DIOXINS IN BREAST MILK AND STEROID HORMONES IN SALIVA OF ONE YEAR OLD CHILDREN IN VIETNAM

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Introduction

From 1961 – 1971, 11.4 million gallons of Agent Orange was sprayed over Southern Vietnam. Agent Orange is contaminated by dioxin, which is not degraded readily and is still causing health problem in Viet Nam.

Since 2002, we have been continuously researching the dioxin and health in Vietnam. Recently, we have focused the steroid hormone as an early indicator of dioxin-induced health effects[1,2]. The relationship between dioxin in breast milk and steroid hormone in Vietnamese lactating women, it was suggested that dioxin influenced steroidogenesis[3]. The relationship between dioxin and steroid hormones in serum of old Vietnamese men, serum dioxin levels were significantly higher in hotspot than non-exposed area, especially in hotspot, the closer to the airbase, the higher dioxin levels, while in non-exposed area, dioxins were not elevated in former soldiers[4]. However, no significant correlations between testosterone, dehydroepiandrosterone (DHEA) and estradiol and dioxins levels were found[5]. As a lipophilic compound, the major exposure route of infants is oral through breast milk from mothers. Although there had been variety of researches focused in health effect of dioxin in previous studies, there is not much studied about how dioxin affected to children. We reported that dioxin induced the inhibition of DHEA synthesis in 3 year old children. The purpose of the study is to clarify the relation between dioxins in maternal breast milk and 1 year old children' salivary steroid hormones in Vietnam.

Materials and methods

Dioxins exposed area is Bien Hoa located about one hour distant from Ho-Chi-Minh city in the South Vietnam and known as hot-spot, where the contamination of dioxin still remains with high level from the severe consequence of storage and mixing herbicide in wartime. Control area is Kim Bang located about one hour distant from Hanoi in the North Vietnam and one rural area that was least suffered by chemical warfare in the past and there are not many industrial areas

All lactating mothers, who have given birth to their first child and full breast-fed for 4 to 16 weeks, consented to donate milk samples (10-20 mL). 54 samples were collected from lactating females aged between 20 and 30 years at Bien Hoa in 2010 and 55 samples were collected at Kim Bang in 2011. Each one year later, in the morning (between 8:00 and 10:00 AM) saliva samples were collected to analyze steroid hormone from 33 one-year infants in Bien Hoa and from 43 one-year infants in Kim Bang district by hormone free cotton.

As indicators of steroid hormone, cortisol and DHEA were analyzed. The analytical procedure of these steroids by LC-MS/MS was reported by Kido et al.

Results and discussion

The Characteristics of subjects are shown in Table 1. Statistical significance differences were indicated on age, body weight and height, chest and head measurement. All of them are larger in exposed area than control area.

Table 1 The Characteristics of subjects.

		Exposed (n = 33)	Control (n = 43)	P Value
Sex	Boys	21 (64%)	22 (51%)	0.27 [3]
	Girls	12 (36%)	21 (49%)	
Age (Months)		15 (14 - 16)	14 (14 - 15)	0.004 [2]
BMI (kg/m2)		15.6 (15.4 - 17.3)	15.4 (14.6 - 16.2)	0.077 [2]
Weight (kg)		10.57 ± 1.86	9.11 ± 1.20	0.0003 [1]
Height (m)		0.80 ± 0.03	0.76 ± 0.03	< 0.0001 [1]

Chest measurement (cm)	46.8 (45.3 - 48.8)	46.0 (44.0 – 47.0)	0.009 [2]
Head measurement (cm)	46.4 ± 1.6	44.9 ± 1.0	< 0.0001 [1]

[1] t-test, [2] Wilcoxon test, [3] Chi-square test.

Dioxins in breast milk and cortisol and DHEA in saliva of 1 year children are shown in Table 2.
All kinds of dioxin levels in lactating mothers' breast milk were significantly higher in subjects of exposed area than in those of control area. On salivary steroid hormones, DHEA showed significantly higher in subjects of exposed area than in those of control area although no significant difference was found on cortisol between two areas.
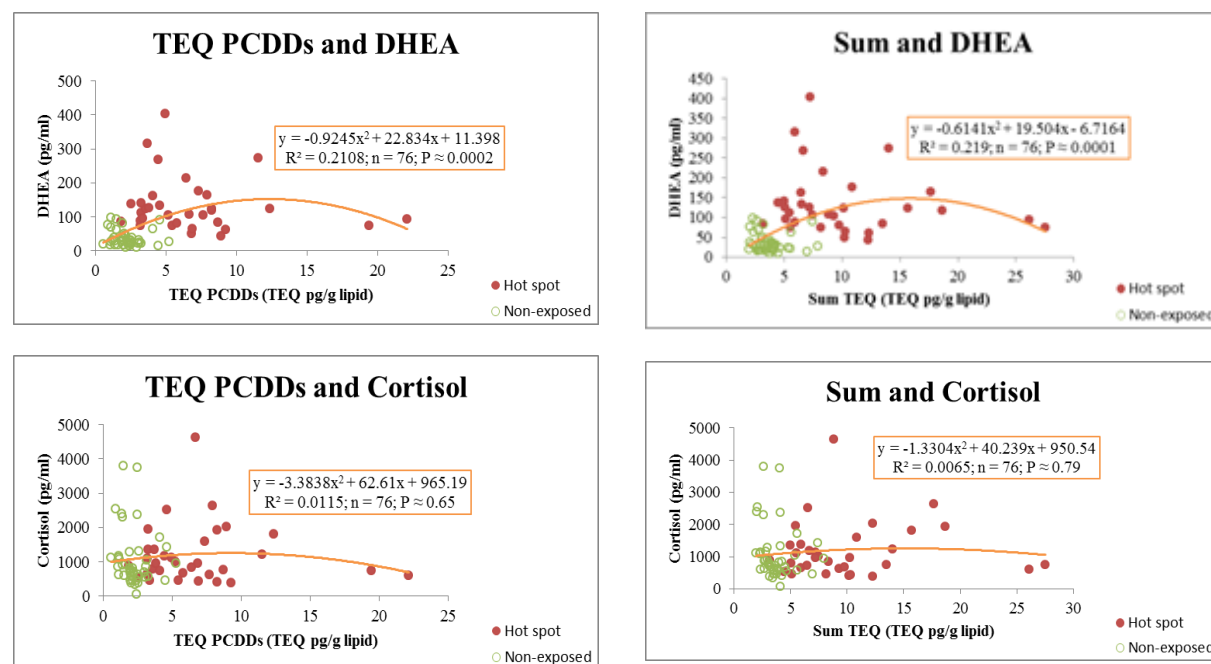
Table 2 Dioxins in breast milk and cortisol and DHEA in saliva of 1 year children

Congener	Exposed (n = 33)		Control (n = 43)		P Value
	Geometric mean (Range)	Mean ± SD / Median (Interquartile)	Geometric mean (Range)	Mean ± SD / Median (Interquartile)	
2,3,7,8-TeCDD	1.78 (0.41 – 15.74)	2.52 ± 2.80	0.58 (0.16 – 2.12)	0.68 ± 0.42	0.0007 [1]
Total TEQs PCDDs	5.80 (1.85 – 22.11)	6.81 ± 4.44	2.03 (0.57 – 5.26)	2.24 ± 1.00	0.0001 [1]
Total TEQs PCDFs	2.80 (1.30 – 10.41)	2.37 (1.94 - 3.40)	1.42 (0.68 – 2.85)	1.48 ± 0.47	0.0001 [2]
Sum TEQs	8.80 (3.16 – 27.53)	10.03 ± 5.76	3.52 (1.97 – 7.93)	3.72 ± 1.37	0.0001 [1]
DHEA	111.24 (42.53 – 403.69)	133.52 ± 80.17	28.94 (9.68 – 98)	36.18 ± 23.53	< 0.0001 [1]
Cortisol	955.1 (388.5 – 4635.6)	921.3 (625.4 - 1363.0)	808.4 (57.7 – 3806.7)	808.42 (570.17 - 1160.40)	0.32 [2]

[1] t-test, [2] Wilcoxon test.

The relations between PCDD or sum (PCDD+PCDF) and DHEA or cortisol were shown in Fig.1.
DHEA significantly associated with PCDD and sum using biphasic curve. However, cortisol showed no relation with PCDD and sum, either.

Fig. 1. The relations between the concentrations of PCDD or sum (PCDD+PCDF) and DHEA or cortisol.

TEQ PCDDs and DHEA

$y = -0.9245x^2 + 22.834x + 11.398$
$R^2 = 0.2108; n = 76; P \approx 0.0002$

Sum and DHEA

$y = -0.6141x^2 + 19.504x - 6.7164$
$R^2 = 0.219; n = 76; P = 0.0001$

TEQ PCDDs and Cortisol

$y = -3.3838x^2 + 62.61x + 965.19$
$R^2 = 0.0115; n = 76; P \approx 0.65$

Sum and Cortisol

$y = -1.3304x^2 + 40.239x + 950.54$
$R^2 = 0.0065; n = 76; P \approx 0.79$

Dioxin total intake (DTI) of each child was calculated by formula below .

$DTI = 30 \times T \times A \times R \times C$

T: Time of full breast feeding (months).   A: Amount of intake milk per day for each child = 800 ml.
R: % lipid content of milk.   C: TEQ concentration (TEQ pg/g lipid).
Suppose each month has 30 days.

DTI of 1 year children in two areas are shown in Table 3. All kinds of DTI levels were significantly higher in exposed area than control area.

Table 3 Dioxin total intake (DTI) of 1 year children in two areas

Congener (TEQ pg/g lipid)	Hot-spot (n = 33) Geometric mean (Range)	Non-exposed (n = 43) Geometric mean (Range)	P Value
2,3,7,8-TeCDD	5093 (523 – 52464)	1167 (360 – 5990)	0.0004 [1]
Total TEQs PCDDs	16553 (2892 – 82541)	4086 (1000 – 15209)	< 0.0001 [1]
Total TEQs PCDFs	7982 (1128 – 65365)	2849 (686 – 8461)	0.0005 [1]
Sum TEQs	25118 (4812 – 117161)	7067 (2185 – 23671)	< 0.0001 [1]

[1] t-test.

The relations between total intakes of PCDD or sum (PCDD+PCDF) and DHEA or cortisol are shown in Fig.2. They also showed the significant relations between DHEA and total intakes of PCDD or sum (PCDD+PCDF). However, cortisol showed no relation with PCDD and sum, either.

Fig. 2. The relations between total intakes of PCDD or sum (PCDD+PCDF) and DHEA or cortisol.
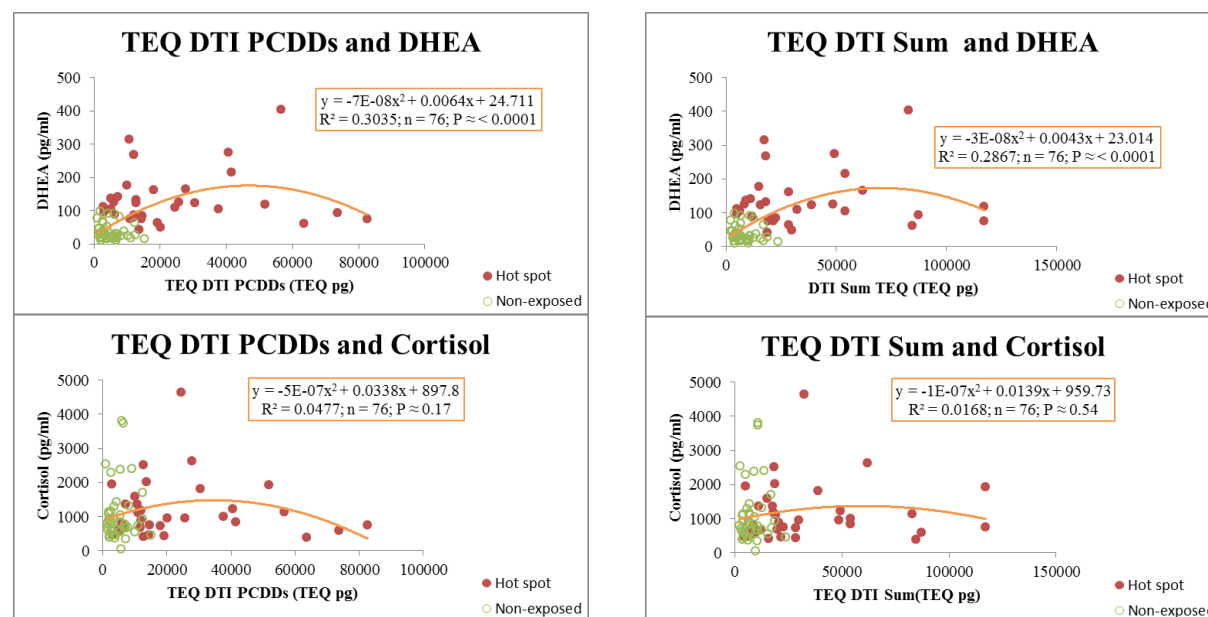
In both areas, bell shaped significant correlations were found between dioxins or DTI levels and DHEA, while there was no relations with cortisol. Consequently, DHEA might be disrupted by dioxins. It is necessary to observe these infants consecutively and also increase the population of children.

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