Neurotoxicology and Teratology 41 (2014) 89–95

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Home environment and prenatal exposure to lead, arsenic and zinc on the neurodevelopment of six-month-old infants living in Chitwan Valley, Nepal

Rajendra P. Parajuli [a,*], Takeo Fujiwara [b], Masahiro Umezaki [a], Hana Furusawa [a], Chiho Watanabe [a]

[a] Department of Human Ecology, Graduate School of Medicine, University of Tokyo, Japan
[b] Department of Social Medicine, National Research Institute for Child Health and Development, Tokyo, Japan

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 20 December 2013
Accepted 30 December 2013
Available online 11 January 2014

Keywords:
Neurodevelopment
Bayley Scale of Infant Development
Nepal
Toxic and essential elements
HOME scale

ABSTRACT

Background: We have previously reported the inverse associations between *in utero* levels of lead (Pb), arsenic (As) (i.e., toxic elements), and neurodevelopmental indicators (i.e., motor and state regulation cluster score) measured by the Brazelton Neonatal Behavioral Assessment Scale, third edition (NBAS III) in this cohort at birth. Using additional follow-up, this study investigated the effects of cord blood levels of Pb, As, and zinc (Zn) (an essential element) and the postnatal environment on the neurodevelopment of 6-month-old infants in Chitwan Valley, Nepal.
Methods: In total, 100 mother–infant pairs were recruited from Chitwan District, Nepal. Pb, As, and Zn concentrations in cord blood were measured. Postnatal raising environment (i.e., HOME score or home environment hereafter) was evaluated using the Home Observation for Measurement of Environment (HOME) scale. Neurodevelopment of infants at 6 months ($n = 94$) was assessed according to the Bayley Scale of Infant Development, second edition (BSID II). Multivariable regression adjusting for covariates was performed to determine the associations of *in utero* levels of toxic and essential elements and the home environment with neurodevelopment scores.
Results: Cord blood levels of Pb, As, and Zn were not associated with any BSID II cluster scores in 6-month-old infants. The total HOME score was positively associated with the Psychomotor Development Index (PDI) score (coefficient = 0.59, 95% confidence interval [CI] = 0.04 to 1.13).
Conclusion: In this cohort, detrimental effects of *in utero* Pb and As on neurodevelopmental indicators observed at birth did not persist at 6 months of age, while it showed an association between the neurodevelopment and home environment.

1. Introduction

Enormous efforts have been devoted to clarify the determinants of human neurodevelopment. There is substantial evidence from animal studies indicating the associations between *in utero* exposure to toxic elements (e.g., Pb and As) and *in utero* deficiencies of essential elements (e.g., Zn), and fetal neurodevelopment [e.g., Bhatnagar and Natchu, 2004; Rodriguez et al., 2002; Toscano and Guilarte, 2005; Wright and Baccarelli, 2007]. However, the results of epidemiological studies on the associations between *in utero* exposure to toxic elements and *in utero* deficiencies of essential elements, and later neurodevelopment are inconsistent.

From 2002 to 2004, a workgroup of the Centers for Disease Control and Prevention (CDC) Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) reviewed 23 scientific studies including 16 separate populations and found that intelligence quotient (IQ) and general cognitive index outcomes among children aged less than 5 years are associated with blood Pb levels (BLLs) less than 100 μg/L. The workgroup concludes that there is no safe level for blood Pb in children (CDC ACCLPP, 2007). In their review, Gilbert and Weiss (2006) propose that the BLL that should prompt public health actions is 20 μg/L. Although the majority of the earlier studies report detrimental effects of Pb in later neurodevelopment, a number of studies do not report signs of neurodevelopmental deficit when the mean BLL exceeded 44 μg/L (Minder et al., 1998), 71 μg/L (Prpic-Majic et al., 2000), or 100–160 μg/L (Ernhart and Greene, 1990; Harvey, 1986; Lansdown

Abbreviations: Pb, lead; As, arsenic; Zn, zinc; CDC, Centers for Disease Control and Prevention; ACCLPP, Advisory Committee on Childhood Lead Poisoning Prevention; IQ, intelligence quotient; EDTA, ethylenediaminetetraacetic acid; BLL, blood Pb level; USA, United States of America; BMI, body mass index; NBAS III, Neonatal Behavioral Assessment Scale, third edition; BSID II, Bayley Scale of Infant Development, second edition; MDI, Mental Development Index; PDI, Psychomotor Development Index; HOME scale, Home Observation for Measurement of Environment scale.

* Corresponding author at: Department of Human Ecology, School of International Health, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel.: +81 3 5841 3806; fax: +81 3 5841 3395.
E-mail address: parajulirp@humeco.m.u-tokyo.ac.jp (R.P. Parajuli).

0892-0362/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ntt.2013.12.006

et al., 1986; Smith et al., 1983). Further, such data from Asian studies are, still, scarce (Kile et al., 2009; Patel et al., 2006).

Despite the evidence of a cross-sectional association between postnatal As exposure and neurodevelopment, the effect of prenatal As exposure on later neurodevelopment remains unclear. The lack of association between maternal As level during pregnancy (i.e., urinary As level during the first, second, and third trimesters as a proxy of prenatal exposure) and neurodevelopmental indicators (BSID II) at 7, 18 and 60 (among boys only) months was reported in Bangladesh (Hamadani et al., 2010, 2011; Tofail et al., 2009). However, no study has investigated the association between cord blood As level, which is considered a better bioindicator of prenatal exposure (Parajuli et al., 2013), and the neurodevelopment of 6-month-old infants, which is an important developmental milestone including mental and psychomotor development (CDC, 2012).

Regarding the association between *in utero* Zn levels and neurodevelopment, positive association was reported between maternal Zn intake during pregnancy and lactation period with a neurodevelopmental indicator, the habituation cluster of the Brazelton scale, at 6 months of age (Kirksey et al., 1991). Randomized controlled trials show that Zn supplementation to pregnant mothers and infants improved the locomotor development scores of infants in Newfoundland (Friel et al., 1993), fine and gross motor skills of children in China (Sandstead et al., 1998), and mental and psychomotor development scores of infants in Chile (Castillo-Duran et al., 2001). In contrast, several studies failed to detect such effects (Black et al., 2004b; Tamura et al., 2003; Taneja et al., 2005). The inconsistencies among these studies might be attributable to different levels of Zn deficiency, which can be attributed to different settings and populations.

In the present study, we targeted the Chitwan Valley in lowland (Terai) Nepal, because we assumed that relatively large amounts of Pb and As are circulating in the soil, water, air, and living organisms that is enough to cause neurodevelopmental deficits (Parajuli et al., 2012). Inverse associations between cord blood levels of Pb and As, and neurodevelopmental indicators (i.e., motor and state regulation cluster score, respectively) measured by the Brazelton Neonatal Behavioral Assessment Scale, third edition (NBAS III) are reported in children in this cohort at birth (Parajuli et al., 2013). The Chitwan District is located at the junction of a highway from Kathmandu and an east–west highway where many vehicles emit Pb into the environment (Shrestha et al., 2003). In addition, the district is a hotspot of As contamination (Pokhrel et al., 2009). In addition to the problem of toxic elements, owing to Zn deficit in soil of lowland Terai (Harrington et al., 1989; Pokharel, 1997), Zn deficiency is reported to be a health problem in the region (Andersen, 2007; Christian et al., 2006). Thus, because of these environmental conditions, it is anticipated that the associations between neurodevelopment and toxic and essential elements will be relatively more detectable in the Chitwan District than in developed countries where exposure to toxic and essential element intakes is well regulated. The objectives of the present study were to investigate the effects of *in utero* exposure to Pb, As (toxic elements), and Zn (an essential element) and home environment on the neurodevelopment scores of 6-month-old infants in Chitwan District, Nepal.

2. Methods

2.1. Study sample

The eligibility criteria in the present study were as follows: residence in Chitwan District for at least 2 years, at term pregnancy when the mothers visited the hospital (more than 37 weeks of gestation), age of 18–40 years, *per vaginam*, singleton, and no reports of diabetes, hypertension, or preeclampsia. In total, 200 pregnant mothers were approached between September and October 2008 in the Bharatpur General Hospital of Chitwan District. Among them, 119 were eligible. Mothers were informed of the background and objectives of the study, what they would experience during the study process, the benefits, and the potential risks, although none were expected. Finally, 100 women signed a letter of informed consent (participation rate, 84%). The study protocol was approved by the ethics committees of the Graduate School of Medicine, the University of Tokyo (approval no #2244) and of the Bharatpur General Hospital, Chitwan, Nepal.

2.2. Measurements of levels of cord blood elements

Cord blood was collected from the placenta by midwives according to the routine aseptic procedure. Cord blood (10 mL) was collected into a trace metal-free cryovial containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Cord blood samples were stored in a freezer at −20 °C for less than 1 month, transported to the laboratory in Tokyo while kept frozen with dry ice, and stored in a deep freezer at −78 °C until analysis.

Pb, As, and Zn levels in the cord blood samples were measured at the Department of Human Ecology at the University of Tokyo which has a clean room to minimize contamination. The methods and research findings from this cohort have been published previously (Parajuli et al., 2012). The certified reference material (CRM), "Seronorm" trace elements whole blood level-1, lot MR 4206 (Sero AS, Billingstad, Norway), was used. The observed values for each element were within the certified range. Randomly selected cord blood samples (20%) were analyzed twice for all elements. For all the elements measured, there was no statistical difference between the two measurements, and the correlations between them ranged from 0.90 to 0.95, depending on the element.

Of the 100 cord blood samples, 94 samples were used to measure Pb, As, and Zn levels. The other 6 cord blood samples were not used due to lack of identity information (i.e., subjects ID) on the sampling vial tag. In addition, due to limited sample volume (as one blood sample was taken from each newborn cord, but the blood sample was measured twice), cord blood Pb levels could not be re-measured in 15 samples; thus, the data of the Pb levels of 79 cord blood samples were used for analysis.

2.3. Interview on the day of delivery

The following information was collected from mothers after delivery by interview: mother's age, parity, gender of baby, gestational age, time and date of delivery, educational level, annual family income, smoking during pregnancy, and alcohol consumption during pregnancy.

2.4. Postnatal home environment

The author (RPP) visited the home of each mother–infant pair after approximately 6 months (192.8 ± 13.2 days after their baby was born) after delivery and evaluated the postnatal home environment according to the HOME scale (Caldwell and Bradley, 1984). The scale includes 45 items and is the total evaluation (i.e., by both observation and interview) of parental response to child's behavior, acceptance of child, organization of environment, learning material, parental involvement with the child, and opportunities of variety for baby. Therefore, the possible scores range from 0 to 45, with scores <25 indicating a "less stimulating" home environment (Torres-Sanchez et al., 2007). Of the 100 mothers enrolled in the cohort, the home environment of 94 homes was evaluated.

2.5. Anthropometry of mothers and infants at birth and at 6 months

The height and weight of the mothers were recorded just before delivery. Height was measured to the nearest 0.1 cm. Body weight was recorded to the nearest 0.1 kg using a portable digital scale (Model BF-046 WH; Tanita, Tokyo Japan). Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). The birth weight of the newborns was obtained from hospital records.