 589–598. [PubMed: 16112324]
- Rohlman DS, Bailey SR, Anger WK, McCauley L. Assessment of neurobehavioral function with computerized tests in a population of hispanic adolescents working in agriculture. *Environ Res* 2001; 85: 14–24. [PubMed: 11161647]

- Rohlman DS, Bodner T, Arcury TA, Quandt SA, McCauley L. Developing methods for assessing neurotoxic effects in Hispanic non-English speaking children. *Neurotoxicology* 2007a; 28: 240–4. [PubMed: 16759705]
- Rohlman DS, Gimenes LS, Eckerman DA, Kang SK, Farahat FM, Anger WK. Development of the Behavioral Assessment and Research System (BARS) to detect and characterize neurotoxicity in humans. *Neurotoxicology* 2003; 24: 523–31. [PubMed: 12900065]
- Rohlman DS, Ismail AA, Abdel-Rasoul G, Lasarev M, Hendy O, Olson JR. Characterizing exposures and neurobehavioral performance in Egyptian adolescent pesticide applicators. *Metab Brain Dis* 2014; 29: 845–55. [PubMed: 24833556]
- Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology* 2007b; 28: 374–80. [PubMed: 17141876]
- Ronfani L, Vecchi Brumatti L, Mariuz M, Tognin V, Bin M, Ferluga V, et al. The Complex Interaction between Home Environment, Socioeconomic Status, Maternal IQ and Early Child Neurocognitive Development: A Multivariate Analysis of Data Collected in a Newborn Cohort Study. *PLoS One* 2015; 10: e0127052. [PubMed: 25996934]
- Rovet JF. The role of thyroid hormones for brain development and cognitive function. *Endocr Dev* 2014; 26: 26–43. [PubMed: 25231442]
- Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle--new insights and potential implications. *Nat Rev Endocrinol* 2014; 10: 206–14. [PubMed: 24322650]
- Sanchez-Pena LC, Petrosyan P, Morales M, Gonzalez NB, Gutierrez-Ospina G, Del Razo LM, et al. Arsenic species, AS3MT amount, and AS3MT gene expression in different brain regions of mouse exposed to arsenite. *Environ Res* 2010; 110: 428–34. [PubMed: 20138265]
- Sander MC, Lindenberger U, Werkle-Bergner M. Lifespan age differences in working memory: a two-component framework. *Neurosci Biobehav Rev* 2012; 36: 2007–33. [PubMed: 22771333]
- Sarne D Effects of the Environment, Chemicals and Drugs on Thyroid Function. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, et al., editors. *Endotext*, South Dartmouth (MA), 2000.
- Schussler GC, Schaffner F, Korn F. Increased serum thyroid hormone binding and decreased free hormone in chronic active liver disease. *N Engl J Med* 1978; 299: 510–5. [PubMed: 98709]
- Shankar S, Shanker U, Shikha. Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. *ScientificWorldJournal* 2014; 2014:304524. [PubMed: 25374935]
- Spear LP. Adolescent neurodevelopment. *J Adolesc Health* 2013; 52: S7–13. [PubMed: 23332574]
- Sun X, Sun Y, Li WC, Chen CY, Chiu YH, Chien HY, et al. Association of thyroid-stimulating hormone and cardiovascular risk factors. *Intern Med* 2015; 54: 2537–44. [PubMed: 26466686]
- Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. *J Clin Endocrinol Metab* 2007; 92: 4575–82. [PubMed: 17911171]
- Suzuki S, Nishio S, Takeda T, Komatsu M. Gender-specific regulation of response to thyroid hormone in aging. *Thyroid Res* 2012; 5: 1. [PubMed: 22280879]
- Tarim O Thyroid hormones and growth in health and disease. *J Clin Res Pediatr Endocrinol* 2011; 3: 51–5. [PubMed: 21750631]
- Tolins M, Ruchirawat M, Landrigan P. The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. *Ann Glob Health* 2014; 80: 303–14. [PubMed: 25459332]
- Triggiani V, Tafaro E, Giagulli VA, Sabba C, Resta F, Licchelli B, et al. Role of Iodine, Selenium and Other Micronutrients in Thyroid Function and Disorders. *Endocr Metab Immune Disord Drug Targets* 2009.
- Tyler CR, Allan AM. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. *Curr Environ Health Rep* 2014; 1: 132–147. [PubMed: 24860722]

- Valenzuela OL, Borja-Aburto VH, Garcia-Vargas GG, Cruz-Gonzalez MB, Garcia-Montalvo EA, Calderon-Aranda ES, et al. Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic. *Environ Health Perspect* 2005; 113: 250–4. [PubMed: 15743710]
- Van Geen A, Cheng Z, Seddique AA, Hoque MA, Gelman A, Graziano JH, et al. Reliability of a commercial kit to test groundwater for arsenic in Bangladesh. *Environ Sci Technol* 2005; 39: 299–303. [PubMed: 15667109]
- von Bromssen M, Jakariya M, Bhattacharya P, Ahmed KM, Hasan MA, Sracek O, et al. Targeting low-arsenic aquifers in Matlab Upazila, Southeastern Bangladesh. *Science of the Total Environment* 2007; 379: 121–132. [PubMed: 17113133]
- Wang Y, Li S, Piao F, Hong Y, Liu P, Zhao Y. Arsenic down-regulates the expression of Camk4, an important gene related to cerebellar LTD in mice. *Neurotoxicol Teratol* 2009; 31: 318–22. [PubMed: 19410645]
- Wasserman GA, Liu X, Loiacono NJ, Kline J, Factor-Litvak P, van Geen A, et al. A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. *Environ Health* 2014; 13: 23. [PubMed: 24684736]
- Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline J, et al. Water arsenic exposure and intellectual function in 6-year-old children in Araihaaz, Bangladesh. *Environ Health Perspect* 2007; 115: 285–9. [PubMed: 17384779]
- Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, et al. Water arsenic exposure and children's intellectual function in Araihaaz, Bangladesh. *Environ Health Perspect* 2004; 112: 1329–33. [PubMed: 15345348]
- Wasserman GA, Liu X, Parvez F, Chen Y, Factor-Litvak P, Loiacono NJ, et al. A cross-sectional study of water arsenic exposure and intellectual function in adolescence in Araihaaz, Bangladesh. *Environ Int* 2018; 118: 304–313. [PubMed: 29933234]
- Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Ahsan H, Levy D, et al. Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology* 2011; 32: 450–7. [PubMed: 21453724]
- Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Kline J, Siddique AB, et al. Child Intelligence and Reductions in Water Arsenic and Manganese: A Two-Year Follow-up Study in Bangladesh. *Environ Health Perspect* 2016; 124: 1114–20. [PubMed: 26713676]
- Watanabe M, Houten SM, Matakaki C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006; 439: 484–9. [PubMed: 16400329]
- Wilson D, Hooper C, Shi X. Arsenic and lead in juice: apple, citrus, and apple-base. *J Environ Health* 2012; 75: 14–20; quiz 44.
- Ye Y, Xie H, Zeng Y, Zhao X, Tian Z, Zhang S. Association between subclinical hypothyroidism and blood pressure - a meta-analysis of observational studies. *Endocr Pract* 2014; 20: 150–8. [PubMed: 24014003]
- Zheng Y, Stute M, van Geen A, Gavrieli I, Dhar R, Simpson HJ, et al. Redox control of arsenic mobilization in Bangladesh groundwater. *Applied Geochemistry* 2004; 19: 201–214.
- Zimmermann MB. Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: a review. *Am J Clin Nutr* 2009; 89: 668S–72S. [PubMed: 19088150]
- Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. *Lancet* 2008; 372: 1251–62. [PubMed: 18676011]
- Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol* 2004; 16: 809–18. [PubMed: 15500540]

Highlights

- Arsenic in groundwater appeared to affect thyroperoxidase antibodies (TPOAb).
- TPOAb was positively associated with latencies in two neurocognitive outcomes.
- Thyrotropin (TSH) was negatively associated with correct match-to-sample count.

Figure 1.

Concentrations of As in tubewells of HEALS participants in Araihaazar, Bangladesh

Note: This should be a single-column fitting image

Table 1.

Sample characteristics by Water Arsenic (WAs) exposure categories (n=32)

Characteristics	Low lifetime WAs exposure group (n=16) Mean $\pm$ SD or Number (%)	High lifetime WAs exposure group (n=16) Mean $\pm$ SD or Number (%)	p-values
Age (years)	16.75 $\pm$ 0.63	16.54 $\pm$ 0.72	0.26
Child BMI (kg/m ²)	19.40 $\pm$ 3.10	19.62 $\pm$ 3.23	0.39
Blood Pressure			
Systolic (mmHg)	111.40 $\pm$ 11.94	106.40 $\pm$ 12.54	0.16
Diastolic (mmHg)	69.20 $\pm$ 9.76	67.00 $\pm$ 10.01	0.44
Maternal education			
No education	10 (62.5)	8 (50.0)	0.48
Elementary school or above	6 (37.5)	8 (50.0)	
Paternal education			
No education	7 (43.8)	7 (43.8)	1.00
Elementary school or above	9 (56.2)	9 (56.2)	
Sex			
Boys	8 (50.0)	8 (50.0)	1.00
Girls	8 (50.0)	8 (50.0)	
Television in house	6 (37.5)	7(43.8)	0.72
House constructions (roof)			
Corrugate/Biomass	16 (100.0)	15 (93.8)	0.33
Concrete	0 (0.0)	1 (6.2)	
WAs (μ g/L)	5.16 $\pm$ 0.80	144.04 $\pm$ 83.46	<0.001
UAs (μ g/g Creatinine)	66.51 $\pm$ 52.27	328.36 $\pm$ 220.47	<0.001
TSH (μ IU/ml)	2.79 $\pm$ 1.02	3.92 $\pm$ 3.10	0.18
Total T ₃ (ng/dl)	108.30 $\pm$ 26.26	100.83 $\pm$ 27.42	0.43
Free T ₄ (ng/dl)	1.01 $\pm$ 0.09	1.00 $\pm$ 0.16	0.89
TPOAb (IU/ml)	32.32 $\pm$ 61.96	47.04 $\pm$ 89.73	0.65
SRT latency (ms)	372.10 $\pm$ 56.21	433.62 $\pm$ 96.22	0.05
SDT latency (ms)	2746.63 $\pm$ 621.09	2922.75 $\pm$ 444.58	0.36
MTS latency (ms)	3839.94 $\pm$ 609.89	3877.96 $\pm$ 625.48	0.85
CPT latency (ms)	399.70 $\pm$ 38.06	417.50 $\pm$ 36.28	0.09
MTS correct count	17.95 $\pm$ 1.61	17.65 $\pm$ 1.88	0.76

Characteristics	Low lifetime WAs exposure group (n=16) Mean $\pm$ SD or Number (%)	High lifetime WAs exposure group (n=16) Mean $\pm$ SD or Number (%)	p-values
FTT (preferred) count	60.06 $\pm$ 17.58	46.25 $\pm$ 18.55	0.05
FTT (non-preferred) count	61.32 $\pm$ 23.94	45.56 $\pm$ 18.77	0.09

Table 2:

Estimated regression coefficient B (standard error se) for the association between As exposure and thyroid hormone biomarkers, with adjustment for age, sex and BMI

Regression Models	Explanatory variables in the models	Total T ₃ (ng/dl) B (95% CI)	Free T ₄ (ng/dL) B (95% CI)	TSH (μIU/mL) B (95% CI)	TPOAb (IU/mL) B (95% CI)
Model 1 ^a	Log UAs (μg/g Cr)	-0.02 (-0.09, 0.06)	-0.19 (-0.08, 0.41)	0.64 (-0.16, 0.14)	32.12 (-15.55, 82.12)
	Age (yrs)	0.16 [*] (0.04, 0.27)	-0.58 (-1.57, 0.40)	-0.89 (-2.24, 0.47)	-12.49 (-96.03, 71.06)
	Boys (compared to girls)	0.26 [*] (0.08, 0.38)	0.62 (-0.60, 1.80)	-0.55 (-2.22, 1.12)	-71.80 (-171.50, -30.90)
	BMI (kg/m ²)	-0.05 [*] (-0.08, -0.02)	-0.04 (-0.28, 0.20)	0.24 (-0.09, 0.56)	21.87 [*] (2.72, 31.00)
Model 2 ^b	Log WAs (μg/L)	-0.02 (-0.07, 0.01)	-0.17 (-0.52, 0.16)	0.22 (-0.27, 0.53)	16.62 (-12.40, 36.87)
	Age (yrs)	0.15 [*] (0.05, 0.28)	-0.62 (-1.16, 0.36)	-0.88 (-2.29, 0.53)	-10.44 (-65.43, 74.25)
	Boys (compared to girls)	0.24 [*] (0.08, 0.39)	0.70 (-0.49, 1.80)	-0.78 (-2.46, 0.92)	-83.57 (-185.75, 18.63)
	BMI (kg/m ²)	-0.05 [*] (-0.07, -0.02)	-0.05 (-0.28, 0.19)	0.24 (-0.10, 0.56)	21.57 [*] (2.30, 41.84)

* p<0.05;

^aModel 1 was constructed for log-transformed urinary As (adjusted for creatinine) as an exposure variable, whereas

^bModel 2 was constructed for log-transformed water As as an exposure variable; same covariates were used in both models.

Table 3:

The association between neurobehavioral outcomes and thyroid hormone biomarkers, with adjustment for age and sex.

BARS Neurobehavioral tests		Thyroid function biomarkers (log-transformed) as explanatory variables			
Test names	Outcomes measured	Total T ₃ (ng/mL) B (95% CI)	Free T ₄ (pmol/L) B (95% CI)	TSH (μIU/mL) B (95% CI)	TPOAb (μIU/mL) B (95% CI)
<i>Higher latency in outcome variable indicates negative effects on brain function</i>					
Simple Reaction Time (SRT)	SRT latency (ms)	−53.34 (−229.21, 114.81)	−63.20 (−310.62, 240.25)	29.49 (−29.84, 96.19)	82.58*** (53.65, 106.36)
Symbol Digit Test (SDT)	SDT latency (ms)	121.80 (−847.26, 762.86)	−801.20 (−362.85, 854.34)	−25.63 (−230.86, 191.87)	276.85** (85.41, 439.44)
Match to Sample Test (MTS)	MTS latency (ms)	−452.73 (−939.20, 355.62)	−224.47 (−2123.35, 1395.34)	180.86 (−118.08, 437.47)	168.81 (−26.68, 391.83)
Continuous Performance Test (CPT)	CPT latency (ms)	−23.00 (−73.62, 42.67)	113.15 (6.68, 212.45)	7.60 (−14.07, 27.82)	3.19 (−11.02, 16.50)
<i>Lower count in outcome variable indicates negative effects on the brain</i>					
Match to Sample Test (MTS)	MTS correct count	1.22 (−0.99, 4.38)	1.51 (−3.24, 7.25)	−0.95* (−1.95, −0.21)	0.04 (−0.68, 0.59)
Finger Tapping Test (FTT)	FTT preferred hand (number of taps)	0.81 (−33.45, 40.98)	29.93 (−36.09, 103.69)	−1.77 (−15.42, 10.50)	−5.30 (−12.51, 1.88)
Finger Tapping Test (FTT)	FTT non-preferred hand (number of taps)	3.12 (−20.55, 30.43)	22.58 (−32.38, 82.21)	−0.29 (−13.88, 11.83)	−5.36 (−13.78, 1.68)

p<0.001,

**
p=0.005,

*
p=0.05