THE RELATIONSHIP BETWEEN VISUAL ACUITY AND DIOXIN LEVELS OF BREAST MILK IN THE HERBICIDE SPRAYED AND NON-SPRAYED AREAS IN VIETNAM

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Abstract

The purpose of this study is to clarify the relationship between visual acuity and dioxin levels of breast milk in Vietnamese mothers considering nutritional status and daily living condition as confounding factors. Subjects consist of 80 mothers in herbicide sprayed area and 53 mothers in non-sprayed area in Vietnam. Dioxin levels in breast milk were significantly higher in mothers of sprayed area than those of non-sprayed area. Visual acuity was lower in mothers of sprayed area than those of non-sprayed area. However, it was not significantly different. There was no significant difference among several biochemical indicators, nutritional status and living condition between sprayed and non-sprayed areas, either. Considering nutritional status and daily living condition as confounding factors, visual acuity had no significant association with explanatory variables such as dioxin levels, nutritional status and living condition using step-wise multiple regression analysis. Further research should be needed at hot spots in Vietnam to confirm their relation.

Introduction

Since 2002, we have been conducted collaborative research on the effect of herbicide to inhabitants in herbicide sprayed and non-sprayed areas in Vietnam. It has been found that dioxin levels in human serum, breast milk and adipose tissues in sprayed area were significantly higher than those in non-sprayed area as well as dioxin levels of soil even after about 40 years passed since herbicide was sprayed[1]. However, human health effects by herbicide were clarified limitedly. As facts, in serum indicators, total bilirubin was significantly higher in sprayed area although alkali phosphatase was significantly lower in sprayed area. In body measurement, chest circumstance of children in sprayed area was significantly lower although no significant difference was seen between body height, weight and head circumstance[1]. Visual acuity of mothers and pupils in sprayed area was significantly lower that in sprayed area[2,3]. The purpose of this study is to clarify the relationship between visual acuity and dioxin levels of breast milk in Vietnamese mothers considering nutritional status and living condition

as confounding factors.

Material and Methods

1. Subjects

The study population consisted of the subjects residing 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. In 2002 and 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. 80 lactating mothers in Cam Chinh commune and 53 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. Among them, blood was taken from 70 mothers in sprayed area and 27 mothers in non-sprayed area.

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

The measurement of the dioxins concentration was preceded 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. 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). 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. Several indicators as follows were analyzed at the commercial laboratories; Total protein and its fraction, iron, vitamin A, B_1, B_2 and B_{12}.

3. Survey on nutritional status and living condition

The Food frequency questionnaire (FFQ) for Vietnamese developed by Kusama was used for survey on nutritional status. The living condition was also interviewed using questionnaire by Vietnamese researchers.

Results and Discussion

Dioxin levels in breast milk were significantly higher in mothers of sprayed area than those of non-sprayed area (Table 1). Visual acuity was lower in mothers of sprayed area than those of non-sprayed area. However, it was

not significant different (Table 2). There were no significant difference among several biochemical indicators, nutritional status and living condition between sprayed and non-sprayed areas, either. Relationship between each indicator was examined and significant simple correlation coefficient was only shown between visual acuity and vitamin A or some of vitamin B. Nutritional Status using FFQ was shown in Table 3. Retinol and total fatty acid (FA) levels are significantly higher in sprayed area than non-sprayed area. Considering nutritional status and living condition as confounding factors, visual acuity had no significant association with explanatory variables such as dioxin levels, nutritional status and living condition even though some variables such as present history of diseases, difference of area and vitamin B_{12} were selected using step-wise multiple regression analysis (Table 4). The reason why significant difference of visual acuity disappeared at the examination in 2007 might be intervened by the former examination in 2005. It means that some persons who showed low acuity improved during two years. The limitation of this study is that sample of breast milk was collected in 2002 and 2003. The time discrepancy has 4 or 5 years. Even though half life of dioxins is 7 – 11 years, it weakens the relationship between dioxin levels and other factors such as visual acuity, nutritional status and living condition. Therefore, further research should be needed at hot spots in Vietnam to confirm their relation.

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Table1: Comparison of dioxin level in breast milk between herbicide sprayed and non-sprayed areas

		Sprayed area N=70	Non-sprayed area N=27	
T4CDD	[pgTEQ/g Fat]	0.95 ± 0.68	0.60 ± 0.22	*[1)]
T4CDF	[pgTEQ/g Fat]	0.06 ± 0.03	0.12 ± 0.05	***[1)]
TEQ-PCDDs	[pgTEQ/g Fat]	5.20 ± 3.43	1.99 ± 0.58	***[1)]
TEQ-PCDFs	[pgTEQ/g Fat]	6.35 ± 4.52	1.88 ± 0.76	***[1)]
TEQ-Total	[pgTEQ/g Fat]	11.54 ± 7.49	3.86 ± 1.28	***[1)]

n.s: not significant, *: p<.05, ***: p<.001

This data is dioxin level in breast milk which was taken in 2002 & 2003

Table2: Comparison of visual acuity between herbicide sprayed area and non-sprayed area

			Sprayed area N=70		Non-sprayed area N=27	
Visual acuity in 2007	Right Eye	***[2)]	1.4 ± 0.4	***[2)]	1.5 ± 0.4	n.s[1)]
	Left Eye		1.3 ± 0.4		1.5 ± 0.5	n.s[1)]
Visual acuity in 2005	Right Eye		1.1 ± 0.4		1.5 ± 0.4	***[1)]
	Left Eye		1.1 ± 0.4		1.5 ± 0.4	***[1)]

[1)] Mann-Whitney test, [2)] Paired t test
n.s: not significant, ***: p<.001

Table3: Comparison of Energy and Nutrient Intakes from FFQ in sprayed and non sprayed.

Nutrients		Energy-adjusted[b] sprayed (n = 80)	non sprayed (n = 53)	P-value[a]	Vietnamese RDA age18-60
Energy	(kcal)	1854 (1440 - 2423)	1885 (1423 - 2253)	n.s.	2100-2600[1)]
Protein	(g)	87.2 (78.5 - 92.9)	86.6 (81.9 - 93.9)	n.s.	55
Lipid	(g)	52.8 (45.3 - 60.0)	57.9 (52.6 - 62.0)	*	
Carbohydrate	(g)	290.4 (270.4 - 312.4)	275.8 (268.9 - 290.2)	*	
Fiber	(g)	12.8 (10.9 - 16.0)	15.0 (12.3 - 19.4)	*	
Ash	(g)	23.9 (22.3 - 27.5)	27.0 (25.0 - 29.2)	**	
Vitamin A[2)]	(μg)	31.9 (27.3 - 34.6)	32.5 (28.6 - 35.4)	n.s.	500
Retinol	(mcg)	341.2 (239.8 - 428.9)	242.8 (143.9 - 345.5)	***	
Carotin	(mcg)	8080.3 (6050.1 - 10015.8)	9027.1 (7083.7 - 10885.1)	n.s.	
Vitamin B_1	(mg)	1.4 (1.2 - 1.6)	1.6 (1.4 - 1.7)	***	0.9
Vitamin B_2	(mg)	1.3 (1.2 - 1.5)	1.5 (1.3 - 1.7)	**	1.3
Niacin	(mg)	16.1 (15.1 - 17.5)	16.6 (14.8 - 18.6)	n.s.	14.5
Vitamin C	(mg)	217.9 (183.0 - 262.6)	238.1 (193.0 - 310.0)	n.s.	70
Calicium	(mg)	898.7 (775.9 - 1046.1)	942.9 (850.0 - 1117.9)	n.s.	500
Phosphorus	(mg)	1122.5 (1034.5 - 1212.8)	1181.8 (1103.4 - 1268.8)	n.s.	
Iron	(mg)	25.9 (22.1 - 29.8)	26.1 (21.9 - 30.2)	n.s.	24
Sodium	(mg)	3733.1 (3336.4 - 4673.7)	4313.1 (3978.3 - 4978.0)	**	
potassium	(mg)	3054.2 (2744.0 - 3365.2)	3419.3 (2883.0 - 3846.5)	*	
Magunesium	(mg)	336.3 (295.2 - 391.1)	346.7 (304.4 - 429.8)	n.s.	
Zinc	(mg)	8.4 (7.5 - 9.5)	9.4 (8.0 - 11.0)	*	
Manganese	(mg)	81.6 (38.8 - 143.6)	58.0 (23.1 - 140.6)	n.s.	
Copper	(mcg)	1200.5 (1110.9 - 1365.4)	1224.1 (1107.9 - 1326.6)	n.s.	
Fluorine	(mcg)	285.6 (200.5 - 439.7)	217.9 (142.0 - 408.1)	n.s.	
Iodine	(mcg)	6.9 (4.8 - 8.8)	9.0 (7.6 - 12.0)	***	
Selenium	(mcg)	59.1 (46.7 - 69.7)	58.8 (48.1 - 69.1)	n.s.	
Total FA	(g)	0.4 (0.2 - 0.7)	0.2 (0.1 - 0.3)	***	
Palmitic	(g)	3.3 (2.6 - 4.3)	3.0 (2.1 - 4.1)	n.s.	
Stearic	(g)	2.0 (1.5 - 2.7)	1.9 (1.4 - 2.6)	n.s.	
Linoleic	(g)	2.5 (2.1 - 3.0)	2.3 (1.9 - 2.9)	n.s.	
Linolenic	(g)	0.2 (0.1 - 0.2)	0.2 (0.1 - 0.2)	n.s.	
Cholesterol	(g)	228.3 (172.3 - 267.6)	178.9 (130.5 - 248.5)	*	

Date sre Median and 25-75th percentile.

[1)]Vietnamese RDA for women (kcal)

Workload	light	medium	heavy
Age 18-30	2200	2300	2600
30-60	2100	2200	2500

[2)]Caluculated conventionally as retinol + 1/6of carotin.
[a]Wilcoxon signed rank test
[b]Nutrient intake were adjusted for energy intake by the residual method.
*P<0.05 **P<0.01 ***P<0.001

Table4: Relation of visual acuity and various explanatory variables using stepwise multiple regression analysis

Right visual acuity N=97	β	P value	Left visual acuity N=97	β	P value
Present history of Eye disease (1=Yes,2=No)	0.18	0.10	Areas (1: Sprayed area, 2: Non-Sprayed area)	0.17	0.12
Fe	-0.17	0.12	Present history of Eye disease (1=Yes,2=No)	0.14	0.19
Vitamin B_{12}	0.16	0.14			
R^2	0.052		R^2	0.053	

β: Standardized partial regression coefficient
R^2: Adjusted R square