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Height and blood chemistry in adults with a history of developmental arsenic poisoning from contaminated milk powder

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ABSTRACT

Background: Arsenic poisoning interferes with bone metabolism in laboratory animal studies, and human studies suggest lowered bone mass density at elevated exposures. As the long-term consequences of developmental arsenic toxicity are poorly known, we carried out a clinical pilot study of survivors of the mass arsenic poisoning of bottle-fed infants in Japan in 1955.

Objectives: The purpose was to evaluate the association between developmental arsenic exposure and physical stature and routine blood chemistry reflecting major organ functions more than 50 years later.

Methods: The study sample consisted of 50 individuals recruited at two hospitals in Okayama Prefecture, Japan: 27 known poisoning victims (14 men and 13 women), and 23 non-exposed local controls of similar age (10 men, 13 women). We collected information from physical examinations that included routine blood counts and blood biochemistry.

Results: The average height of the exposed group was 6.5 cm below that of the unexposed group (p=0.02), while the latter was in accordance with national data for both sexes. In addition, the exposed participants had a higher mean (SD) serum concentration of alkaline phosphatase (ALP) of 233 (63) U/L than the unexposed participants (191 (44) U/L) (p=0.01). No other statistically significant difference was observed, and liver enzymes were within normal ranges.

Conclusions: Adults who had suffered arsenic poisoning during infancy showed decreased height and elevated ALP that suggests abnormalities in bone metabolism possibly induced by arsenic incorporated in the bone matrix.

1. Introduction

Chronic exposure to arsenic via inhalation or drinking water ingestion is linked to cancer of the lung, skin, kidney, and urinary bladder (Straif et al., 2009) and an increased risk of a variety of other non-communicable diseases (National Research Council, 1999). Apart from neurotoxicity, evidence on long-term effects of developmental toxicity of arsenic is limited (Vahter, 2008), but possible adverse effects on birth outcomes include low birth weight (Bloom et al., 2014; Gilbert-Diamond et al., 2016; Kile et al., 2016; Rahman et al., 2009). Further, a prospective cohort study from Bangladesh suggests an inverse association between early-life arsenic exposure from contaminated drinking water and children's growth during infancy (Gardner et al., 2013; Saha et al., 2012). Growth inhibition due to arsenic exposure has been supported by laboratory animal studies that show decreased body length and femur length (Hu et al., 2012), as well as decreased bone mass density (Wu et al., 2014). A cross-sectional study of adult males reported an inverse association between environmental arsenic exposure and bone mass density (Akbal et al., 2014). *In vitro* studies have reported arsenic-induced decreased mineralization of osteoblasts in primary cell cultures (Hu et al., 2012), decreased osteoblast differentiation (Wu et al., 2014), and increased differentiation of preosteoclasts (Szymczyk et al., 2006). Arsenate likely substitutes phosphate in the skeletal apatite crystals (Lindgren et al., 1982), and arsenic exposure causes a decreased expression of alkaline phosphatase (ALP) (Hu et al., 2012), an enzyme that hydrolyzes pyrophosphate and provides inorganic phosphate needed for bone mineralization (Millan, 2013).

As further evidence is needed regarding the long-term consequences of developmental arsenic toxicity, we carried out a pilot study

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of surviving patients who were poisoned by arsenic in infancy (Yorifuji et al., 2016). The mass arsenic poisoning of bottle-fed infants happened in the western part of Japan during the summer of 1955. The cause was contaminated milk powder, and the estimated arsenic concentration in the reconstituted milk was about 4–7 mg/L (Dakeishi et al., 2006; Hamamoto, 1957). The exposures lasted for about four months from the change in formulation of the milk powder in April 1955 until the recall on August 24, 1955 (Hamamoto, 1957). Approximately 13,000 children were affected and manifested skin pigmentation, diarrhea, and fever; more than 100 died from acute poisoning (Hamamoto, 1957). Unfortunately, long-term follow-up studies of the victims have not been carried out, and the absence of accessible registries hampers any follow-up studies (Yorifuji et al., 2011).

We recently conducted a pilot study of a small group of poisoned victims and non-exposed controls of similar age; the primary focus was on the long-term effects of developmental arsenic exposure on neurological outcomes more than 50 years later (Yorifuji et al., 2016). We found that exposed participants had more unfavorable neuropsychological outcomes than the unexposed controls, which is consistent with previous studies suggesting developmental neurotoxicity of arsenic exposure (Smith and Steinmaus, 2009; Tolins et al., 2014; Vahter, 2008). We are now reporting the results of the general physical examination and routine blood chemistry, where we explored a variety of parameters that may reflect organ dysfunctions, including bone metabolism. This unique incident of arsenic exposure may provide useful information to elucidate whether developmental arsenic exposure results in adverse effects that persist through mature ages.

2. Materials and methods

2.1. Study setting

During the period from April 2012 to February 2013, we conducted a retrospective cohort study of participants at two hospitals (Okayama Kyoritsu Hospital and Mizushima Kyodo Hospital) in Okayama Prefecture, Japan. We selected Okayama Prefecture because the Prefecture was the most severely affected area in the country (Hamamoto, 1957). We collected information on physical status and blood examination in connection with the regular health examinations offered to registered victims and conducted at the two participating hospitals. We also collected a urine specimen for arsenic analysis. The unexposed subjects were examined by the same methods; blood and urine samples were coded, but blinding of the clinical examiner was not possible. Demographic characteristics including past medical history were obtained from a questionnaire, but arsenic exposure levels in infancy, duration of breastfeeding, and detailed residential history were unavailable. The study setting and methods are described in further detail in our previous report (Yorifuji et al., 2016). The study was approved by the Institutional Review Boards of Okayama University (protocol No. 487) and at each hospital.

2.2. Study participants

We included 50 participants who provided written, informed consent for participation in the study: 27 among known poisoned victims (14 men and 13 women) in Okayama Prefecture and 23 local, non-exposed controls (10 men and 13 women). Among those invited, three exposed (10.0%) and three non-exposed individuals (11.5%) refused to participate in the study.

The exposed participants were identified in part from the list of victims known to the Hikari Association to receive free regular health examinations at the two hospitals (n=15). The Hikari Association is a public-interest foundation dedicated to providing permanent relief for the victims of the contaminated milk powder. It supports victims through various efforts such as benefits, including living allowance, and regular health examinations for those who have disabilities or a relevant medical diagnosis. According to the Hikari Association, it supports regular health examinations for victims with disorders specific to arsenic poisoning, such as skin lesions, mental disorder, or intellectual disability. Among the 15 participants identified through the Association, 12 received benefits for their disorders, while three participants did not, because they did not suffer from qualifying disorders. The list of exposed participants was supplemented by a list of victims who did not receive regular health examinations offered by the Hikari Association but who could be reached through the two local hospitals (n=12); the hospitals offered health care to these victims regardless of their current health status and certification.

We also recruited 23 non-exposed controls from medical staff at the two hospitals, including clerks, nurses, and laboratory technicians, at ages similar to the exposed participants. None of them had been exposed to the contaminated milk powder during infancy, but information whether they were breastfed or formula-fed with other milk powder could not be obtained.

2.3. Medical examination

The regular health examinations recorded physical status (height, weight, blood pressure at rest, visual acuity, hearing acuity), blood analyses (complete blood count and biochemical blood tests), urine analysis (urine protein, blood, and sugar), chest X-ray, and electrocardiogram. The same standardized procedures were used at the two participating hospitals. In the present study, we selected physical status including height, weight, and blood pressure at rest as well as blood counts and biochemistry as the parameters most relevant in regard to childhood arsenic exposure. Neurobehavioral outcomes were analyzed in a previous report (Yorifuji et al., 2016).

2.4. Urine arsenic analysis

During the health examinations, we collected 10 mL spot urine samples for immediate freezing at −80 °C. The frozen samples were couriered to the US laboratory for arsenic analysis. The samples were acidified with nitric acid before determination of arsenic species by atomic absorption spectrophotometry.

2.5. Statistical analyses

We compared demographic characteristics between exposed and unexposed participants to identify possible confounders. We then compared the clinical results on physical status, blood analyses, and urine arsenic analysis between the two groups. Because alcohol status may affect blood biochemistry, we repeated the analyses after exclusion of participants who reported drinking alcohol every day. In addition, as arsenic is reported to increase risks of liver disease, diabetes mellitus, and neurocognitive dysfunctions (Das et al., 2012; Islam et al., 2011; Maull et al., 2012; Yorifuji et al., 2016), we excluded those diagnosed with these diseases before repeating the analyses. We also carried out the same analyses after stratification by sex.

In our previous study, we observed that exposed participants had more unfavorable neuropsychological outcomes than the unexposed participants, in particular for Design memory subtest from Wide Range Assessment of Memory and Learning 2 and Grooved pegboard test (both dominant and non-dominant hands) (Yorifuji et al., 2016). As an exploratory analysis not included in the original protocol, we examined the correlation of these tests with other clinical outcome parameters significantly associated with exposure status in order to ascertain the possible phenotypic pattern of sequelae from arsenic poisoning during infancy.

We used Chi-square tests for binary outcomes and *t*-tests for continuous outcomes after having confirmed that the residuals approached a Gaussian distribution. PASW Statistics software (SPSS Japan Inc., version 18.0J) was used for the analyses. We report two-