DIOXIN EXPOSURE AND INFANT NEURODEVELOPMENT – A FOLLOW UP STUDY IN VIETNAMESE INFANTS

Tai PT[1,2*], Nishijo M[1], Nakagawa H[1], Maruzeni S[1], Anh NTN[1], Luong HV[2], Anh TH[2], Nhat TV[3], Thanh TT[4], Phuong PT[5], Nguyet LTM[6], Son LK[7], Morikawa Y[8], Kido T[9], Nishijo H[10]

[1]Department of Epidemiology and Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa, 920-0293, Japan; [2]Biomedical and Pharmaceutical Research Center, Vietnam Military Medical University, Ha Noi, Vietnam; [3]Department of Health of Da Nang City, 103 Hung Vuong Street, Da Nang City, Vietnam; [4]Preventive Medicine Center of Da Nang City, 315 Phan Chu Trinh Street, Da Nang City, Vietnam; [5]Thanh Khe District Hospital, K62/32 Ha Huy Tap Street, Da Nang City, Vietnam; [6]Son Tra District Hospital, 770 Ngo Quyen Street, Da Nang City, Vietnam; [7]Environment Administration, Ministry of Natural Resources and Environment, 67-Nguyen Du street, Hanoi, Vietnam; [8]School of Nursing, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa, 920-0293, Japan; [9]Faculty of Health Sciences, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa, 920-0942, Japan; [10]System Emotional Science, Graduate School of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

Introduction

Brain development during the fetal and infant periods is highly sensitive to environmental neurotoxicants[1]. Polychlorinated biphenyls (PCBs) and dioxins are of particular concern due to their adverse impact on human health. Even though it is highly nutritious for infants, breast milk is the primary source of postnatal exposure to PCBs and dioxins in early infancy[2]. Nursing mothers may expose their infants to PCBs and dioxins through breast milk, which is exceptionally rich in lipids. In South Vietnam, the use of the herbicides during the Vietnam War represents a major source of dioxin exposure[3]. We recently showed that dioxin levels in the breast milk of mothers residing near hot spots were 3 to 4 fold higher than in the breast milk of mothers living in unsprayed areas[4]. These results raised concerns about the effect of dioxin exposure on infant development in these areas. The present study thus investigated the effects of perinatal exposure to dioxins on early neurodevelopment in a mother-infant population residing in a dioxin-contaminated area of Vietnam.

Materials and methods

The former U.S. Air Force base at Da Nang is characterized as a hot spot of dioxin contamination[5]. The Thanh Khe and Son Tra districts of Da Nang city are located about 5-10 km from the airbase and were thus chosen as the location for the present study. The subjects for the study were 138 mother-infant pairs living in the Thanh Khe and 78 mother-infant pairs in Son Tra districts. The criteria for recruitment were as follows: mothers must have resided in one of the study districts for a period encompassing at least the duration of their pregnancy; mothers must have given birth to full-term babies; and there must have been no complications during childbirth. Information was collected for both the mothers (age, residence history, parity, height, weight, smoking habits of the mother and family members, alcohol consumption, employment, education, and economic status) and the infants (gestational age, gender, and breastfeeding status).

A breast milk sample was collected from each nursing mother at 1 month after birth and was used to quantify the levels of 17 different 2,3,7,8-substitued polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) congeners. Toxic equivalent factors for use in calculating the toxic equivalents (TEQ) of PCDDs (PCDDs-TEQ), PCDFs (PCDFs-TEQ), and PCDDs/PCDFs (PCDDs/PCDFs-TEQ) were referenced from the WHO 2005-TEF[6]. Daily dioxin intake (DDI) for nursing infants at 1 month of age was estimated by the following equation; (800 ml; estimated milk volume/day) × (% lipid content of milk/100) × (PCDDs/PCDFs-TEQ in milk in pg/g of lipid) / (infant weight in kg at 1 month of age). When the infants in the present study were divided into 2 groups according to dioxin levels of their maternal breast milk, the cutoff values for 2,3,7,8-tetrachlorinated dibenzo-p-dioxins (TetraCDD), PCDDs/PCDFs-TEQ, and the DDI were 1.8 pg/g lipid, 7.9 pg/g lipid, and 49.8 pg TEQ/kg/day, respectively. These values were estimated from upper range of dioxin levels in the breast milk of 138 mothers living in unsprayed areas calculated using the following equation: (geometrical

mean) × (geometrical standard)^2. Infants were followed up until they were approximately 4 month old, and examined health and developmental condition. The Bayley Scales of Infant and Toddler Development, third edition (Bayley III), was assessed to evaluate infant neurodevelopmental status, cognitive, language, and motor development. Simple correlations between dioxin parameters and infant neurodevelopmental scores were analyzed by Spearman's rho, and comparisons of neurodevelopmental scores between 2 groups at different dioxin exposure levels were performed using generalized linear model in The SPSS software package (ver. 11.0).

Results and discussion

The average characteristics of the 216 mother-infant pairs are presented in Table 1. The mean age of mothers, including 64 primiparae and 152 multiparae, was 27.8 years. Almost one in five (18.5%) mothers consumed alcohol during pregnancy. No mother was a smoker, but 82.9% of mothers stayed with family members who smoked. All infants were breast fed until reaching at least 4 months of age. The breast milk levels of 2,3,7,8-TetraCDD, PCDDs/PCDFs-TEQ, and the infant DDI in the present study were 1.4 pg/g fat, 12.5 pg/g fat and 75.9 pg TEQ/KG/day, respectively. The simple correlations between breast milk dioxin levels and infant DDI and neurodevelopmental scores of infants were illustrated in Table 2. Level of 2,3,7,8-TetraCDD showed significant associates with fine motor ($P = 0.019$) and composite motor scores ($P = 0.011$). These relationships were still significant after adjusting for covariates including age, parity, family member smoking habits, alcoholic consumption, education, and the economic status of the mothers, and gestational age, age (in days) at examination, gender, and birth weight of infants and resident area (Thanh Khe or Son Tra). The associations between 2,3,7,8-TetraCDD and cognitive score was in borderline of significant ($P = 0.056$). However, it became significant after adjusting for above covariates (data is not shown). DDI showed significant associates with Cognitive, fine motor and composite motor scores in both simple correlation model (Table 2) and covariates adjusted models (data is not shown). PCDDs/PCDFs-TEQ showed significant association with fine motor score in adjusted model, but not with other aspects of neurodevelopment in both simple correlation model (Table 2) and covariates adjusted models (data is not shown). To confirm that dioxin exposure exceeding the levels found in unsprayed areas has a negative impact on neurodevelopment, infants were divided into 2 groups at different breast milk levels of 2,3,7,8-TetraCDD, PCDDs/PCDFs-TEQ and the infant DDI, defined by cut off-values in the exposure levels in unsprayed areas, and the neurodevelopmental scores were compared between these 2 infant groups. Comparisons were made using a general linear model after adjustment for covariates (Figure 1). Infants in the group exposed to higher levels of 2,3,7,8-TetraCDD had significantly lower cognitive scores than infants in the lower exposure group. Moreover, the higher DDI group showed significantly lower cognitive, composite motor, and fine motor scores. The present study provides evidence that perinatal dioxin exposure is inversely correlated with the neurodevelopment of 4-month-old infants whose mothers reside in dioxin contamination hot spots in Vietnam. Dioxins predominantly affect cognitive ability and fine motor skills of these infants.

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Table 1 Characteristics of mothers and infants (N = 216)

Characteristics	Value	(Min - Max , SD)
Mothers		
Age (years)[1]	27.8	(17 - 43 , 6.0)
Residency (years)[1]	19.2	(1 - 43 , 11.7)
Parity (% of Primiparae)	29.6	
Weight (kg)[1]	57.7	(41 - 75 , 7.0)
Height (cm)[1]	154.0	(141 - 176 , 5.0)
Education (years)[1]	8.6	(0 - 17 , 3.5)
Types of Job (%)		
Housewives	37.5	
Unskillful laborers	26.9	
Skillful laborers	35.6	
Alcohol consumption during pregnancy (%)	18.5	
Smoking habit of family members (%)	82.9	
Family income (Millions VNDs/month)[1]	3.0	(0.5 - 10 , 1.6)
Dioxin levels in breast milk (pg/g fat)[2]		
2,3,7,8-TetraCDD	1.40	(0.1 - 10.0 , 2.3)
PCDDs-TEQ	7.1	(1.6 - 39.4 , 1.7)
PCDFs-TEQ	5.3	(1.2 - 25.7 , 1.7)
PCDDs/PCDFs-TEQ	12.5	(2.9 - 65.1 , 1.6)
Infants		
Gestational age (weeks)[1]	39.5	(37.0 - 42.0 , 0.8)
Gender (% of boys)	57.4	
Duration of breastfeeding ≥ 4 months (%)	100.0	
Daily dioxin intake via breast feeding (pgTEQ/kg/day)[2]	75.9	(8.6 - 579.6 , 2.0)

Abbreviations: Min, minimum; Max, maximum; SD, standard deviation

[1] Arithmetic mean value; [2]Geometrical mean value

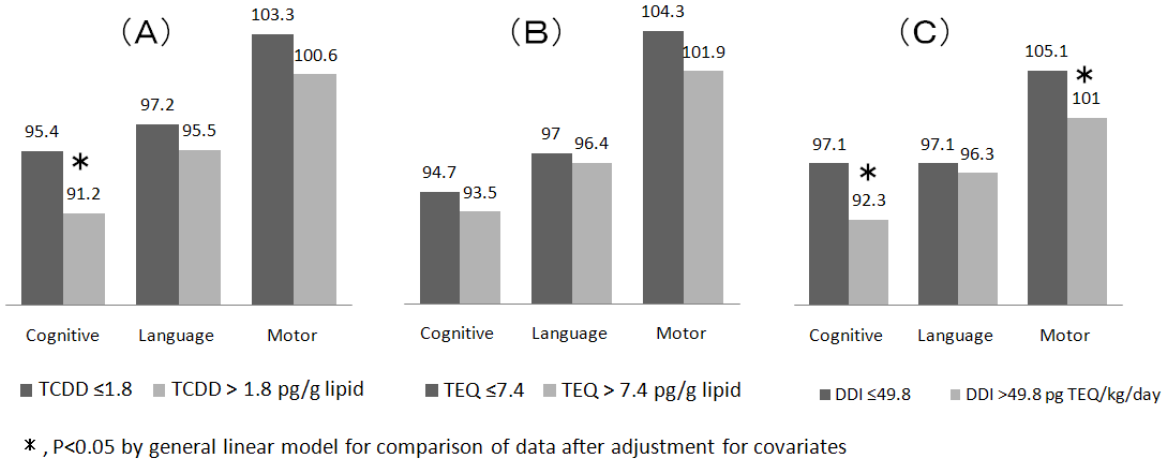
Table 2 Simple correlations between breast milk dioxin level and DDI and infant developmental scores

	Cognitive score		Language score						Motor score					
	Composite		Composite		RC		EC		Composite		FM		GM	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
PCDD congeners														
2,3,7,8-TetraCDD	-0.130	0.056	-0.121	0.076	-0.091	0.182	-0.089	0.194	-0.172	0.011	-0.160	0.019	-0.117	0.087
1,2,3,7,8-PentaCDD	-0.066	0.338	-0.048	0.484	-0.012	0.856	-0.040	0.562	-0.127	0.063	-0.135	0.047	-0.053	0.439
1,2,3,4,7,8-HexaCDD	-0.054	0.430	-0.037	0.593	-0.024	0.726	-0.015	0.826	-0.050	0.464	-0.062	0.363	-0.003	0.960
1,2,3,6,7,8-HexaCDD	-0.039	0.572	-0.071	0.300	-0.046	0.503	-0.048	0.487	-0.108	0.113	-0.093	0.174	-0.065	0.342
1,2,3,7,8,9-HexaCDD	-0.040	0.560	-0.107	0.116	-0.068	0.317	-0.087	0.202	-0.097	0.157	-0.113	0.099	-0.018	0.793
1,2,3,4,6,7,8-HeptaCDD	-0.023	0.736	-0.056	0.411	-0.047	0.494	-0.037	0.589	-0.060	0.380	-0.051	0.453	-0.027	0.694
OctaCDD	-0.069	0.311	-0.014	0.841	-0.036	0.600	0.011	0.876	-0.075	0.274	-0.066	0.336	-0.057	0.405
PCDF congeners														
2,3,7,8-TetraCDF	-0.115	0.092	-0.215	0.002	-0.097	0.156	-0.243	0.000	-0.164	0.016	-0.127	0.063	-0.128	0.060
1,2,3,7,8-PentaCDF	0.053	0.442	-0.101	0.138	-0.002	0.972	-0.161	0.018	0.028	0.679	-0.003	0.970	0.074	0.280
2,3,4,7,8-PentaCDF	-0.047	0.490	-0.028	0.684	-0.009	0.900	-0.009	0.897	-0.068	0.322	-0.093	0.174	0.006	0.926
1,2,3,4,7,8-HexaCDF	-0.023	0.736	-0.099	0.145	-0.046	0.500	-0.093	0.172	-0.063	0.358	-0.051	0.458	-0.021	0.755
1,2,3,6,7,8-HexaCDF	-0.045	0.508	-0.107	0.115	-0.064	0.347	-0.088	0.197	-0.070	0.305	-0.067	0.329	-0.019	0.783
1,2,3,7,8,9-HexaCDF	0.033	0.633	-0.225	0.001	-0.097	0.154	-0.263	0.000	-0.065	0.339	-0.017	0.806	-0.058	0.400
2,3,4,6,7,8-HexaCDF	0.020	0.767	-0.056	0.409	-0.023	0.734	-0.048	0.480	-0.005	0.938	0.004	0.950	0.007	0.913
1,2,3,4,6,7,8-HeptaCDF	0.029	0.668	-0.096	0.162	-0.011	0.876	-0.132	0.052	0.000	0.995	0.017	0.808	0.024	0.724
1,2,3,4,7,8,9-HeptaCDF	-0.038	0.579	-0.182	0.007	-0.085	0.215	-0.188	0.006	-0.010	0.880	-0.014	0.835	0.034	0.623
OctaCDF	0.094	0.170	-0.050	0.461	0.017	0.799	-0.093	0.172	0.044	0.521	0.072	0.292	0.022	0.743
TEQs														
PCDDs-TEQ	-0.072	0.294	-0.078	0.252	-0.038	0.579	-0.065	0.339	-0.142	0.037	-0.136	0.046	-0.076	0.266
PCDFs-TEQ	-0.030	0.665	-0.075	0.275	-0.032	0.639	-0.064	0.346	-0.064	0.347	-0.067	0.325	-0.009	0.899
PCDDs/PCDFs-TEQ	-0.049	0.473	-0.074	0.277	-0.031	0.648	-0.066	0.338	-0.105	0.126	-0.106	0.120	-0.040	0.561
Daily dioxin intake														
DDI	-0.220	0.001	-0.081	0.238	-0.077	0.260	-0.035	0.612	-0.199	0.003	-0.236	0.000	-0.078	0.256

Abbreviations: RC, receptive communication; EC, expressive communication; FM, fine motor; GM, gross motor

r, Correlation Coefficient; P-value, by Spearman's rho

Figure 1 Comparisons of neurodevelopmental scores between infant groups with different levels of 2,3,7,8-TetraCDD (A) and PCDDs/PCDFs-TEQ (B) in breast milk and the infant DDI (C) defined by cut off-values in the exposure levels in unsprayed areas



*, P<0.05 by general linear model for comparison of data after adjustment for covariates