ASSOCIATION BETWEEN DIOXIN CONCENTRATIONS IN BREAST MILK AND DIETARY NUTRIENT INTAKE IN VIETNAM

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Abstract

Dioxins have been reported to enter human breast milk through the food chain. However, in Vietnam the relationship between the consumption of different foods in the diet and dioxin concentrations in breast milk remains unclear. Therefore we investigated the association between dioxin concentrations in breast milk and dietary nutrient intake in herbicide sprayed and non-sprayed areas in Vietnam. Eighty mothers of sprayed area and 42 mothers of non-sprayed area participated in the study and dietary intake was assessed by food frequency questionnaire. Breast milk was analyzed for the concentration of PCDDs and PCDFs. It was made clear that past exposure rather than present dietary intake may affect present dioxin concentrations in breast milk in the sprayed area in Vietnam. In contrast, it is suggested that present dietary intake and BMI might affect those in the non-sprayed area. Therefore, data do not indicate the influence of exposure largely via usual dietary intake in the sprayed area in comparison with the non-sprayed area in Vietnam. Hence, this study suggests that present dioxin concentrations in breast milk were maintained by continuous past exposure even after 30-40 years had passed.

Introduction

During the Vietnam War (1961-1971), herbicide mixtures were sprayed over forests and villages in Central and Southern Vietnam to defoliate the vegetation. Previous studies have reported that huge amount of dioxins were still involved in samples of soil, sediment, food, human blood and breast milk in southern Vietnam, even though the Agent Orange contamination occurred 30-40 years before sampling[1-3]. There is a potential for continuous dioxin exposure through the consumption of fish and meat from contaminated areas. It has been reported that the largest source of dioxin contaminants is dietary intake, which accounts for greater than 90% of total exposure[4]. Previously in Vietnam animal meat was examined; however, the nutritional investigations in herbicide sprayed areas in Vietnam have been inadequate. For these reasons, more detailed information is needed to elucidate the role of routine dietary intake in human exposure to dioxin 30-40 years after Agent Orange use was discontinued in Vietnam. The purpose of this study is to clarify the association between dioxin concentrations in breast milk and nutrient intake in herbicide sprayed and non-sprayed areas in Vietnam and also to investigate the specific factors influencing dioxin levels in breast milk.

Materials and Methods

Study areas designated in the north-central area of Vietnam, namely the Cam Chinh commune in the Cam Lo

district of Quang Tri province, where herbicides were sprayed during the war, and the Cam Phuc commune in the Cam Xuyen district of Ha Tinh province, which was not sprayed with herbicides during the war. These two communes were once separated by the demilitarized zone (DMZ), the 17th parallel that divided the country during the Vietnam War. These two communes are similar in terms of their socio-economic make-up, customs, ethnic groups, and health care systems[5]. Subjects were 158 mothers, namely 90 mothers in sprayed and 68 mothers in non-sprayed areas. All of them attended the survey of long term effects of dioxin on human health held in 2002-2003. They were between 20-30 years old and provided 10-20 ml of breast milk for analysis of dioxin concentrations. Among them 122 mothers, namely 80 mothers (88.9%) in herbicide sprayed area and 42 mothers (61.8%) in non-sprayed area were participated in this study conducted in August of 2007. Dietary intake was assessed by a food frequency questionnaire (FFQ) for Vietnamese. This questionnaire was developed by Kusama[6] as a tool to estimate the habitual nutrient intake of the Vietnamese population. The FFQ is a standard tool in nutritional epidemiology and calculates the intake of nutrients. The intake of calories and nutrients were computed by multiplying the frequency of intake for each food item by the nutrient content of the specified portion size. The reproducibility and validity of this FFQ were established by using 24-hour dietary recalls (24HRs) and repeated FFQ.

Results and Discussion

Table 1 summarizes the median intake (with 25 and 75 percentiles) of energy and each nutrient per day in sprayed and non-sprayed areas and a comparison of the difference between the two areas. The intake of retinol, total FA and cholesterol in the sprayed area were higher than those in the non-sprayed area. By contrast, the intake of non-animal protein, lipid, non-animal lipid, fiber, ash, vitaminB_1, vitaminB_2, phosphorus, sodium, potassium, zinc and iodine in the non-sprayed area were higher than those in the sprayed area. The concentrations of TEQ levels of PCDDs and PCDFs and Total (PCDDs + PCDFs) in breast milk in sprayed and non-sprayed areas were shown in Table 2. There were statistical differences ($p<0.001$) in TEQ-PCDDs, TEQ-PCDFs and TEQ-Total between sprayed and non-sprayed areas, respectively. Simple correlations of dioxin concentrations in breast milk with the nutrient intake were not significant in the two areas, respectively. Table 3 shows multiple linear regression in all subjects (sprayed and non-sprayed areas). Multiple linear regression analysis found that only area was associated with TEQ-PCDDs TEQ-PCDFs. In the sprayed area small adjusted R^2 values of regression were revealed in Table 4. In the non-sprayed area the adjusted R^2 values of regression were higher than those in the sprayed area in Table 5. In the non-sprayed area all models found an association between BMI and TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total. TEQ-PCDDs were highly associated with intake of ash and TEQ-PCDFs and TEQ-Total were associated with intake of linoleic. These results showed that location (sprayed or non-sprayed areas) was the highest contribution to dioxin concentrations in breast milk. It is noted that difference of location is a greater factor than present dietary intake; that is, it is the strongest contributor to dioxin concentrations in breast milk, regardless of type. Incidentally, because the average age of subjects in this study was 31.8±5.5, it is suggested that present dioxin

accumulation in human tissue was influenced by breast-feeding by mothers who were exposed directly to a herbicide, and that dioxin concentrations in breast milk were influenced by intake of foods with high concentrations of dioxin, even if almost all subjects were born after 1977, well after the cessation of herbicide spraying. In consequence it was made clear that past exposure rather than present dietary intake affect present dioxin concentrations in breast milk in the sprayed area in Vietnam. In contrast, it is suggested that present dietary intake and BMI might affect those in the non-sprayed area. Therefore, data do not indicated the influence of exposure largely via usual dietary intake in the sprayed area in comparison with the non-sprayed area in Vietnam. Hence, this study suggests that present dioxin concentrations in breast milk were maintained by continuous past exposure even after 30-40 years had passed.

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Table 1 Energy and nutrient intake from FFQ in herbicide sprayed and non-sprayed areas

Nutrients (per day)		Sprayed area（n = 80）			Non sprayed area (n =42)			p[a)]
Energy	(kcal)	1854	1440	2423	1793	1389	2225	0.620
Protein	(g)	87.2	78.5	92.9	87.6	83.3	95.7	0.227
Animal Protein	(g)	46.8	39.7	56.6	50.2	43.5	57.0	0.460
Non-animal Protein	(g)	34.7	31.3	37.8	37.8	33.4	41.1	0.037
Lipid	(g)	52.8	45.3	60.0	56.7	52.3	61.7	0.036
Animal Lipid	(g)	24.8	19.4	31.0	24.8	19.1	30.7	0.842
Non-animal Lipid	(g)	24.9	21.1	28.5	29.5	24.6	33.9	<0.001
Carbohydrate	(g)	290.4	270.4	312.4	276.9	269.1	294.7	0.059
Fiber	(g)	12.8	10.9	16.0	15.0	12.4	19.4	0.018
Ash	(g)	23.9	22.3	27.5	27.1	25.3	29.2	0.002
Retinol	(μg)	341.2	239.8	428.9	235.0	139.6	344.5	0.001
Carotene	(μg)	8080.3	6050.1	10015.8	9174.3	6958.1	11047.5	0.152
Vitamin B1	(mg)	1.4	1.2	1.6	1.6	1.4	1.8	0.001
Vitamin B2	(mg)	1.3	1.2	1.5	1.5	1.3	1.7	0.007
Niacin	(mg)	16.1	15.1	17.5	16.8	15.1	18.7	0.110
Vitamin C	(mg)	217.9	183.0	262.6	227.6	188.3	301.8	0.518
Calcium	(mg)	898.7	775.9	1046.1	976.1	844.3	1119.4	0.153
Phosphorus	(mg)	1122.5	1034.5	1212.8	1207.7	1139.4	1278.4	0.008
Iron	(mg)	25.9	22.1	29.8	27.1	22.6	31.1	0.647
Sodium	(mg)	3733.1	3336.4	4673.7	4240.6	3968.2	4957.5	0.022
Potassium	(mg)	3054.2	2744.0	3365.2	3419.7	2947.9	3858.0	0.020
Magnesium	(mg)	336.3	295.2	391.1	341.8	304.5	431.6	0.568
Zinc	(mg)	8.4	7.5	9.5	9.5	8.2	10.8	0.005
Manganese	(mg)	81.6	38.8	143.6	62.1	26.7	140.1	0.221
Copper	(μg)	1200.5	1110.9	1365.4	1239.9	1123.1	1328.1	0.880
Fluorine	(μg)	285.6	200.5	439.7	218.2	148.8	399.3	0.125
Iodine	(μg)	6.9	4.8	8.8	9.0	7.7	12.3	<0.001
Selenium	(μg)	59.1	46.7	69.7	60.5	52.2	70.4	0.269
Total FA	(g)	0.4	0.2	0.7	0.2	0.1	0.3	<0.001
Palm tic	(g)	3.3	2.6	4.3	2.8	2.1	4.0	0.074
Stearic	(g)	2.0	1.5	2.7	1.7	1.4	2.6	0.126
Linoleic	(g)	2.5	2.1	3.0	2.2	1.9	2.9	0.126
Linolenic	(g)	0.2	0.1	0.2	0.2	0.1	0.2	0.572
Cholesterol	(mg)	228.3	172.3	267.6	177.3	123.9	247.6	0.006

Data are shown at median (25-75th percentile).

Nutrient intake were adjusted for energy intake by the residual method.

[a)]Wilcoxon signed rank test.

Table 2 Comparison of dioxin concentrations in breast milk between herbicide sprayed and non-sprayed areas

	Sprayed area (n=80)	Non sprayed area (n=42)	p
TEQ-PCDDs[pgTEQ/gFat]	1.51 (1.08 - 1.86)	0.63 (0.44 - 0.85)	<0.001[a)]
TEQ-PCDFs[pgTEQ/gFat]	1.62 (1.21 - 2.07)	0.69 (0.34 - 0.87)	<0.001[b)]
TEQ-Total[pgTEQ/gFat]	2.32 (1.87 - 2.65)	1.34 (1.09 - 1.57)	<0.001[b)]

Data are shown at median (25-75th percentile).

Data were log-transformed to improve normality.

[a)] t-test, [b)] Wilcoxon signed rank test.

Table 3 Stepwise multiple linear regression of TEQ-PCDDs, TEQ-PCDFs, TEQ-Total levels in breast milk and nutrient intake in all subjects (n=122)

	Standardized coefficients	p	Adjusted R^2
TEQ-PCDDs			
Constant		<.0001	.367
Area	.610	<.0001	
TEQ-PCDFs			
Constant		.320	
Area	.686	<.0001	.472
Fluorine	.257	.001	
Non-animal Lipid	.223	.004	
Zinc	-.138	.068	
TEQ-Total			
Constant		.605	
Area	.683	<.0001	
Fluorine	.236	.002	.469
Non-animal Lipid	.203	.008	
BMI	.177	.013	
Zinc	-.146	.054	

Data of dioxin level were log-transformed for analysis.

Variables : Code of Area (1,sprayed area ; 0,non-sprayed area),Age,BMI,Monthly income of husband, Energy,Protein,Animal Protein,Non-animal Protein,Lipid,Animal Lipid,Non-animal Lipid, Carbohydrate,Fiber,Ash,Retinol,Caroten,VitaminB_1,VitaminB_2,Niacin,VitaminC,Calcium, Phosphorus,Iron,Sodium,Potassium,Magnesium,Zinc,Manganese,Copper,Fluorine,Iodine, Selenium,Total FA,Palmitic,Stearic,Linoleic,Linolenic,Cholesterol.

Table 4 Stepwise multiple linear regression of TEQ-PCDDs, TEQ-PCDFs, TEQ-Total levels in breast milk and nutrient intake in sprayed area (n=80)

	Standardized coefficients	p	Adjusted R^2
TEQ-PCDDs			
Constant		<.0001	
Fluorine	.267	.027	.065
Fiber	-.212	.080	
Total FA	.168	.147	
TEQ-PCDFs			
Constant		.874	
Fluorine	.389	.002	.119
Zinc	-.300	.017	
Non-animal Lipid	.247	.025	
BMI	.203	.078	
TEQ-Total			
Constant		.288	
Fluorine	.442	.001	
Zinc	-.335	.010	.120
BMI	.221	.056	
Non-animal Lipid	.221	.044	
Total FA	.199	.080	

Data of dioxin level were log-transformed for analysis.

Variables : Age,BMI,Energy,Protein,Animal Protein,Non-animal Protein,Lipid,Animal Lipid, Non-animal Lipid,Carbohydrate,Fiber,Ash,Retinol,Caroten,VitaminB_1,VitaminB_2,Niacin, VitaminC,Calcium,Phosphorus,Iron,Sodium,Potassium,Magnesium,Zinc,Manganese,Copper, Fluorine,Iodine,Selenium,Total FA,Palmitic,Stearic,Linoleic,Linolenic,Cholesterol.

Table 5 Stepwise multiple linear regression of TEQ-PCDDs, TEQ-PCDFs, TEQ-Total levels in breast milk and nutrient intake in non-sprayed area (n=42)

	Standardized coefficients	p	Adjusted R^2
TEQ-PCDDs			
Constant		.082	
BMI	.428	.010	.221
Ash	.346	.024	
Non-animal Lipid	.258	.122	
Copper	-.254	.080	
TEQ-PCDFs			
Constant		.010	
Linoleic	-.726	<.0001	
Selenium	-.518	.001	.399
Energy	.443	.008	
Protein	-.435	.006	
Animal Lipid	-.404	.007	
BMI	.291	.032	
TEQ-Total			
Constant		.245	
Linoleic	-.495	.004	.212
Energy	.466	.011	
BMI	.379	.014	
Selenium	-.300	.061	

Data of dioxin level were log-transformed for analysis.

Variables : Age,BMI,Energy,Protein,Animal Protein,Non-animal Protein,Lipid,Animal Lipid, Non-animal Lipid,Carbohydrate,Fiber,Ash,Retinol,Caroten,VitaminB_1,VitaminB_2,Niacin, VitaminC,Calcium,Phosphorus,Iron,Sodium,Potassium,Magnesium,Zinc,Manganese,Copper, Fluorine,Iodine,Selenium,Total FA,Palmitic,Stearic,Linoleic,Linolenic,Cholesterol.