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Neurobehavioral effects of arsenic exposure among secondary school children in the Kandal Province, Cambodia

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ABSTRACT

The research was carried out at 3 study sites with varying groundwater arsenic (As) levels in the Kandal Province of Cambodia. Kampong Kong Commune was chosen as a highly contaminated site (300–500 μg/L), Svay Romiet Commune was chosen as a moderately contaminated site (50–300 μg/L) and Anlong Romiet Commune was chosen as a control site. Neurobehavioral tests on the 3 exposure groups were conducted using a modified WHO neurobehavioral core test battery. Seven neurobehavioral tests including digit symbol, digit span, Santa Ana manual dexterity, Benton visual retention, pursuit aiming, trail making and simple reaction time were applied. Children's hair samples were also collected to investigate the influence of hair As levels on the neurobehavioral test scores. The results from the inductively coupled plasma-mass spectrometry (ICP-MS) analyses of hair samples showed that hair As levels at the 3 study sites were significantly different ($p<0.001$), whereby hair samples from the highly contaminated site ($n=157$) had a median hair As level of 0.93 μg/g, while the moderately contaminated site ($n=151$) had a median hair As level of 0.22 μg/g, and the control site ($n=214$) had a median hair As level of 0.08 μg/g. There were significant differences among the 3 study sites for all the neurobehavioral tests scores, except for digit span (backward) test. Multiple linear regression clearly shows a positive significant influence of hair As levels on all the neurobehavioral test scores, except for digit span (backward) test, after controlling for hair lead (Pb), manganese (Mn) and cadmium (Cd). Children with high hair As levels experienced 1.57–4.67 times greater risk of having lower neurobehavioral test scores compared to those with low hair As levels, after adjusting for hair Pb, Mn and Cd levels and BMI status. In conclusion, arsenic-exposed school children from the Kandal Province of Cambodia with a median hair As level of 0.93 μg/g among those from the highly contaminated study site, showed clear evidence of neurobehavioral effects.

1. Introduction

There are thousands of dugs and tube wells being built in Cambodia every year. Well water is in common use throughout Cambodia in order to provide rural Cambodians with groundwater for drinking, cleaning, bathing and agricultural activities. Unfortunately, groundwater in Cambodia is contaminated with naturally occurring arsenic. Arsenic is a ubiquitous element found in the atmosphere, soils, rocks, and natural waters. It is mobilized in the environment through a combination of natural processes such as weathering and anthropogenic activities (Acharyya, 2002). Arsenic from various sources in the environment and drinking water can pose a great threat to human health.

Arsenic is a well-known neurotoxicant that affects neurobehavioral development (Tsai et al., 2003), as well as a dermatotoxicant (Bimal et al., 2006). Various animal studies using different species have explored the mechanism involved in arsenic mediated developmental toxicity. Rodriguez et al. (2002) showed that rats exposed to 36.7 mg/L of arsenic in drinking water during development present deficits in spontaneous locomotors activity and alterations in a spatial learning task. Another study on pregnant rats injected with 30 or 40 mg/kg of arsenic and their off springs showed increased incidence of neural tube, eye, skeletal, renal, and gonadal defects (Beaudoin and Fisher, 1981). Several studies on humans reported that arsenic exposure was associated with neuropsychological impairments including poor cognitive performance and disturbances in visual perception, psychomotor

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speed, attention, speech and memory (Rosado et al., 2007; Bolla-Wilson and Bleeker 1987). Morton and Caron (2007) reported that neurotoxicity due to acute and chronic arsenic exposure in adults and children are prevalent.

Children worldwide are exposed to arsenic in drinking water at concentrations that exceed the guideline recommended by the World Health Organization and the US. Environmental Protection Agency, which is a maximum arsenic level of 10 μg/L or ppb (WHO, 2004). Children are particularly at risk of high exposure in arsenic-affected areas of South Asia such as West Bengal in India (UNICEF, 2004). Arsenic poisoning related to occupational exposure causes central nervous system alterations, including impairments of recent memory, learning, and concentration (Bolla-Wilson, 1987). Recently, there has been increasing use of scalp hair as a biomarker of arsenic exposure because of the ease of sample collection, storage and transportation (Phan et al., 2010). In addition, arsenic has the affinity to bind with abundant sulfhydryl groups in keratin of hair tissues (Hall et al., 2006; Mandal et al., 2003).

Children may be particularly susceptible to neurotoxic substances as suggested by findings from studies on the effects of arsenic and manganese exposure on children's intellectual function (Wasserman et al., 2004). One recent study conducted on 2011 children in Bangladesh suggested that arsenic concentrations in drinking water as low as 10 μg/L were likely to cause reductions in intellectual function in 10-year-old children (Wasserman et al., 2011). Although arsenic is a well-known neurotoxicant affecting the central nervous system, some studies have reported the combined effect of arsenic and lead on the neuropsychological development of children (Calderon et al., 2001). However, in a birth cohort study in the Terai region of lowland Nepal, the cord blood levels of lead, arsenic, and zinc were not associated with the Bayley Scale of Infant Development, second edition (BSID II) cluster scores of 6-monthold infants, while it showed an association between the neurodevelopment and home environment (Parajuli et al., 2014). Among Bangladeshi children whose urine contained 35 μg/L of arsenic, no significant effect of arsenic exposure was found on any of the children's development measures after controlling for social and economic confounders, age and sex (Hamadani et al., 2010).

Neurobehavioral testing is commonly recognized as a sensitive and valid method for detecting early dysfunction of the nervous system resulting from low-level exposure to neurotoxic agents (Liang et al., 1997), and is used for early detection of subclinical dysfunction (Hanninen, 1982). The World Health Organization (WHO) neurobehavioral core test battery (NCTB) is used widely in assessing the neurobehavioral effects of environmental and occupational exposures at a subclinical stage and is the most widely used battery administered by a human tester (Anger, 1990; Cassitto et al., 1990). Neurotoxicity associated with arsenic and lead exposure in children is one of the best examples in which neurobehavioral tests have been used to evaluate effects on central nervous system's function (Calderon et al., 2001).

The objectives of this study are to measure the level of arsenic concentration in the school children's hair; to determine the effects of arsenic exposures on the neurobehavioral performance of school children by using a modified World Health Organization's-neurobehavioral core test battery (WHO-NCTB); and to investigate the influence of hair arsenic (As) levels as well as that of other metals, namely hair lead (Pb), manganese (Mn), and cadmium (Cd) levels on the children's neurobehavioral test scores.

2. Materials and methods

2.1. Study design and respondents

The design of this study is a cross-sectional epidemiological study to assess the effects of arsenic exposures on the neurobehavioral performance of secondary school children, as a result of exposure to arsenic contaminated groundwater in the Kandal Province, Cambodia. Arsenic exposure is believed to occur through direct consumption of the groundwater through drinking or cooking or indirect consumption of contaminated foods irrigated in, or aquatic organisms reared in contaminated groundwater. The research was carried out at 3 chosen study sites with varying groundwater arsenic levels in the Kandal Province. Based primarily on studies of arsenic contamination in groundwater, Kampong Kong Commune in Koh Thom District was chosen as a highly contaminated site (300–500 μg/L), Svay Romiet Commune in Khsach Kandal District as a moderately contaminated site (50–300 μg/L), and Anlong Romiet Commune in Kandal Stueng District as a control site (Fig. 1). This research protocol was approved by the Ethics Committee of the National University of Malaysia (Ref no. UKM 1535/244/SPP3 dated 13/03/2012) and the National Ethics Committee for Health Research (NECHR) under the Ministry of Health, Cambodia (Ref no. 039 NECHR dated 12/03/2012). Informed consent was obtained from all respondents and participation in the research was voluntary. Each respondent had the right to withdraw from participating in this research at any time he or she felt uncomfortable during any stage of the research.

In this study, a total of 535 respondents from 3 secondary schools were selected from the study sites with different arsenic levels in the Kandal Province, Cambodia. The respondents consist of 170 respondents (45% females and 55% males) selected from the heavily contaminated site, 151 respondents (48% females and 52% males) from the moderately contaminated site, and 214 respondents (45% females and 55% males) from the control site. The inclusion criteria of the respondents were children who have lived in the study area for at least 5 years, children (females and males) aged between 12 and 16 years, and children from secondary school grade 7–9. One secondary school in each study site was selected.

2.2. Hair sampling

Children's hair samples were cut from the nape of the neck, as near as possible to the scalp using stainless steel scissors (Phan et al., 2010). The collected hair samples were kept in labeled polyethylene ziplock bags and stored in darkness until analysis. For each hair sample, the respondent's background, was surveyed with respect to daily groundwater consumption, exposure duration, and dietary habits. Collected hair samples were investigated for the association between arsenic accumulation and neurobehavioral test scores.

2.3. Hair samples preparation and analyses

Hair samples were first washed in a beaker with deionized water and a magnetic stirrer for 15 min, after which they were alternately washed with acetone and deionized water as recommended by the International Atomic Energy Agency (IAEA, 1985). The washed samples were placed in individual glass beakers and allowed to dry at 60 °C overnight in a drying oven (Phan et al., 2010).

A microwave digestion system (Milestone Start D) with a rotor for 12 Teflon digestion vessels was used for hair sample digestion. As much as 0.5 g of dry hair sample was weighed in a dry, clean, Teflon digestion vessel. To this, 1.5 ml of concentrated HNO_3 (GR grade, produced by Merk, Germany) and 0.5 ml of H_2O_2