FIVE YEARS STUDIES ON THE LONG TERM EFFECTS OF WAR AGENT ORANGE / DIOXIN ON HUMAN HEALTH IN VIETNAM

Kido T[1], Maruzeni S[1,2], Suzuki H[1], Odamae Y[1], Muranaka M[1], Naganuma R[1], Tawara K[2], Nishijo M[2], Nakagawa H[2], Hung TM[3], Thom LTH[3], Dung PT[3], Nhu DD[1,3], Oka H[4], Noguchi K[4]

[1]Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Japan; [2]Department of Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa, Japan; [3]Division for Mitigation of the Consequences of the Chemicals used during the War on Human Health (10-80 Division), Vietnam Ministry of Health, 35 Nguyen Huy Tuong, Thanh Xuan, Ha Noi, Viet Nam; [4]Environmental Science Department, Ishikawa Prefectural Institute of Public Health and Environmental Science, 1-11 Taiyogaoka, Kanazawa, Japan

Abstract

Over 35 years have passed since herbicides were sprayed during Vietnam War. To clarify the relation of dioxins levels in human breast milk with health effects, epidemiological studies have been conducted in sprayed and non-sprayed areas in Vietnam since 2002. Breast milk samples were collected from lactating females aged between 20-30 years old in both communes. Biochemical indications in serum and the measurement data on bodies of their infants who were fed by their breast milk were compared between two areas. As results, significant difference between the two communes was recognized in TEQ for total PCDDs, total PCDFs) and sum of total PCDDs and PCDFs for both primiparae and multiparae ($p<0.001$). No significant difference was found between biochemical indicators in sera or body measurements of mothers and their children except for only a few indicators. Statistically significant relations were seen between total PCDD/Fs and CH-E or chest measurements of infants. Furthermore, we will have to pursue a critical indicator for dioxin-induced human health effects in the epidemiological study in Vietnam.

Introduction

Between 1962 and 1971 in Vietnam War, the US military sprayed herbicides mainly in the south part of Vietnam; the operation was named Ranch Hand. Organic chlorine herbicides including Agent Orange was contaminated with dioxin, which is a kind of endocrine disruptors chemicals (EDCs). In 1974, the US National Academy of Sciences published estimates of the extent and distribution of herbicides sprayed, but a recent study reported that the spray inventory with heavily dioxin-contaminated herbicides is estimated to be seven million liters more and that the amount of dioxin sprayed in estimated to be almost doubled.[1] There are more than 220 kinds of isomers in dioxins and each isomer has different strength of toxicity. The international Agency for Research on Cancer Committee classified TCDD as a carcinogen in humans. There were some studies on health effect due to sprayed herbicides in the Vietnam War, but their study subjects were American or Korean veterans who were exposed to dioxins in Vietnam during the Vietnam War.[2,3] However, studies in Vietnamese have rarely been published internationally. Furthermore, in most former studies, dioxin concentrations were analyzed by pooling samples. Breast milk were collected from some people and serum samples were from about one hundred people.[4] Therefore, it is very difficult to show an individual exposure from the dioxin concentrations. But, it is still important to make clear an individual concentration of internal dioxins in order to investigate health effects. Besides, the dioxin studies of herbicides in the Vietnam War reported mainly TCDD and did not take note to other isomers and appropriate control areas where socioeconomic back ground was consistent with exposed areas have not been selected in the former studies. In this study, dioxins in human breast milk and their relation with health effects are shown.

Materials and Methods

1. Subjects

The study population consisted of the subjects resident in sprayed area or non-sprayed area which had been once separated by the demilitarized zone of latitude 17 degrees north line, a military boundary in the Vietnam War. Cases attributed to exposed area to herbicide operation were obtained from Cam Chinh commune located in Quang Tri province. Otherwise the control group was Cam Phuc commune in Ha Tinh province, which had not experienced herbicide operation during the War. The present outline of sprayed and non-sprayed areas of herbicides in the Vietnam War is shown in Table 1. In September 2002 and July 2003, breast milk samples were collected from lactating females aged between 20-30 years old in both communes. Subjects were explained on the study purpose by each local committee people. 84 lactating mothers in Cam Chinh commune and 72 lactating mothers in Cam Phuc commune donated milk samples after all of them consented to cooperation using document written in Vietnamese. The domiciles of all volunteers in each province were confirmed. Participants provided 10-20 ml volume of milk. Samples were collected by hands of mothers themselves, local medical staffs or training researchers at each local clinic. All samples were frozen immediately after collection. At the same time, 27 soil samples including 4 sediment samples were collected from 2 areas to determine the dioxin levels. In August 2004, to compare the laboratory results of biochemical indications in serum of each nursing mothers in the both areas, 88 people in 119 lactating mothers in sprayed area and 49 people in 100 mothers in non-sprayed area participated and 90 their infants in sprayed area and also 53 their infants in non-sprayed area participated to show the relation of dioxins concentration in breast milk with the measurement data on bodies of infants who were fed by those breast milk.

2. The measurement of the dioxins concentration in breast milk and biochemical indications

The measurement of the dioxins concentration was proceeded with reference to “the conditional manual for the measurement of dioxins in breast milk” (Ministry of Health, Labour of chemical substance research group in Japan.1999). All extracts were subject to a series of chromatographic clean-up steps prior to analysis for PCDDs and PCDFs by a high resolution mass spectrometer (JEOL MStation-JMS700) equipped with a gas chromatograph (HP-6890), and measurements were performed by selected ion monitoring (SIM) method. Regarding with the sensitivity of the HRMS, at a signal-noise ratio (S/N) = 3, detection limit of 0.3 pg/g fat and absolute measurement limit of 0.08 pg/g fat were achieved. In addition, revised pretreatment procedure, which used only less than 10g of breast milk and kept high accuracy, was introduced in this study, while about 50g of breast milk was necessary in the broadly accepted method.[5] Recoveries of the ^{13}C-2,3,7,8-substitued PCDDs and PCDFs were 75-90% for a 10ml breast milk sample, which agreed with the recovery range regulated by the Japanese Industrial Standard (JIS). Concentration levels of dioxins were showed by actual measurement values and ones converted to 2,3,7,8-TCDD toxic equivalents (TEQ), submitting the internal Toxicity Equivalent Factor (TEF) of WHO-TEF (1997). Depending on isomer, concentration value in some milk samples was not detectable (ND) or did not meet quantification criteria (NDR). In either occurrence, half the detection level was employed to estimate value in lipid basis. Blood samples were collected by local medical staffs or researchers and they were soon centrifuged to obtain serum. All sera were immediately frozen after collection, kept and sent to Japan. Researchers measured the height, weight, circumference of head and chest on each infant. A Research Report of Body Growth of Infant: 2000 by Ministry of Health, Labour and Welfare in Japan was referred for instruments and methods of body measurement. To measure height of infants, supine position type was used. Digital baby scale for weight with minimum scale of 2 g at least, certified by national authorization, was also used. This scale was adjusted by gravity difference of latitude for Vietnam.

Results and Discussion

In the soil samples, geometric mean of ΣPCDDs/DFs (TEQ for total PCDDs/DFs) in sprayed area was significantly higher than that in non-sprayed area. In the breast milk samples, significant difference between the two communes was recognized in ΣPCDDs (TEQ for total PCDDs), ΣPCDFs (TEQ for total PCDFs) and ΣPCDDs/DFs (TEQ for total PCDDs/DFs) for both primiparae and multiparae ($p<0.001$). Furthermore ΣPCDDs level was approximately same as ΣPCDFs level for either commune independently of their parity. In the laboratory results of sera in mothers between two areas, statistically significant difference was only showed in creatinine although mean creatinine of non-sprayed area was higher than that in sprayed area ($p<0.01$). In simple correlation coefficients between TEQ- PCDD/Fs in breast milk and laboratory results in serum of mothers, significant correlations were shown between total PCDD/Fs and CH-E or LDH. Both of them were negative correlation ($p<0.05$). Results of multiple regression analysis of mothers' CH-E or LDH in sera as dependent variables and TEQ-PCDD/Fs in breast milk or mothers' age as explanatory variables were shown as significant relation between total PCDD/Fs and CH-E ($p<0.05$) in all samples. When excluding two outliers, statistically significant relations were seen between total PCDD/Fs and CH-E ($p<0.01$) or LDH ($p<0.05$) In mean age, body measurement of mothers (height, weight and BMI) and children (height, weight, circumference of head and chest measurement) in two areas, mothers' mean age in the sprayed area was significantly higher than that in the non-sprayed area ($p<0.01$) while mean height and weight in the non-sprayed area were higher than those in the sprayed area ($p<0.01$). But, no significant difference in BMI of mothers, age and all of body measurement values of infants was shown between two areas. In simple correlation coefficients among age, results of body measurements of mothers and children, and TEQ-PCDD/Fs in breast milk, statistically significant negative correlations with Total PCDD/Fs were showed between weight and chest measurements of infants ($p<0.05$). Results of multiple regression analysis with weight or chest measurements of children as dependent variables and TEQ-PCDD/Fs in breast milk (geometric mean) and age of infants as explanatory variables were shown as significant relations between Total PCDD/Fs and weight or chest measurements of infants ($p<0.01$) in all subjects. In addition, statistically significant relation was only seen between total PCDD/Fs and chest measurement of infants ($p<0.01$) when excluding two outliers. Although some indicators associated with dioxin levels, we will have to pursue a critical indicator for dioxin-induced human health effects in the epidemiological study in Vietnam.

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Table1 The outline at present of sprayed and non-sprayed area of herbicides in the Vietnam War

	Sprayed area (QT)	Non-sprayed area (HT)
Rate of agricultural pursuer	63%	83%
Annual income	1,274,390VND	1,180,000VND
The amount of the agricultural chemicals used (/ family / year)	74 ℓ	374 ℓ
Food source of supply	Within district	Without district
Cooking oil	Lard	Vegetable oil
Rate of smoker	20 %	13 %

VND: Vietnam Dong (Vietnamese currency)

QT: Cam Chinh commune, Quang Tri province; HT: Cam Phuc commune, Ha Tinh province

Table2 PCDDs/DFs-TEQ levels in breast milk from mothers in QT and HT

parturient woman	Primiparae					Multiparae				
Areas	QT		HT			QT		HT		
Number of subjects	N = 26		N = 36			N = 58		N = 36		
Age	24.0±3.97		23.1±3.29			29.7±4.88		23.1±3.08		
Isomers (pg TEQ/g lipid)	Mean	S.D	Mean	S.D		Mean	S.D	Mean	S.D	
ΣPCDDs	5.60	(1.58)	2.31	(1.39)	***	3.68	(1.81)	1.63	(1.71)	***
ΣPCDFs	6.15	(1.52)	2.19	(1.43)	***	4.00	(2.02)	1.55	(1.49)	***
ΣPCDDs/Fs	11.75	(1.51)	4.49	(1.36)	***	7.68	(1.88)	3.19	(1.54)	***

Values are geometric mean (geometric SD) except for age.

TEQ was calculated by TEF of WHO-TEF (1997).

TEQ: Toxic Equivalent, TEF: Toxic Equivalency Factor.

QT: Cam Chinh commune, Quang Tri province; HT: Cam Phuc commune, Ha Tinh province

***: $p<0.001$

Table 3 Multiple regression analysis of dependent variables for weights and chest measurement of children with TEQ-PCDD/Fs in breast milk and age of children

	N	TEQ-PCDDs/Fs	p-value	Age of children	p-value	R
CH-E	43	-0.372	*	0.174		0.380
LDH	43	-0.297		-0.155		0.359
Weights of children	43	-0.296	**	0.703	**	0.770
Chest measurement of children	43	-0.359	**	0.530	**	0.648
CH-E	41	-0.434	**	0.090		0.462
LDH	41	-0.374	*	-0.198		0.384
Weights of children	41	-0.187		0.742	**	0.745
Chest measurement of children	41	-0.333	**	0.580	**	0.635

β: standardized partial regression coefficient, R: Multiple correlation coefficient.

*: $p<0.05$, **: $p<0.01$.