ANALYSIS OF DDT AND ITS METABOLITES IN HUMAN CORD BLOOD IN NEPAL

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Abstract

Adverse effects on fetal development due to DDT, DDE, and DDD have been concerned. We surveyed the levels of DDT and its metabolites, DDE and DDD, in cord blood samples obtained from Nepal. The relationships between DDTs and some parameters of fetus and their mother such as MDI, PDI, head circumference (HC), birth weight (BW) and mothers BMI etc. DDT and its metabolites were detected in cord blood samples except for *o,p'*-DDT. We found that the QuEChERS procedure is an effective method for analysis of biological samples. There are no significant association between characteristics and indexes of the fetuses and their mother except for fetal HC and BW. It was suggested that DDTs might have other influences on the fetus and their developments.

1. Introduction

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane （DDT） , one of the persistent organic pollutants (POPs), is an organochlorine insecticide that had been used all over the world. DDT and its metabolites (Fig.1), 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDE)、1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), have been concerned about human health effects through global-scale environmental pollution and food chain because of their long-term persistence and long-distance mobility. Therefore, production and use of DDT have been internationally banned except for areas such as India where malaria has been an issue.[1)]

DDT and its metabolites (DDTs) have been found in human tissues (blood, adipose tissue, breast milk etc.) because of their chemical characteristic property. Also, it is known that DDTs can easily through out the placenta from mother to fetus. Humans are known to be unable and easily affected by external factor such as chemical substance in fetal stage. DDTs are endocrine disrupting compounds and are concerned that affect fetal mental development and adverse effects on the development of immune function. There are some studies in many countries reporting DDTs levels in cord blood.[2)3)] However, there are few reports of DDTs levels in cord blood in developing countries especially in Nepal.

The purpose of the study is to survey on the levels of DDT and its metabolites, DDE and DDD, from 59 cord blood samples obtained from Nepal, and to investigate relationships between DDTs and some parameters of fetus and their mother. Analytical procedures were consisted with GC-MS after using QuEChERS pretreatment procedure.

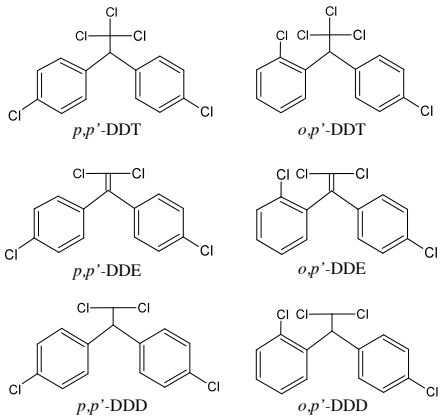

Fig.1. Structural formulae of DDTs

2. Materials and methods

2.1. Human cord blood samples

This research is carried out within the scope of the program, "Clarifying the Relevance of Bread-and-Butter Job

Conversion and Chemical Environment Conversion in Asia" supported by Global Environment Research Fund of Ministry of the Environment, Japan. Human cord blood samples were collected from Nepal. Totally, 59 samples were subjected for the analysis. All samples were reserved under -20◦C until the analysis.

2.2. Sample preparation

Before analysis, the stored frozen cord bloods were brought to room temperature. One mL of a blood sample was spiked with the ^{13}C-labeled surrogate at the final concentration of 20 ng/mL. The sample was extracted three times with 10 mL of n-hexane/acetone (1:1) by liquid-liquid extraction. The obtained supernatant was washed with extra-pure water, and lipid was removed by the sulfuric acid treatment. The QuEChERS method applied to the preparation of the cord blood samples was used for the process of a cleanup. After adding 200 μL of acetic acid, n-hexane layer was transferred a tube containing 1.5 grams of sodium acetate and 6 grams of magnesium sulfate (QuEChRS first tube), and the tube was shaken for one minute, and centrifuged for one minute at 3000 rpm. The whole n-hexane layer was transferred to another tube, and then was completely evaporated under vacuum pressure. One mL of n-hexane was added to the residue, and transferred to second tube containing 150 milligrams of magnesium sulfate and 500 milligrams of PSA packing. The second tube was shaken for one minute, and then centrifuged for one minute at 3000 rpm. To a gas chromatography-mass spectrometry (GC-MS) system, 4 μL of the obtained supernatant was injected.

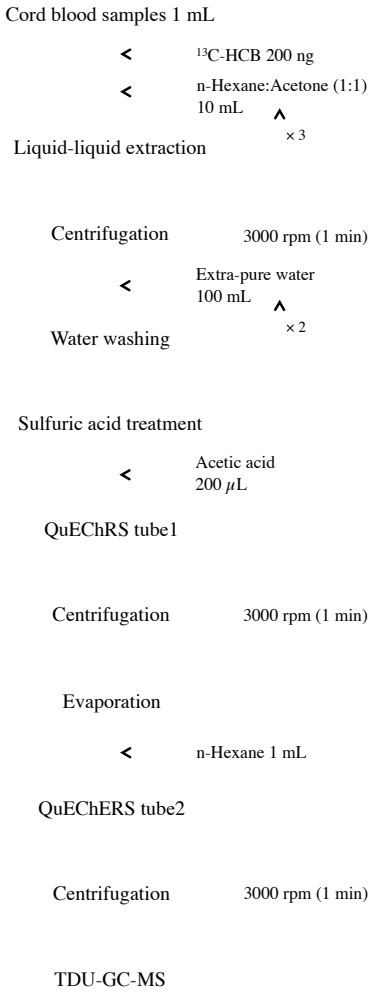

Fig.2. Detail flow chart of the analysis method of human cord blood samples by using QuEChERS.

2.3. GC-MS analysis

GC-MS analysis was performed using 6890 Agilent (Agilent Technologies, Wilmington, USA) gas chromatograph equipped with a quadruple mass filter 5973 network mass selective detector (MSD). Operating was the electron impact (EI) ion source and selected ion monitoring (SIM) mode. An HP-5 MS capillary column (30 m× 0.25 mm i.d.; 0.25 μm film thick) from Agilent was used for qualitative determinations, applying the SIM mode. Samples (4 μL) were automatically injected using the splitless-injection mode. The transfer line of the GC to the MS was kept at 280 °C, the EI ion source of the MS at 230°C. The ionization energy was 70 eV. The GC oven program was: initial temperature at 70 °C for 2 min and then increased to 160 °C at 60 °C min^{-1}, then increased to 240 °C at 5 °C min^{-1} and to 300 °C at 50 °C min^{-1}. The carrier gas was high-purity helium (99.999%) with a constant flow of 1 mL min^{-1}.

Quantification was carried out in the SIM mode. The selected masses were m/z 235 and 165 for DDT and DDD, m/z 246 and 176 for DDE at dwell time of 100 ms/ion/scan.

3. Results and Discussions

All compounds were detected in cord blood samples except for o,p'-DDT. The mean of recovery efficient of DDTs was 86.8%. We found that the QuEChERS procedure is an effective method for analysis of biological samples. The levels of DDT in cord blood from Nepal seems to be higher than previous study[4)] reported in Spain by Sala et al. As listed in Table 1, the most frequently residue was *p,p'*-DDE(71.2%). *p,p'*-DDT, *o,p'*-DDE, *p,p-'*DDD *o,p-'*DDD were detected in 11.9%, 20.3%, 15.3%, 3.4% among the cord blood samples, respectively. It suggested that DDT is accumulated in the mother's body as its main metabolite, DDE, and has transferred to the fetus. *p,p'*-DDT was almost detected from cord blood of primiparous fetus. Due to the low frequency of detection of *p,p'*-DDT and *o,p'*-DDE, *p,p'*-DDD, their relationships with

Table 1

Mean, median and max of DDTs levels (ng/mL) in cord blood.

	Total (n=59)				Male (n=28)				Female (n=31)			
	n(%)	Mean ± S.D.	Median	Max	n(%)	Mean ± S.D.	Median	Max	n(%)	Mean ± S.D.	Median	Max
p,p'-DDT	7(11.9)	0.53 ±1.52	0.00	5.60	2(7.1)	0.24 ±0.92	0.00	4.47	5(16.1)	0.80 ± 1.88	0.00	5.60
o,p'-DDT	0 (0)	-	-	-	0(0)	-	-	-	0(0)	-	-	-
p,p'-DDE	42 (71.2)	1.61 ± 2.20	1.1	10.9	19(67.1)	1.01 ±1.11	0.88	4.53	23(74.2)	2.15 ± 2.75	1.23	10.9
o,p'-DDE	12 (20.3)	0.31 ± 0.85	0.00	5.10	5(17.9)	0.13 ±0.31	0.00	1.14	7(22.6)	0.47 ± 1.13	0.00	5.10
p,p'-DDD	9 (15.3)	0.38 ± 0.91	0.00	3.59	2(7.1)	0.12 ±0.43	0.00	1.80	7(22.6)	0.61 ± 1.14	0.00	3.59
o,p'-DDD	2 (3.4)	0.13 ± 0.73	-	4.00	0(0)	-	-	-	2(6.5)	0.26 ± 0.99	0.00	4.00

Table 2

Characteristics and indexes of the 59 fetuses and their mother.

	Total (n=59)			Male (n=28)			Female (n=31)		
	Mean ± S.D.	median	Range	Mean ± S.D.	Median	Range	Mean ± S.D.	median	Range
Age of mother	23.14 ± 3.62	23	18-37	22.7 ± 3.50	22.5	18-35	23.6 ± 3.73	23	18-37
Mothers BMI	23.0 ± 3.00	22.5	16.8-32.7	22.5 ± 2.71	22.0	19.2-31.0	23.6 ± 3.19	22.9	16.8-37.2
Birth weight (g)	3064 ± 446	3200	2400-4000	2989 ± 446	2950.0	2400-3750	3130 ± 434	3200	2500-4000
PI	2.53 ± 0.38	2.54	1.78-3.51	2.50 ± 0.37	2.48	1.78-3.16	2.57 ± 0.39	2.56	1.85-3.51
Head circumference (cm)	33.58 ± 1.45	33.50	30.0-37.5	33.6 ± 1.13	34.0	32.0-36.0	33.6 ± 1.72	33.0	30.0-37.5
Parity	1.53 ± 0.88	1	1-5	1.61 ± 0.88	1	1-5	1.45 ± 0.89	1	1-4
MDI	104.6 ± 4.97	104.5	93-113	104.6 ± 5.09	105.0	93-113	104.6 ± 4.96	104	96-113
PDI	102.4 ± 9.53	103.0	81-126	103.7 ± 10.3	104.0	81-126	101.3 ± 8.81	100	81-117

BMI : body mass index = body weight (kg) / height (m)

PI : ponderal index (fetus) = (body weight (g) × 100) / (height (cm))^3

MDI : mental development index

PDI : physical development index

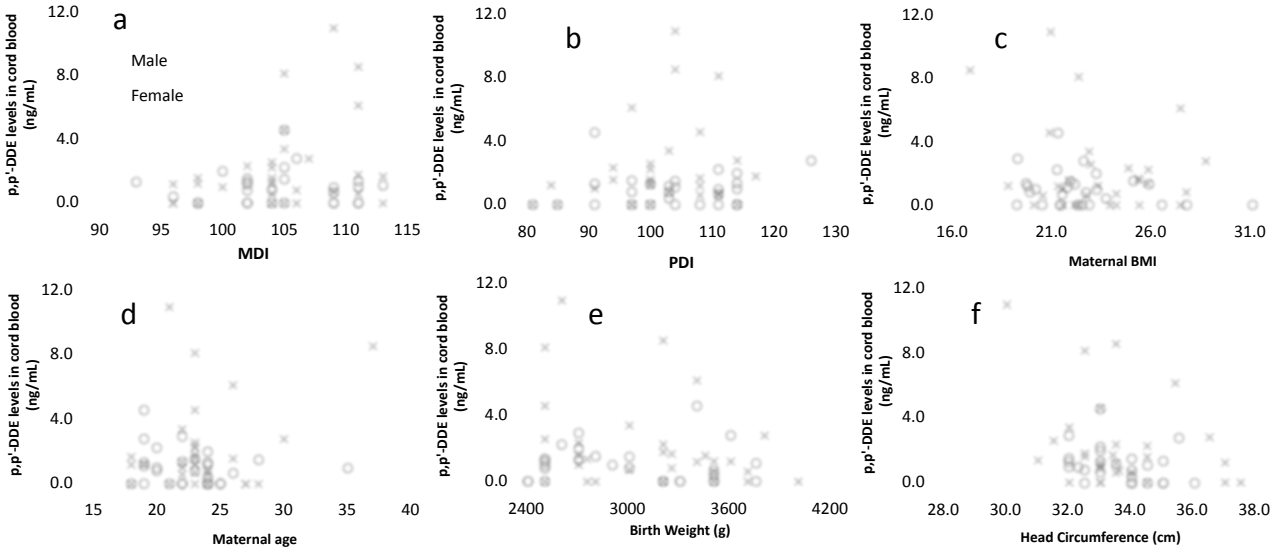

Fig.3. (a) Relationship between MDI and *p,p'*-DDE levels in cord blood. (b) Relationship between PDI and *p,p'*-DDE levels in cord blood. (c) Relationship between maternal BMI and *p,p'*-DDE levels in cord blood. (d) Relationship between aternal age and *p,p'*-DDE levels in cord blood. (e) Relationship between BW and *p,p'*-DDE levels in cord blood. (f) Relationship between HC and *p,p'*-DDE levels in cord blood.

characteristics of the 59 fetuses and their mother was not evaluated in our study.

Table 1 shows that female's detected ratio and detected levels of DDTs are higher than male's. However, as listed in Table 2, no significant differences of each parameter between male and female were observed. It was suggested that DDTs

might have some kinds or another influence on the fetus and their developments.

In our study, no significant relationships between *p,p'*-DDE, MDI and PDI were observed (Fig. 3). These results reflected the influence gained for infancy because MDI and PDI were not measured after birth but for infancy typically. So it may be difficult only for DDTs levels in cord blood samples to evaluate influences of DDTs on fetus.

There are no significant association between maternal characteristics and *p,p'*-DDE in our study (Fig.3). In general, it is reported that DDTs levels in maternal blood tend to increase in proportion to maternal BMI.[5] Although we examined only the correlation between DDTs in cord blood and maternal BMI, *p,p'*-DDE levels ware not associated with BMI. It suggested that the rate of transfer of DDTs from pregnant woman to fetus was not invariable. Because of narrow range of maternal age that we investigated in this study, *p,p'*-DDE levels were also not associated with maternal age.

We found a week negative relationship between *p,p'*-DDE concentration and fetal head circumference and birth weight. These results suggested that DDTs might have potential influences on fetal developments.

We found the mean of *p,p'*-DDE levels in cord blood tend to decrease with increase of parity (Fig.4). About 0.4 ng/mL of mean *p,p'*-DDE levels decreased with increase of one time of parity. This result shows that DDTs levels in woman may decrease by transfer to fetus and breast-feeding experience. It suggested that DDTs risks for fetus might decrease with increase of parity.

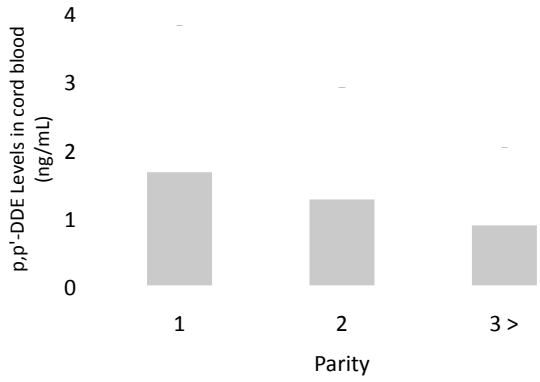

Fig. 4. Relationship between mean p,p'-DDE levels and parity.

4. Reference

[1] G. Devanathan, A. Subramanian, M. Someya, A. Sudaryanto, T. Isobe, S. Takahashi, P. Chakraborty, S. Tanabe, Env.iron Pollution 157 (2009) 148.

[2] M. Mariscal-Arcas, C. Lopez-Martinez, A. Granada, N. Olea, M.L. Lorenzo-Tovar, F. Olea-Serrano, Food and Chem. Toxicology 48 (2010) 1311.

[3] Enrique M. Ostrea Jr., Dawn M. Bielawski, Norberto C. Posecion Jr., Melissa Corrion, Esterlita Villanueva-Uyb, Yan Jina, James J. Janissec, Joel W. Ager, Environ. Research 106 (2008) 277.

[4] M. Falcón, J. Oliva, E. Osuna, A. Barba, A. Luna, Toxicology 195 (2004) 203.

[5] M. S. Wolff, J. A. Britton, Susan L. Teitelbaum, S. Eng, E. Deych, K. Ireland, Zhisong Liu, A. Neugut, R. M. Santella, M. D.Gammon, Cancer Epidemiol Miomakers Prev. 9 (2005) 14