

DIOXINS IN MILK OR BLOOD IN RELATION TO THYROID HORMONES AND OTHER POSSIBLE BIOMARKERS AMONG PEOPLE IN A HIGHLY POLLUTED AREA IN JAPAN

Michinori Kabuto, Hideko Sone, Hideki Imai, Yoshika Kurokawa and Junzo Yonemoto

Environmental Risk Research Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-0053, JAPAN

Introduction

Several suggested biomarkers such as thyroid hormones, CYP-family gene expressions or estrogen metabolites as well as expression of AhR (Aryl hydrocarbon receptor) and estrogen receptor genes have been related to biologically-effective exposures to or body burden of dioxins mainly based on acute animal experiments, although no definitive evidence has been observed in humans yet. In the present study, possible relationship between blood dioxins in terms of TEQ per g-fat and thyroid hormones in sera as well as expression of lymphocyte P-4501A1 (CYP1A1) mRNA were studied. Moreover, the ratio of 2/16 f_2 -hydroxystroene (2/16 f_2 -OHE1) in urine was also examined for its possible quantitative relation to dioxins in milk among lactating mothers. These subjects were recruited from people living in one of the areas with high air pollution of dioxins due to incinerators.

Methods and Materials

Subjects consisted of two groups for milk and blood studies recruited from the residents in T city, S Prefecture, Japan, where air pollution of dioxins from several incinerators have been a serious public concern. The milk study group was 120 post-delivery women (100 in 1997 and 20 in 1998) aged 20s-40s, from whom up to 100 ml of milk was collected. For 20 women studied in 1998, spot urine was also collected for measurements of estrogen metabolites (2- and 16 f_2 -OHE1). The blood study group subjects were 35 in the 1st round in 1997 and 17 in the 2nd round in 1998, from whom whole blood of up to 100 ml was collected via venipuncture. Serum levels of T3, T4 and TSH were measured for all of 52 blood study subjects with radioimmunoassay. CYP1A1 mRNA expressions (copies/ng RNA) in lymphocytes were determined with a quantitative real-time RT-PCR¹ for the former 35 subjects, while it could not be determined for the latter due to failure of lymphocyte culture.

Dioxins in milk and whole blood specimens were separately measured with slightly different methods with using GC-MS. With respect to congeners, however, 9 PCDDs and 11 PCDFs and 19 PCDDs and 5 PCDFs were determined for milk and blood, respectively. Coplanar PCBs (4 non- (#77, #81, #126, #169) and 8 mono- (#123, #118, #114, #105, #167, #156, #157, #189) ortho PCBs) were determined for both milk and blood obtained in 1998. In calculating total TEQ value, TEF values by WHO were used and half value of each detection limit was applied to congeners when they were not detected. Statistical analyses were performed with PC-SAS and GLM procedure was used for adjusting age and other variables.

Results and Discussion

1) Dioxins in milk and blood

Mean TEQ values of dioxins (PCDDs and PCDFs only) for milk and blood of 1997 were 15 and 18 pg TEQ/g-fat with the max values of 30 and 30 pg TEQ/g-fat, respectively, while those of dioxins (PCDDs+PCDFs+cPCB) in milk and blood of 1998 were 26 and 21 with the max values of 49 and 48, respectively. Each of them showed weakly ($p < 0.1$) or significantly correlated with age, respectively,

while no gender difference was noted in case of blood samples. These values were almost consistent with the recently reported values for other non-polluted areas in Japan (reports from Ministry of Health & Welfare available on HP), indicating little contribution of air pollution from the incinerators to their body burden. Moreover, it is indicated that these values are around half compared to the levels observed in Netherlands' study².

2) Dioxins in milk and urinary estrogen metabolites

The ratio 2/16*f*_i-OHE1 was determined for 20 urine samples from the lactating women of 1998, which was 3.1 on the average with a range from 1.2 to 7.8. These ratios did not correlate with TEQ values (PCDDs+PCDFs+cPCBs) in milk, regardless of adjustment for age. According to our previous study on non-lactating Japanese women, the mean ratio was 2.3 (0.3–6.9) for those aged <40 years.

3) Dioxins in whole blood and thyroid hormones in sera

Serum levels of thyroid hormones, or T3, T4 and TSH, were determined for 52 blood study subjects of 1997 and 1998, all of which were within each of the normal ranges reported. Blood dioxins (PCDDs+PCDFs only) in terms of TEQ per g-fat value showed a significant negative correlation only with T3 levels ($r = -0.341$; $p < 0.05$). However, since both dioxins values and T3 levels also correlated negatively with age significantly and weakly, respectively, age adjusted mean T3 levels by quartiles of dioxins values were calculated. As shown in Fig. 1, there was no significant differences among the quartiles, although T3 level tended to be lower in the highest quartile.

4) Dioxins in whole blood and lymphocyte CYP1A1 mRNA expression

As for 35 subjects of 1997, CYP1A1 mRNA expression values were obtained. Although its age and sex adjusted mean values tended to be elevated compared to those in other 3 categories as shown in Fig. 2, no statistically significant difference was observed. The results were similar, if small number of smokers were excluded.

5) Conclusive remarks

Body burdens of dioxins among the present subjects were generally lower (almost half) compared to those in some European countries. In case of the Netherlands' epidemiological study, risk for neuro-behavioral anomalies among children as well as T3 and T4 level decreases and TSH level elevation in both children and their mothers have been related to the higher levels of dioxins in milk or blood when the lower levels, which are compatible with the present values, were used as reference. In the present study, milk or blood dioxins were not associated with serum thyroid hormones, lymphocyte CYP1A1 mRNA expression or

estrogen metabolites was observed, although it could not be denied that T3 and T4 levels tended to decrease and CYP1A1 mRNA expression did to increase in the highest quartile of blood dioxins values.

The ratio of 2/16*f*_i-OHE1 observed for the present 20 lactating women was slightly higher compared to those among not lactating women studied previously. However, it was suggested that milk dioxins may not decrease its value in a direction to increase the risk as has been suggested experimentally.

Thus, it seems important to increase the subjects in the same area to confirm the above tendencies as well as to investigate people with more body burden of dioxins, or more dietary intake especially through eating fish, meat, milk products and so on.

Acknowledgement

The authors thank for all members of the committee for "a study on dioxin accumulation in human milk" organized by T City, S Prefecture, all participants to this survey and staffs in the Health Center of T City.

References

1. Suzuki, C., Kabuto, M., Watanabe, S., Yonemoto, J., Tohyama, C. and Sone, H.: Analysis of CYP1A1, CYP1A2 and CYP1B1 gene expression levels in blood using a real-time reverse transcriptional-PCR system (in preparation).
2. Akkermans, PWC: Effects of environmental exposures to polychlorinated biphenyls and dioxins on growth and development in young children, Rotterdam, 1999

Table 1: Numbers of Subjects by Year in Milk and Blood Studies

Year	Subjects	
	Milk Study (lactating mothers only)	Blood Study (No. of males)
1997	100	35 (9)*, ***
1998	20**	17 (4)***
total	120	52 (13)

Note) Dioxins were measured for all milk and whole blood samples basically. Moreover, * CYP1A1 mRNA gene expression was also determined with a quantitative real-time RT-PCR, ** spot urine samples were collected and subjected to estrogen metabolites measurements and *** serum thyroid hormones were measured

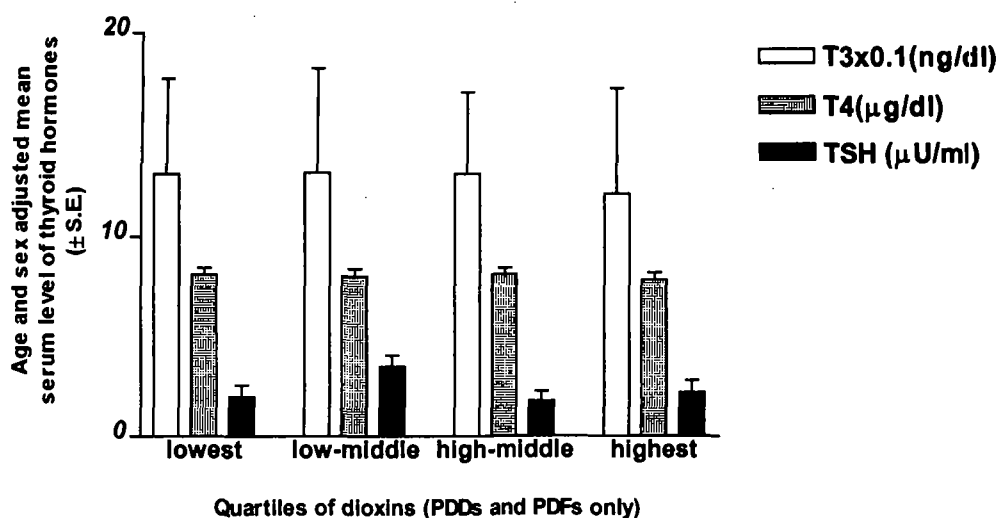


Fig.1 Age and sex adjusted mean thyroid hormone levels according to quartiles of blood dioxins values in TEQ (PCDDs and PCDFs only). Although the mean total T3 and T4 levels tended to be lowered, the differences from those in the lowest or reference category were not significant.

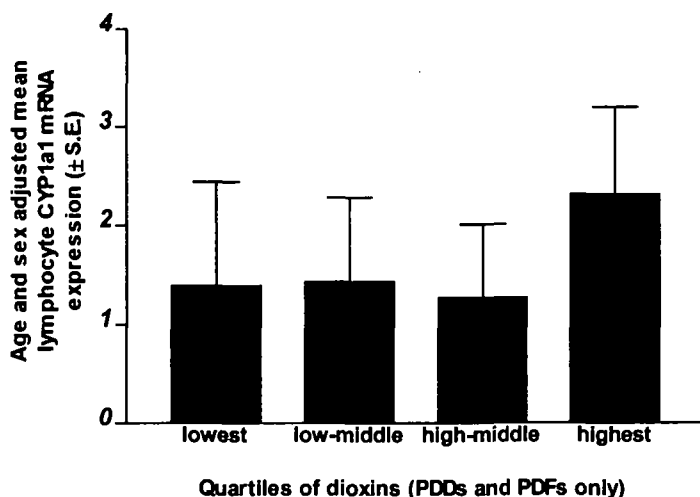


Fig.2 Age and sex adjusted mean CYP1A1 mRNA expression according to quartiles of blood dioxins values in TEQ (PCDDs and PCDFs only). Although the mean value in the highest quartile tended to be elevated, the difference from that of the lowest or reference category was not significant.