EFFECTS OF DIOXIN EXPOSURE ON THYROID HORMONES IN POPULATIONS LIVING NEAR HOT SPOTS OF DIOXIN CONTAMINATION IN VIETNAM

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Introduction

Dioxins [polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs)] are highly toxic environmental contaminants, which are lipophilic and resistant to biodegradation. These compounds enter the human body through the food chain and finally accumulate in adipose tissue[1]. A series of deleterious health effects are thought to be associated with dioxins exposure. From some studies on animal, as well as on some populations worldwide, dioxins were considered as endocrine disruptors, in which thyroid gland was one prominent target. In animal models, maternal exposure to TCDD induces elevated b-TSH and neonatal primary hypothyroidism. TCDD and other related compounds have been shown to accelerate thyroid hormone clearance by increasing metabolic enzyme activity and competing with plasma binding proteins[2]. The effects dioxin on endocrine system may trigger other health outcomes such as infant growth retardation and developmental abnormalities, altered reproductive capacity[3, 5]. Danang and Bienhoa airbases were considered as hot spots of dioxin contamination in Vietnam. These former US airbases had served as sites for transportation and storage of great amount of herbicides for a herbicide spraying program in southern Vietnam that extended from 1962 to 1971. These two airbases were extremely contaminated with dioxins due to the tremendous amount of herbicide stored and spilled during mixing and loading there. Populations residing around these airbases were at high risk of exposure. Tai et al (2011) recently showed that levels of PCDDs/Fs in the breast milk of mothers residing near hot spots were threefold to fourfold higher than those in the breast milk of mothers living in unsprayed areas, and that infant daily dioxin intake (DDI) of Vietnamese infants in hot spots were estimated to be twofold to threefold higher than the recently documented values in US and Japanese infants[6]. In this study, we want to clarify the effects of dioxin exposure on thyroid function of general population who living near Danang and Bienhoa airbases.

Materials and methods

Subject: Subjects were recruited from general populations living around Danang and Bienhoa airbases. In 2012, total 114 people (including 57 males and 57 females) who were born before or during and after the period of herbicide spraying (1962-1971) participated in the survey. Demographic information of subjects was collected by an interview. A blood sample from each subject was collected in the morning of examination day. The serum was extracted from whole blood and store at -20^{o}C until analysis of hormones and dioxin levels.

T3, T4, FT3, FT4, TSH measurement: The estimation of T3, T4, FT3, FT4, TSH were done by electrochemiluminance method on Roche Elecsys 2010 instrument, with guidelines for the normal ranges of serum TSH, T3, T4, FT4, FT3 were as 0.23-4 pmol/l, 1.1 – 3, 71.5-158 mmol/l, 12.7-20.8 pmol/l, 3.89-6.66 pmol/l, respectively. For the analysis purpose, the values that were below detection limits were set as half of detection limit.

Dioxin level determination in serum by DR CALUX. The DR CALUX-bioassay analysis was performed in Dioxin laboratory of Vietnamese Military Medical University, which had been certified for approval to perform DR CALUX analysis by BioDetection System, Amsterdam, Netherland. Approximately 2 ml of serum was used for fat extraction by n-hexane and 2-propanol. Fat content was weighted before clean-up step, in which acid-labile matrix components were removed by passage through a silica column containing two layers: 20% and 33% (w/w) concentrated H2SO4. This extract was dried and then diluted in dimethylsulphoxide (DMSO) before exposing to rat H4IIE hepatoma (H4L1.1c4) cells. These cells stably transfected with an AhR-controlled luciferase reporter gene construct (pGudluc1.1) and were grown confluent in 96-well view plates. Samples,TCDD standards and internal control were exposed in triplicate for 24 h in the same plate, using DMSO (0.8% v/v) as a vehicle. After removal of the medium, cells were washed twice with phosphate-buffered saline (Oxoid, Hampshire, UK). The cells were harvested in 30 µl cell lysis reagent (Luciferase Assay System; Promega, Leiden, The Netherlands). For measurements of luciferase activity, 100 µl luciferin assay mix at room temperature was added. After thorough mixing, the light production was measured in a Centro LB 960 Microplate Luminometer. A linear standard curve was built and used to calculate dioxin level in samples, which finally expressed in a unit of total pg bioanalytical equivalent (BEQ)/ g fat.

Statistical analysis. In general analysis, data were expressed as frequency with percentage for categorical variables. For comparison of difference in percentage between groups, OR, CI, χ^2 was performed. All comparisons were considered under a significance level 0.05. All data were analyzed using STATA version 12.0.

Results and discussion:

Table 1. Demographic characteristics by BEQ category

	High > 69,16 BEQ/g	**Low < 69,16 BEQ/g**	**Total**
	n (%)	n (%)	n (%)
n	**57**	**57**	
Mean BEQ	106.38 (35.34)	38.67 (18.4)	
Range	70.58 - 209.54	5.40 - 67.75	
Age group			
20 - 30	2 (50%)	2 (50%)	**4 (100%)**
31 - 40	3 (50%)	3 (50%)	**6 (100%)**
41 - 50	16 (48,8%)	17 (51.52%)	**33 (100%)**
51 - 60	26 (54.17%)	22 (45.83%)	**48 (100%)**
>60	10 (43.48%)	13 (56.52%)	**23 (100%)**
Total	**57 (50%)**	**57 (50%)**	**114 (100%)**
	p > 0.05		
Gender			
Male	25 (43.86%)	32 (56.14%)	**57 (100%)**
Female	32 (56.14%)	25 (43.86%)	**57 (100%)**
Total	**57 (100%)**	**57 (100%)**	**114 (100%)**
	p > 0.05		
BMI			
<18.5	4 (6.25%)	4 (8%)	**8 (7,01%)**
18.5 - 25	42 (76.56%)	41 (68%)	**83 (72.81%)**
≥ 25	11 (17.19%)	12 (24%)	**23 (20,18%)**
Total	**64 (100%)**	**50 (100%)**	**114 (100%)**
	p > 0.05		

The subjects were divided into two groups based on level of dioxins in serum blood. The cutoff value was set equal to absolute mean of dioxin concentration (69.19 pg BEQ/g fat). Demographic characteristics of the participants in both groups were presented in Table 1. Distribution of age, gender, BMI between high and low groups was not different ($p > 0.05$). The mean dioxin level in high group was approximately 3 times as much as that in low group (106.38 vs 38.67 pg BEQ/ g fat).

Effect of Dioxin on thyroid function was showed in table 2, number of abnormal of TSH, T3, T4, FT3, FT4 in High BEQ category were higher than the number in Low category. But number abnormal of TSH, T4, FT3, FT4 were significantly difference between two groups. The odd ratios of all thyroid hormone exception of T3 did not increase or decrease significantly between groups. Odd ratio of T3 showed significant association between high category and low category (OR = 2.407, CI: 1.222 - 5.163, $\chi^2 = 0,5.19$, $p = 0.023$).

This study, there were no different distribution of age, gender and BMI between two groups. There was significant association between T3 and BEQ category (table 2) OR = 2.407. That mean Dioxin effect on thyroid function.

Findings from studies that examined effects of serum TCDD levels on thyroid function in two cohorts of chemical workers exposed to TCDD in the production of 2,4,5-trichlorophenol were inconsistent[7]. A recent study on approximately 225,000 veterans of the Vietnam era found that those who served in Vietnam or were otherwise exposed to defoliants had a 2.5-fold to 3.0-fold higher prevalence of the diagnosis of Graves' disease, compared to Veterans who served elsewhere[8]. Some reports Dioxin decrease total T3 serum level[9]. But there were not any effects from other result[10].

Table 2. Abnormal thyroid hormone levels by BEQ category

		High < 69,16 BEQ/g	**Low > 69,16 BEQ/g**	**Total**
		n (%)	**n (%)**	**n (%)**
TSH	Normal	8 (14.04%)	10 (17.54%)	18 (15,79)
	Abnormal	49 (85.96%)	47 (82.46%)	96 (84.21%)

	Total	57 (100%)	57 (100%)	114 (100%)
		OR = 0.767, CI: 0.279 - 2.111, χ^2 = 0,2639, p = 0.607		
T3	Normal	18 (31.58%)	30 (52.63%)	48 (42.11%)
	Abnormal	39 (68.42%)	27 (47.37%)	66 (57.37%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR = 2.407, CI: 1.222 - 5.163, χ^2 = 0,5.19, p = 0.023		
FT3	Normal	25 (43.86%)	32 (56.14%)	57 (50%)
	Abnormal	32 (56.14%)	25 (43.86%)	57 (50%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR = 1.638, CI: 0.782 - 3.434, χ^2 = 0.4298, p = 0.1898		
T4	Normal	16 (28.07%)	18 (31.58%)	34 (29.82%)
	Abnormal	41 (71.93%)	39 (68%)	80 (70.18%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR = 1.183, CI: 0.53 - 2.642, χ^2 = 0.0588, p=0.682		
FT4	Normal	7 (12.28%)	8 (14.04%)	15 (13.16%)
	Abnormal	50 (87.72%)	49 (85.96%)	99 (86.84%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR = 1.166, CI: 0.393 - 3.462, χ^2 = 0,0768, p = 0.782		

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