ORIGINAL ARTICLES

Effects of Perinatal Dioxin Exposure on Development of Children during the First 3 Years of Life

Pham The Tai, MD, PhD[1], Muneko Nishijo, MD, PhD[2], Tran Ngoc Nghi, MD[2], Hideaki Nakagawa, MD, PhD[2], Hoang Van Luong, MD, PhD[1], Tran Hai Anh, MD, PhD[1], and Hisao Nishijo, MD, PhD[3]

Objective To investigate the longitudinal effects of perinatal exposure to dioxin on neurodevelopment and physical growth of a birth cohort during the first 3 years of life.

Study design A total of 217 mother–infant pairs living in a dioxin-contaminated area in Vietnam were followed up. Perinatal dioxin exposure of infants was estimated by the measurement of dioxin levels in breast milk of nursing mothers. Neurodevelopment of infants and children, including cognitive, language, and motor development, was determined at 4 months, 1 year, and 3 years of age. Physical growth, including weight, height, and head and abdominal circumferences, was measured at birth, 1 and 4 months, and 1 and 3 years of age. Multivariate mixed models were applied for analyzing repeated measures.

Results In boys, composite motor and gross motor scores were decreased with increasing exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TetraCDD). The high toxic equivalent of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs-TEQ) group showed a significant decrease in expressive communication score. In girls, there was no decreased score in any neurodevelopment aspects in high-exposure groups. All body size measures in boys were decreased in the high-exposure groups of 2,3,7,8-TetraCDD and PCDDs/PCDFs-TEQ. In girls, high 2,3,7,8-TetraCDD and PCDDs/PCDFs-TEQ exposure was associated with increased head and abdominal circumferences.

Conclusions Perinatal dioxin exposure affects physical growth and neurodevelopment of infants and children in the first 3 years of life in a sex-specific manner. *(J Pediatr 2016;175:159-66)*.

The use of herbicides for defoliant and crop destruction purposes during the Vietnam war from 1961 to 1972 remains a problematic postwar issue. After several decades of spraying of herbicides, levels of dioxin in the environment and in humans residing in the sprayed areas remain elevated,[1-3] which is particularly the case around areas of former US airbases that were used for storing herbicides during the war, called hot spots of dioxin contamination.[4]

We previously investigated levels of dioxin in breast milk of lactating mothers in a hot spot in Da Nang, Vietnam. We found that these levels were approximately 4-fold greater than those of lactating mothers residing in unsprayed areas.[5] We have followed up a birth cohort in Da Nang, Vietnam, since 2008-2009 and investigated the effects of perinatal exposure to dioxin indicated by dioxin levels in maternal breast milk to development of infants and children, including physical growth and neurodevelopment.

Cross-sectional study results of neurodevelopmental effects have been reported in 4-month-old infants and 1-year and 3-year-old children. High dioxin exposure decreased scores of cognitive, expressive communication, and fine motor skills, as tested by Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) in 4-month-old infants,[6] as well as social emotional scores in toddlers at 1 year of age.[7] In 3-year-old children, exposure to high toxic equivalents of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs-TEQs) also decreased Bayley-III scores of all domains in boys.[8] No study, however, has clarified the longitudinal effects of dioxins on the neurodevelopment of these children.

2,3,7,8-TetraCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
Bayley-III	Bayley Scales of Infant and Toddler Development, Third Edition
BMI	Body mass index
GM	Geometrical mean
GSD	Geometrical SD
IGF-I	Insulin-like growth factor I
PCB	polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDDs/PCDFs-TEQ	Toxic equivalent of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans
PCDF	Polychlorinated dibenzofuran
WHO	World Health Organization

From the [1]Biomedical and Pharmaceutical Research Center, Vietnam Military Medical University, Ha Dong, Ha Noi, Vietnam; [2]Department of Epidemiology and Public Health, Kanazawa Medical University, Uchinada, Ishikawa, Japan; and [3]System Emotional Science, Graduate School of Medicine, University of Toyama, Toyama, Japan

Supported by High-Technology Center of Kanazawa Medical University (H2006-9), Mitsui & Co. Ltd. Asian Core Program, and the Japan Society for the Promotion of Science (17406016 and 22240051). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2016.04.064

We previously reported that dioxin affects infant growth at cross-sectional examinations, 1 month, and 4 months of age[9]; however, the effects of dioxins on physical growth of infants older than 4 months old have not been reported in our birth cohort. Therefore, this study aimed to investigate the longitudinal effects of perinatal dioxin exposure on physical growth and neurodevelopment in our birth cohort in Da Nang, Vietnam, during the first 3 years of the life.

Methods

Two districts, Thanh Khe and Son Tra, which are located around Da Nang Air Base, a former US Air Force base, were studied. The most recent update of dioxin-contaminated conditions in the environment inside and around the airbase has been described in detail in a final report of Hatfield Consultants. They reported significant quantities of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TetraCDD) in some soil samples (858-361 000 pg/g dry weight) and in sediment samples (674-8580 pg/g dry weight of 2,3,7,8-TetraCDD) collected at Da Nang airbase.[3]

In 2008 and 2009, 159 mother–infant pairs in the Thanh Khe district and 82 pairs in the Son Tra district were enrolled in the present study. When the pregnant mothers gave birth in these 2 district hospitals, obstetricians recruited them on the basis of the following criteria: (1) mothers who resided in the target areas during their pregnancies; (2) infants who were studied were full term; and (3) mothers and infants who experienced no complications during birth.

Written informed consent was obtained from all of the mothers according to a process reviewed and approved by the Health Department of Da Nang City and the Vietnamese Military Medical University. The institutional ethics board for epidemiologic studies at Kanazawa Medical University approved the study design.

Follow-up surveys of the subjects were performed at 1 month, 4 months, 1 year, and 3 years of age. Among the 241 mother–infant pairs at baseline, 227 (94.2%) pairs participated at 1 month of age (30.5 ± 1 days), 219 (90.9%) pairs participated at 4 months of age (4.1 ± 0.6 months), 214 (88.8%) pairs participated at 1 year of age (11.1 ± 1 months), and 198 (82.2%) pairs participated at 3 years of age (36.4 ± 1.6 months) in surveys. Nonparticipation was attributed to movement to other places or being absent on the examination days, except for 2 mother–infant pairs in whom the infants died in the first postnatal month. There were no significant differences in the characteristics of mother–infant pairs and levels of dioxin in breast milk among participants and nonparticipants.

Body size of infants, including weight, height, and head and abdominal circumferences, were measured at birth, 1 month, 4 months, 1 year, and 3 years of age. At birth, the medical staff of the obstetrics department measured body size of newborns in district hospitals. At 1 month of age, medical staff in community health centers measured infant body size at home. At 4 months, 1 year, and 3 years of age, all measurements of body size were performed by researchers in community health centers. For analysis, absolute values of body size, as well as age-adjusted z scores, were used after calculation for weight, height, head circumference, and body mass index (BMI) by use of the World Health Organization (WHO) standards (www.who.int/childgrowth). Because WHO standards are unavailable for abdominal circumference, we used the z score of the present subjects for abdominal circumference.

The Bayley-III is an individually administered instrument that is used to assess developmental functioning of infants and young children between 1 and 42 months of age across 5 scales: cognitive, receptive and expressive language, and fine and gross motor skills. Additionally, social-emotional and adaptive behavior scales were used, whereas infants' caregivers' responses to the questionnaire were based on their daily observation of the infants. In the present study, the Bayley-III was administered 3 times at 4 months, 1 year, and 3 years of age. A single, well-trained examiner who was blind to the dioxin exposure level of the infants and children performed the test every time.

Mothers were interviewed face-to-face by medical staff with the use of a structured questionnaire when children were 4 months of age. Information was collected for the mothers (age, history of residency, parity, height, weight, smoking history of the mother and family members, alcohol consumption, employment, education, and economic status) and the infants (gestational age, sex, and breastfeeding status).

Breast milk samples were collected at 1 month after birth with the assistance of midwives or medical workers. Approximately 10 mL of breast milk from each sample was used to quantify the levels of 17 2,3,7,8-substituted polychlorinated dibenzo-p-dioxin (PCDD)/polychlorinated dibenzofuran (PCDF) congeners. A series of purifying operations including alkali digestion, hexane extraction, and chromatography on a multilayered silica gel column was performed. A single-layered column of activated carbon dispersed on silica gel was used to separate and collect PCDD/PCDF fractions. Quantification was performed with a gas chromatograph (HP-6980; Hewlett-Packard, Palo Alto, California) equipped with a high-resolution mass spectrometer (high-resolution-gas chromatography/mass spectrometry; MStation-JMS700, JEOL, Tokyo, Japan) operating in the selected ion-monitoring mode. The established method of analysis have been described previously in detail.[5] The calculations of PCDDs/PCDFs-TEQ were referenced from the WHO 2005 toxic equivalent factor.[10] Levels of dioxin in breast milk were considered as perinatal dioxin exposure in infants. For exposure indices, we used 2,3,7,8-TetraCDD, as well as PCDDs/PCDFs-TEQ levels in the breast milk of nursing mothers.

Statistical Analyses

SPSS (version 21.0) software package for Windows (SPSS Inc, Armonk, New York) was used for statistical analysis. Levels of 2,3,7,8-TetraCDD and PCDDs/PCDFs-TEQ were