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Chemosphere 62 (2006) 1135–1141

CHEMOSPHERE

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Human milk survey for dioxins in the general population in Japan

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Received 26 October 2004; received in revised form 13 May 2005; accepted 27 May 2005

Available online 9 August 2005

Abstract

Background: Much attention has been paid to the level of dioxins in breast milk in Japan but few large-scale studies have been conducted on the subject.

Methods: From 1997 to 2002, we collected 839 samples of breast milk from primiparas residing throughout Japan. Starting in 1999, breast milk was also collected from secundiparas. Seven isomers of polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 of polychlorinated dibenzofurans (PCDFs), 4 of coplanar polychlorinated biphenyls (Co-PCBs) and 8 of mono-ortho-chlorinated polychlorinated biphenyls (mono-ortho PCBs) were analyzed by employing gas chromatography and mass spectrometry. A correlation between the level of dioxins in human milk and the age of the mothers was noted for the primiparas and the secundiparas; and the levels were compared between the first and the second deliveries. Grouped by parity and prefecture in each year, observations were also made on the trends in these levels. Dioxin levels are shown by using geometric means because their distributions were skewed to the left.

Results: The sum of PCDDs and PCDFs, Co-PCBs, mono-ortho PCBs, and total dioxins in the breast milk of primiparas were 13.9, 5.4, 3.4, and 22.7 pg TEQ/g fat, respectively. In the samples obtained from secundiparas, these levels were 63–68 percents of those taken from the primiparas. The correlation coefficients between the PCDDs/DFs, Co-PCBs, mono-ortho PCBs, and total dioxins and the age of the primiparas were 0.19, 0.17, 0.36, and 0.24, respectively. All these correlations were statistically significant ($p < 0.001$). The positive correlations between these contaminants and the age of the secundiparas were also examined. The total dioxins as well as PCDDs/DFs, Co-PCBs, and mono-ortho PCBs in the breast milk of the primiparas declined significantly between 1998 and 2002 (regression coefficients: -0.04 , -0.05 , -0.03 , and -0.03 , respectively). However, no significant decline in these levels was observed when sorted by prefectures.

Conclusions: Much attention should be paid to the age and parity of nursing mothers when investigating the relationship between the level of dioxins in breast milk and the body burden of infants.

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Keywords: Dioxins; Polychlorinated biphenyls; Human milk; Epidemiology; Population surveillance

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1. Introduction

The toxicity of dioxins such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) is extremely high and their effects on reproductive systems, thyroid function, the immune system, and development in some animal models are significant (Yonemoto, 2000). In humans, however, it is controversial whether reproductive and hormonal systems and the developmental mechanism are affected by these contaminants (Yonemoto, 2000; Matsuura et al., 2001; Vreugdenhil et al., 2002). The effects of exposure to dioxins are most prominent in fetuses and infants; therefore studies have focused on this segment of the population.

Food intake, inhalation of polluted air and skin contact with contaminated soil and materials are possible routes of human exposure (Watanabe et al., 1999). It is estimated that for the general population in Japan, most dioxins originate in food, primarily fish and meat. For the majority of infants, breast milk is the most important source of their nutrition and immunologically active substances. Because breast milk is rich in fat and dioxins are highly lipophilic, consumption of breast milk constitutes the main route of dioxin exposure for breast-fed infants. The dioxin content in human breast milk has been measured in Japan and western countries (Iida et al., 1999; Nakagawa et al., 1999; Tajimi et al., 2004;

Takekuma et al., 2004). However, few large-scale studies have been conducted and sampling of breast milk was limited geographically in earlier studies. For the current study, starting in 1997, samples of human breast milk was measured throughout Japan and the levels of dioxins in these samples were measured. Thus the pattern of distribution of the dioxin levels in breast milk in this country was observed and described from the viewpoints of time and geography.

2. Methods

In 1997, 20 primiparas each from four prefectures (Saitama, Tokyo, Ishikawa and Osaka) were enrolled; and in 1998, the same number of primiparas from 19 prefectures (Iwate, Miyagi, Akita, Ibaragi, Gunma, Chiba, Kanagawa, Niigata, Ishikawa, Yamanashi, Shizuoka, Aichi, Osaka, Shimane, Hiroshima, Yamaguchi, Fukuoka, Kumamoto and Okinawa) and Yokohama City were enrolled (Fig. 1). Starting in 1999, the same numbers of new primiparas from six prefectures (Iwate, Chiba, Niigata, Ishikawa, Osaka and Shimane) were enrolled. There are 47 prefectures in Japan and all were invited to participate in this study but no response was received from other prefectures. When 20- to 39-year-old healthy pregnant women visited their obstetricians, they were asked by the public health nurses in their

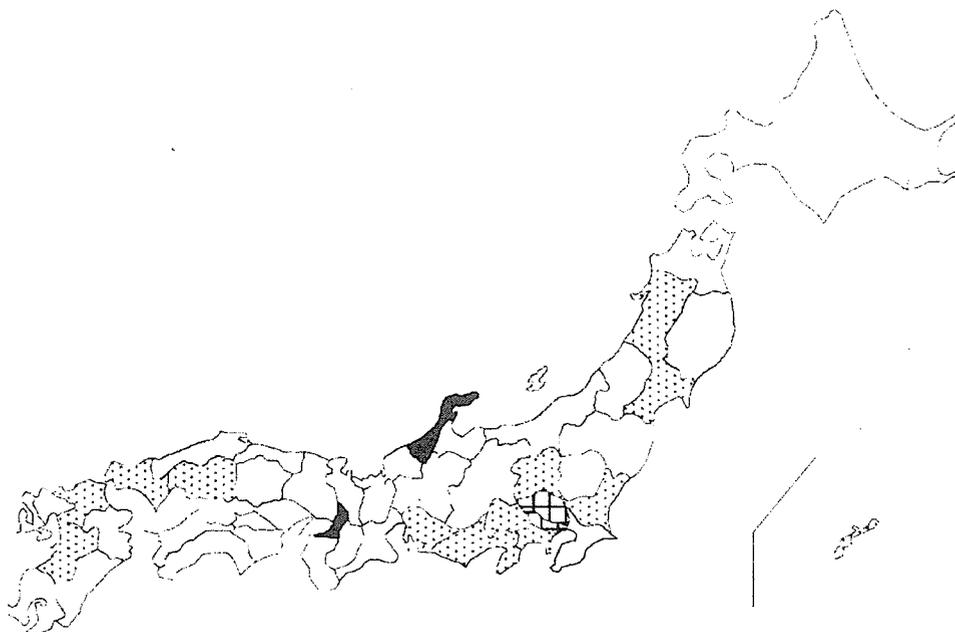


Fig. 1. Title: The map of prefectures in Japan that participated in our study from 1997 to 2002. Crosses represent ones that participated in our study in 1997 (Saitama and Tokyo). Dots represent prefectures and a city that participated in 1998 (Miyagi, Akita, Ibaragi, Gunma, Kanagawa, Yamanashi, Shizuoka, Aichi, Hiroshima, Yamaguchi, Fukuoka, Kumamoto, Okinawa, and Yokohama City). Blacks represent ones that participated from 1997 to 2002 (Ishikawa and Osaka). Grays represent ones that participated from 1998 to 2002 (Iwate, Chiba, Niigata, and Shimane).

prefecture or city if they take part in this study. All participants submitted a signed informed consent form to the public health nurses, who then collected about 50 ml of breast milk that was manually expressed by each of these mothers 30 days after the delivery. The timing of the collection of the breast milk was not defined. To measure the concentration of dioxins, these milk samples were sent to the Japan Food Research Laboratory in Tokyo. Starting in 1999, the breast milk was also collected from the same mothers after they delivered their second infants.

Seven isomers of PCDDs, 10 of PCDFs, 4 of Co-PCBs, and 8 of mono-ortho-chlorinated PCBs (mono-ortho PCBs) were analyzed by using gas chromatography and mass spectrometry at the Japan Food Research Laboratory. In brief, the milk samples were mixed with an aqueous solution of sodium oxalate, diethyl ether and ethanol and the mixture was extracted with hexane. The fat content was determined gravimetrically. Thereafter, a three-step clean-up procedure was performed by using a column filled with silica gel, followed by another column containing aluminum oxide, then by an activated charcoal column. After concentrating the sample, gas chromatography and mass spectrometry were employed to measure the contents. A mixture of ^{13}C -labelled PCDDs, PCDFs, Co-PCBs, and mono-ortho PCBs was used as an internal standard. The levels of dioxins were described on a fat basis and toxic equivalences (TEQs) were calculated by using toxic equivalent factors (TEFs) of 2,3,7,8-tetrachlorodibenzodioxin, which had been reported by WHO (Van den Berg et al., 1998). First, the distributions of the sum of PCDDs and PCDFs (PCDDs/DFs), Co-PCBs, mono-ortho PCBs, and the total of these compounds in breast milk from primiparas and secundiparas were shown. The data on Co-PCBs and the total dioxin levels in the breast milk of primiparas in 1997 were excluded because only three isomers of Co-PCBs were measured in that year. Second, the correlation between the levels of dioxins in breast milk and the ages of all mothers (both primiparas and secundiparas) was determined. The levels of dioxins were converted into natural logarithms. Among the secundiparas, the changes in the levels of dioxins in the breast milk that was obtained after the first and second deliveries were observed. Finally, the changing trends in the levels of dioxins in breast milk during a 6-year period (from 1997 to 2002) were examined, as well as the trends in the total level of dioxins from 1998 to 2002 in 6 prefectures (Iwate, Chiba, Niigata, Ishikawa, Osaka and Shimane).

2.1. Statistical analysis

The correlation coefficients were shown by Pearson's correlation. A paired *t*-test was used to compare the levels of dioxins after the first and second deliveries.

Regression coefficients were obtained by using a simple regression analysis to evaluate the natural logarithms of the levels of dioxins against age and the trends observed in the levels of dioxins for all subjects in each prefecture. The calendar year was the independent variable for the analysis of the changing trends. Probabilities less than 0.05 were considered to be statistically significant. The statistical analyses were performed by using SPSS 11.0J for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

From 1997 to 2002, a total of 839 primiparas participated in the study. Co-PCBs, mono-ortho PCBs and the total level of dioxins were measured in 767 samples of primiparas during this period. Both arithmetic and geometric means of the levels of dioxins are shown because their distributions were skewed to the left (Table 1). The geometric means of the levels of PCDDs/DFs, Co-PCBs, mono-ortho PCBs and total of dioxins in breast milk of primiparas were 13.9, 5.4, 3.4, and 22.7 pg TEQ/g fat, respectively. Similarly, the geometric means of the levels of these dioxins in breast milk of 89 secundiparas from 1999 to 2002 were 8.8, 3.6, 2.3, and 14.7 pg TEQ/g fat, respectively. The ratios of levels of these compounds for the secundiparas to those for primiparas were 0.63, 0.67, 0.68 and 0.65, respectively.

There was a correlation between the levels of dioxins in human milk and the age of the mother. The correlation coefficients between PCDDs/DFs, Co-PCBs, mono-ortho PCBs, and total dioxins and the age of the primiparas were 0.19, 0.17, 0.36, and 0.24, respectively. All were statistically significant ($p < 0.001$). Positive correlations were also observed between these contaminants and the age of the secundiparas, the correlation coefficients being; PCDDs/DFs = 0.19, Co-PCBs = 0.24, mono-ortho PCBs = 0.36, and total dioxins = 0.24. The regression coefficients for the level of the dioxins against the age of the primiparas and secundiparas are shown in Table 2. Except for the PCDDs/DFs against the age of the secundiparas, the regression coefficients were statistically significant.

Among the secundiparas, the levels of total dioxins, as well as PCDDs/DFs, Co-PCBs, and mono-ortho PCBs significantly decreased after the first delivery and the trend continued after the second delivery ($p < 0.001$, Table 3). The ratios of the levels of PCDDs/DFs, Co-PCBs, mono-ortho PCBs, and total dioxins after the second delivery to those after the first delivery were 0.61, 0.66, 0.67, and 0.62, respectively.

A changing trend in the geometric means of dioxins in breast milk was seen in Table 4. Among the primiparas, the levels of the total dioxins declined significantly from 1998 to 2002 (regression coefficient: -0.04 ,

Table 1
Distributions of dioxins in breast milk of Japanese from 1997 to 2002 (unit: pg TEQ/g fat)

	<i>n</i>	Arithmetic mean	Standard deviation	Geometric mean	Median	Maximum	Minimum
<i>Primipara</i>							
PCDDs/DFs ^a	839	14.8	5.4	13.9	14.2	56.0	3.7
Co-PCBs ^{b,c}	767	5.9	2.7	5.4	5.4	29.5	1.2
Mono-ortho PCBs ^c	767	3.7	1.6	3.4	3.4	16.1	0.9
Total ^c	767	24.1	8.3	22.7	23.0	59.0	7.0
<i>Secundipara</i> ^d							
PCDDs/DFs ^a	89	9.8	5.7	8.8	8.6	43.6	0.0
Co-PCBs ^b	89	4.1	2.3	3.6	3.5	13.3	1.3
Mono-ortho PCBs	89	2.6	1.4	2.3	2.3	10.2	0.7
Total	89	16.5	8.8	14.7	15.0	64.0	5.0

^a PCDDs/DFs: the sum of 7 polychlorinated dibenzo-*p*-dioxins and 10 polychlorinated dibenzofurans.

^b Co-PCBs: the sum of 4 coplanar polychlorinated biphenyls.

^c These values represent the data from 1998 to 2002 because 4 Co-PCBs and 8 mono-ortho PCBs were measured from 1998.

^d Dioxins in breast milk of secundiparas were collected from 1999 to 2002.

Table 2
Regression coefficients for the levels of dioxins against ages of mothers

	Regression coefficients (95% confidence interval)	Standard error	<i>p</i> -Value
<i>Primiparas</i>			
ln (PCDDs/DFs ^a)	0.026 (0.017–0.035)	0.005	<0.001
ln (Co-PCBs ^{b,c})	0.028 (0.016–0.039)	0.006	<0.001
ln (mono-ortho PCBs ^c)	0.056 (0.045–0.066)	0.005	<0.001
ln (total dioxins ^c)	0.031 (0.022–0.040)	0.005	<0.001
<i>Secundiparas</i> ^d			
ln (PCDDs/DFs ^a)	0.037 (–0.005–0.078)	0.021	0.08
ln (Co-PCBs ^b)	0.047 (0.006–0.088)	0.021	0.025
ln (mono-ortho PCBs)	0.069 (0.030–0.108)	0.020	0.001
ln (total dioxins)	0.044 (0.006–0.083)	0.020	0.026

^a PCDDs/DFs: the sum of 7 polychlorinated dibenzo-*p*-dioxins and 10 polychlorinated dibenzofurans.

^b Co-PCBs: the sum of 4 coplanar polychlorinated biphenyls.

^c These values represent the data from 1998 to 2002 because 4 Co-PCBs and 8 mono-ortho PCBs were measured from 1998.

^d Dioxins in breast milk of secundiparas were collected from 1999 to 2002.

Table 3
Comparison between the levels of dioxins in breast milk after the first delivery and those after the second delivery among secundiparas (unit: pg TEQ/g fat)

	After the first delivery			After the second delivery			<i>p</i> -Value
	<i>n</i>	Geometric mean	95% CI	<i>n</i>	Geometric mean	95% CI	
PCDDs/DFs	89	14.4	13.2–15.8	89	8.8	7.9–9.7	<0.001
Co-PCBs(4) ^a	72	5.3	4.7–5.9	72	3.5	3.1–4.0	<0.001
Mono-ortho PCB(8) ^a	72	3.3	2.9–3.7	72	2.2	1.9–2.5	<0.001
Total ^a	72	23.1	20.9–25.5	72	14.4	12.8–16.2	<0.001

^a These values represent the data from 1998 to 2002 because 4 isomers of Co-PCBs and 8 isomers of mono-ortho PCBs were measured from 1998.

$p < 0.001$). The levels of PCDDs/DFs, Co-PCBs, and mono-ortho PCBs also decreased significantly (regres-

sion coefficients: –0.05, –0.03, and –0.03, respectively). Among the secundiparas, however, no significant decline

Table 4
Time trends of geometric means of dioxins in breast milk of Japanese from 1997 to 2002 (unit: pg TEQ/g fat)

	Calendar year						Regression coefficient	<i>p</i>
	1997	1998	1999	2000	2001	2002		
<i>Primipara</i>								
PCDDs/DFs	16.6	14.2	14.6	12.9	12.8	11.6	-0.05	<0.001
95% CI	15.0–18.2	13.6–14.6	13.9–15.5	12.3–13.7	11.9–13.7	10.5–12.7		
Co-PCBs ^a		5.8	4.7	4.3	5.9	5.1	-0.03	0.016
95% CI		5.5–6.0	4.4–5.2	3.9–4.7	5.4–6.4	4.5–5.8		
Mono-ortho PCBs ^a		3.5	3.2	3.0	3.2	3.2	-0.03	0.002
95% CI		3.4–3.7	3.0–3.5	2.8–3.3	3.0–3.5	2.8–3.6		
Total		23.6	22.9	20.5	22.0	20.1	-0.04	<0.001
95% CI		22.9–24.5	21.8–24.3	19.3–22.0	20.5–23.8	18.2–22.2		
<i>Secundipara</i> ^b								
PCDDs/DFs			9.3	9.4	9.3	6.4	-0.13	0.03
95% CI			7.4–11.6	8.0–11.0	7.0–12.3	5.4–7.4		
Co-PCBs			3.0	3.4	4.6	3.3	0.06	0.35
95% CI			2.3–4.0	2.9–4.0	3.6–5.9	2.6–4.1		
Mono-ortho PCBs			2.1	2.3	2.4	2.0	-0.01	0.80
95% CI			1.7–2.5	2.0–2.7	1.9–3.2	1.6–2.5		
Total			14.3	15.2	16.4	11.7	-0.06	0.29
95% CI			11.5–18.0	13.1–17.6	12.7–21.5	10.0–13.7		

CI: confidence interval.

^a These values represent 4 isomers of Co-PCBs and 8 isomers of mono-ortho PCBs that were measured from 1998.

^b Dioxins in breast milk of secundiparas were collected from 1999 to 2002.

was noted in the levels of Co-PCBs, mono-ortho PCBs, and total dioxins. A changing trend was also noted in the total levels of dioxins in the breast milk of the primiparas residing in 6 prefectures (Table 5). The changing patterns in the level of total dioxins were diverse in these prefectures, with no significant decline in any particular prefectures.

4. Discussion

The distribution of dioxin levels in breast milk obtained from Japanese mothers was observed. The small sample size and geographical limitation were the main issues associated with earlier studies on dioxins in breast milk. In the current study, these were overcome by

Table 5
Time trends of geometric means of total dioxins in breast milk of primiparas by 6 prefectures (unit: pg TEQ/g fat)

Prefectures	Calendar year					Regression coefficient	<i>p</i> -Value
	1998	1999	2000	2001	2002		
Iwate	18.9	20.9	17.0	19.9	18.4	6.9E-04	0.36
95% CI	16.8–21.5	17.3–25.0	14.3–19.9	17.0–23.6	14.7–23.1		
Chiba	25.5	23.8	21.3	22.2	22.2	1.3E-03	0.05
95% CI	22.0–30.0	21.3–26.6	19.1–23.6	19.3–25.8	14.6–33.5		
Niigata	21.1	22.9	20.9	19.5	17.5	1.2E-03	0.08
95% CI	18.5–24.1	19.9–26.6	17.8–24.8	16.4–22.9	14.3–21.1		
Ishikawa	15.5	23.6	20.9	17.5	19.1	2.3E-04	0.84
95% CI	11.2–22.0	19.3–28.8	17.0–25.5	14.4–21.1	11.4–32.1		
Osaka	27.7	23.1	21.3	27.7	22.7	6.8E-04	0.34
95% CI	24.3–31.5	20.5–26.1	17.1–26.3	23.8–32.5	19.3–26.6		
Shimane ^a	30.9	24.1	22.0	27.9	–	1.1E-03	0.14
95% CI	26.6–35.9	21.1–27.4	19.1–25.0	23.8–32.5	–		

^a We excluded a value in 2002 in Shimane prefecture because we could only acquire a sample of primipara in 2002.

sequential collection of breast milk from those mothers who resided throughout Japan. The skewed distribution of dioxin levels in breast milk had been demonstrated in our earlier study (Nakamura et al., 2000) and by others (Beck et al., 1994; Nakagawa et al., 1999; Tajimi et al., 2004). The geometric means of the level of dioxins were calculated in this study; but the arithmetic means were also included because the latter was commonly used in other studies (LaKind et al., 2001). The arithmetic means of the levels of PCDDs/DFs, Co-PCBs, mono-ortho PCBs, and total dioxins in breast milk of primiparas during the period in question were 14.8, 5.9, 3.7, and 24.1 pg TEQ/kg fat, respectively. These were similar to what had been reported in earlier studies conducted in Japan (Tajimi et al., 2004; Takekuma et al., 2004). The levels of dioxins were also examined in the breast milk of the secundiparas and found to be significantly lower than those of the primiparas. The dioxin levels in the secundiparas were 0.63–0.67 of those of the primiparas, an observation that was lower than that was cited in other studies (Beck et al., 1994; Iida et al., 1999; Nakagawa et al., 1999; Yang et al., 2002; Tajimi et al., 2004), which was explained by the difference between the geometric and arithmetic means.

The positive correlations were observed between the level of dioxins in breast milk and the ages of both primiparas and secundiparas. These findings were similar to those reported in other studies (Chao et al., 2004; Tajimi et al., 2004). Among the secundiparas, dioxin levels after the second delivery were significantly lower than those after the first delivery. Similar findings were reported in a study on the kinetics of dioxin in those mothers who were nursing their infants (Abraham et al., 1998).

From the current trends, it was concluded that the level of dioxins in the breast milk of the primiparas in the general population had declined significantly. It was reported that when the general public in one country consumed less fish, there was a decline in the dioxin levels in human milk (Schuhmacher et al., 2004); however, no evidence for this relationship between a decline of the level of dioxins in human milk and reduced consumption of fish has been established in Japan.

Consideration must be given to whether the level of dioxins in breast milk in this study are representative of the current levels in Japan. Only six prefectures participated in the current study continually from 1998 and the number of participants in each prefecture for each year was small. However, these six prefectures appear to be relatively uniformly distributed throughout the main part of Japan. Because of the small sample size, the changing trends in the level of dioxins in each prefecture were diverse and no statistical significance was noted. It was concluded that the dioxin level in breast milk should be evaluated among a sufficiently large population.

We emphasize the importance of the age and parity of the mothers who supplied their breast milk in investigating the relationship between the level of dioxins in breast milk and the body burden borne by infants or children. WHO continues to support breast feeding because scientific evidence is still insufficient to warrant a change in its recommendation (Brouwer et al., 1998). The Ministry of Health, Labour and Welfare of Japan also recommends breast feeding to mothers who raise their infants. Based on the results of this study, the relationship between the level of dioxins in breast milk and the body burden of breast-fed children in Japan should be clarified.

Acknowledgement

This study was supported in part by Grants from the Ministry of Health, Labour and Welfare in Japan.

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Haplotype Analysis Reveals Founder Effects of Thyroglobulin Gene Mutations C1058R and C1977S in Japan

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Context: Thyroglobulin (Tg) mutations were previously believed to be rare, resulting in congenital goitrous hypothyroidism. However, an increasing number of patients with Tg mutations, who are euthyroid to mildly hypothyroid, have been identified in Japan.

Objectives: The purpose of this study was to investigate whether the three frequently found Tg mutations, namely C1058R, C1245R, and C1977S, were caused by a founder effect.

Results: We found 26 different mutations within the Tg gene in 52 patients from 41 families. Thirty-five patients were homozygous for the mutations, whereas the others were compound heterozygous. The occurrence of Tg mutation within the general Japanese population is

one in 67,000. Patients with the C1245R mutation were found throughout Japan, whereas those with the C1058R mutation were confined to a small village on a southern island, and those with the C1977S mutation were restricted to a city. The eight patients with the C1058R mutation and the seven patients with the C1977S mutation all showed the same combinations of 18 single-nucleotide polymorphisms in the coding region of the Tg gene, which would appear in one in 810 million and one in 37 billion, respectively, control subjects.

Conclusions: The frequently found mutations, C1058R and C1977S, were caused by founder effects. This result suggests that Tg mutations may provide a genetic basis for the cause of familial euthyroid goiter. (*J Clin Endocrinol Metab* 91: 3100–3104, 2006)

MUTATIONS OF THE thyroglobulin (Tg) gene are one of the genetic causes of dysmorphogenesis, which is characterized by the development of goiters with autosomal recessive inheritance (1). Previously, only a few cases of Tg mutations had been reported in subjects displaying severe clinical manifestations, including both impaired physical development and mental retardation (2–8). However, we (9) and others (5) have reported mild cases, where the clinical manifestations include longstanding enlarged goiter from childhood with mild hypothyroidism to euthyroidism.

In this paper, we report on 52 Japanese patients with Tg mutations who were euthyroid to mildly hypothyroid. In addition to the previously reported Tg mutations C1058R, C1245R, C1977S, and G2356R (9, 10), we identified 22 novel

mutations. Among them, three mutations, C1058R, C1245R, and C1977S, were frequently identified. In particular, two of the mutations, C1058R and C1977S, were found only in specific regions of Japan. Haplotype analysis confirmed that these two mutations were caused by a founder effect.

Subjects and Methods

Subjects and Tg gene analysis

Seventy-six patients from 64 families were screened for mutations within the Tg gene. Twenty patients from 16 families showed high serum TSH at neonatal mass screening and goiters in early childhood. Fifty-six adult patients from 48 families were suspected of having Tg mutations because of clinical symptoms, laboratory tests, and histological findings. This study was approved by the ethical committee of Dokkyo University School of Medicine, and the patients or their parents expressed willingness to enroll in the study by signing informed consents.

Direct sequencing of Tg gene

Genomic DNA was isolated from the peripheral leukocytes using a QIAamp blood kit (QIAGEN, Hilden, Germany). All 48 exons and neighboring introns of the Tg gene were amplified using the Expand High-

First Published Online May 23, 2006

Abbreviations: SNP, Single-nucleotide polymorphism; Tg, thyroglobulin; TPO, thyroid peroxidase.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

Fidelity PCR System (Roche, Mannheim, Germany) and directly sequenced by the Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Norwalk, CT) (10).

Haplotype analysis

To study allelic frequencies of single-nucleotide polymorphisms (SNPs) in the coding region of the Tg gene, the nucleotides at 2330 (exon 10), 2443 (exon 10), 2963 (exon 11), 3906 (exon 18), 4493 (exon 21), and 7920 (exon 46) in the Tg cDNA were sequenced in 10 healthy subjects (20 chromosomes) and that at 426 (exon 4) in 50 healthy subjects (100 chromosomes). The nucleotide frequency of the other 11 SNPs was reported previously (9). The likelihood of the haplotypes of the patients with the mutation C1058R, C1245R, and C1977S in a Japanese population was calculated by allelic frequencies of each SNP. First, the likelihood of occurrence of the two nucleotides at a particular SNP position was calculated by multiplying the likelihood of the two nucleotides. Then, the overall likelihood of the haplotype of each patient was calculated by multiplying the likelihood at all of the 18 SNP locations in individual patients.

Results

Tg gene mutations (Table 1)

Direct sequencing of genomic DNA showed mutations within the Tg gene in 52 patients from 41 families. We detected 26 different mutations: 18 missense mutations, four splice mutations, three nonsense mutations, and one single-nucleotide deletion. Thirty-five patients were homozygous for the mutations, whereas 17 patients were compound heterozygous. The mutations are located across the full length of the Tg gene.

Clinical characteristics (Tables 1 and 2)

Among 16 patients born after 1979 when mass screening of hypothyroidism was initiated in Japan, 12 patients were

positive at neonatal mass screening. Among the remaining four patients, only one patient born in 2001 with the mutation Q310P/C2135Y was confirmed negative at mass screening by her medical record. The other three patients who were born in 1980 or 1981 had no medical record. Seven patients positive at mass screening were maintained on thyroid hormone replacement therapy. Their serum TSH level was high and free T₄ was low at mass screening (Table 2). Five patients positive at mass screening were either transiently treated with thyroid hormones or not treated at all. Their thyroid function tests showed subclinical hypothyroidism after 5 yr of age.

Patients born before 1979 suffered from longstanding voluminous goiters from childhood. Their thyroid functions were euthyroid to mildly hypothyroid (Table 2). The serum TSH levels were less than 10 mU/liter, except for one patient with the mutation C1245R/Q2638X who had a serum TSH of 51 mU/liter. Thyroid autoantibodies against Tg, thyroid peroxidase (TPO), and TSH receptor were negative in all the patients. Their serum Tg concentrations were low when compared with the size of goiter. Radioactive iodine uptake by the thyroid glands was high. In 12 patients who underwent an operation, abnormal Tg was suspected by typical histological findings of Tg mutations, which include empty-looking colloid in small follicles covered by tall (or cuboidal) follicular cells containing yellow-green granules (11).

Prevalence of Tg mutations

The prevalence of Tg mutation was analyzed in the Kumamoto Prefecture of Japan. The total number of infants who underwent mass screening from 1990–1999 was 199,826.

TABLE 1. Tg mutations

Nucleotide	Amino acid	No. of families	No. of patients	Born after 1979			
				MS(+) on therapy	MS(+) therapy-free	MS(-)	No record
580T→G/580T→G	C175G/C175G	1	1	0	0	0	0
986A→C/IVS30+1G→A	Q310P/Δexon30	1	2	2	0	0	0
986A→C/6461G→A	Q310P/C2135Y	1	1	0	0	1	0
2969G→A/3035C→T	S971L/P993L	1	1	0	0	0	1
IVS10-1G→A/IVS10-1G→A	Δexon11/Δexon11	1	1	0	0	0	0
3229T→C/3229T→C ^a	C1058R/C1058R ^a	5	8	0	0	0	0
3790T→C/3790T→C ^{a,b}	C1245R/C1245R ^{a,b}	11	12	0	2	0	1
3790T→C/2131C→T	C1245R/Q692X	1	1	0	1	0	0
3790T→C/4537delG	C1245R/FS1513→1566X	1	2	2	0	0	0
3790T→C/IVS24+1G→C	C1245R/Δexon24	1	1	0	0	0	0
3790T→C/7123G→A ^a	C1245R/G2356R ^a	1	1	0	0	0	0
3790T→C/7969C→T	C1245R/Q2638X	1	1	0	0	0	0
4397G→A/3022C→T	S1447N/R989C	1	1	0	0	0	0
4397G→A/7123G→A	S1447N/G2356R	1	1	0	0	0	1
4820G→T/4820G→T	C1588F/C1588F	2	4	0	0	0	0
4820G→T/4310G→A	C1588F/W1418X	1	1	0	0	0	0
5690G→A/7007G→A	C1878Y/R2317Q	1	1	1	0	0	0
5791A→G/6017G→A	I1912V/C1987Y	1	2	2	0	0	0
5986T→A/5986T→A ^{a,b}	C1977S/C1977S ^{a,b}	5	7	0	2	0	0
5986T→A/4820G→A	C1977S/C1588F	1	1	0	0	0	0
6956G→A/6956G→A	G2300D/G2300D	1	1	0	0	0	0
7121G→T/IVS45+2T→A	G2355V/Δexon45	1	1	0	0	0	0
		41	52	7	5	1	3

Nucleotides are numbered denoting the A of the initiator ATG codon as +1. Amino acids are numbered in the mature protein after cleavage of the 19-amino-acid signal peptide. MS(+), Positive at mass screening; MS(-), negative at mass screening.

^a Patients reported in Ref. 10 were included.

^b Patients reported in Ref. 9 were included.

TABLE 2. Clinical characteristics of patients with Tg mutations

	Normal range	Born after 1979			Born before 1979
		MS(+) on therapy	MS(+) therapy-free	MS(-)	
TSH (mU/liter)	0.1–4.0	167 ± 145 (n = 7) ^a	6.30 ± 8.75 (n = 5)	4.26 ± 3.76 (n = 4)	4.20 ± 9.22 (n = 29)
Free T ₄ (pmol/liter)	8–28	4.68 ± 2.27 (n = 7) ^a	13.10 ± 1.78 (n = 5)	12.55 ± 2.48 (n = 4)	10.94 ± 3.27 (n = 24)
Tg (mg/liter)	1–30	BDL	5.3 ± 2.7 (n = 4)	44.4 ± 41.3 (n = 4)	25.5 ± 34.4 (n = 33)
RAIU (%)	5–20	54.6 ± 19.2 (n = 4)	55.3 ± 18.9 (n = 5)	56.4 ± 3.3 (n = 2)	53.6 ± 16.9 (n = 27)

n, Number of patients whose thyroid function tests were available. BDL, Below detection limit; MS(+), positive at mass screening; MS(-), negative at mass screening; RAIU, radioactive iodine uptake.

^a *P* < 0.01 *vs.* born before 1979.

Among 155 infants whose serum TSH was more than 10 mU/liter, 111 infants were subsequently evaluated. Twenty-two infants suffered from abnormal thyroid development, six suffered from dyshormonogenesis, 25 suffered from mild persistent hypothyroidism without known causes, and 12 suffered from transient hypothyroidism. Because we identified three infants with Tg mutations, the incidence of Tg mutations is one in 67,000.

Founder effects

C1058R, C1245R, and C1977S mutations were frequently found in this study (Table 1). We found eight cases from five families homozygous for the C1058R mutation, 12 cases from 11 families homozygous for the C1245R mutation, and seven cases from five families homozygous for the C1977S mutation. To distinguish whether these mutations were derived from a common ancestral chromosome or an independently recurrent mutation, we analyzed 18 SNPs within the coding region of the Tg gene (Table 3). Eight patients homozygous for the C1058R mutation and seven patients homozygous for the C1977S mutation showed the same haplotypes, which based on statistical analysis would be expected to occur at a frequency of one in 810 million and one in 37 billion individuals, respectively. Therefore, our results suggest that the homozygous occurrence of these mutations is a result of a

founder effect. However, the haplotypes of three patients homozygous for the C1245R mutation were different from the others, suggesting that some of the C1245R alleles were a result of independently recurrent mutations.

Discussion

We have detected 26 different mutations of the Tg gene in 52 patients from 41 families. Because three nucleotides (*i.e.* CAG, after nucleotide 2952) were missing in the original report (12), the numbering of the nucleotides and, hence, the amino acid residues was changed accordingly (9, 13). Moreover, because the signal peptide consisting of 19 amino acids was usually excluded in the description of the Tg protein (1), C1263R and C1995S in our previous report (9) were changed to C1245R and C1977S, respectively, in this report. Among these Tg mutations, 69% (18 of 26) were missense mutations, which in many cases occurred as homozygous and compound heterozygous states.

In our series of patients, the symptoms were mild. Among 12 patients who were positive at neonatal mass screening, five were either transiently treated with thyroid hormones or not treated at all. In adult patients, the only clinical manifestation was longstanding goiter with euthyroidism or mild hypothyroidism. No patients developed mental retardation, even without treatment. Laboratory tests were compatible with Tg gene abnormalities, and the serum Tg concentrations

TABLE 3. Eighteen SNPs in patients homozygous with C1058R, C1977S, and C1245R mutations

Exon	cDNA	Nucleotide	Frequency in general population	C1058R, patients 1–8	C1977S, patients 1–7	C1245R			
						Patients 1–9	Patient 10	Patient 11	Patient 12
e4	426	C;T	99;1	TT	CC	CC	CC	CC	CC
e10	2200	T;G	37;63	GG	TT	TT	GG	TT	TT
	2330	C;T	90;10	CC	CC	CC	CC	CC	CC
	2334	T;C	31;69	CC	TT	TT	CT	TT	TT
	2443	G;A	85;15	GG	GG	GG	GG	GG	GG
	2488	C;G	98.6;1.4	CC	GG	CC	CC	CC	CC
e11	2963	G;C	85;15	GG	GG	GG	GG	GG	GG
e12	3082	A;G	66;44	GG	AA	AA	GA	AA	AA
e18	3906	G;A	90;10	GG	GG	GG	GG	GG	GG
	3935	G;A	31;69	AA	GG	AA	AA	AA	AA
e21	4493	C;T	90;10	CC	CC	CC	CC	CC	CC
	4506	C;T	69;31	TT	CC	TT	CT	TT	TT
e29	5512	A;G	50;50	GG	AA	GG	AG	AG	GG
e33	5995	C;T	79;21	CC	TT	CC	CC	CT	TT
e43	7408	C;T	81;19	CC	CC	CC	CC	CC	CC
	7501	T;C	91;19	TT	TT	TT	TT	TT	TT
e44	7589	G;A	63;37	GG	GG	GG	GG	GG	GG
e46	7920	C;T	90;10	CC	CC	CC	CC	CC	CC

Changed nucleotides in patients with C1245R are indicated in *italics*.

of the patients were low compared with the size of goiter. No antibodies against Tg and TPO were detected. Radioactive iodine uptake was increased without organification defect under perchlorate discharge test. Histological studies pointed to abnormal protein trafficking from endoplasmic reticulum to Golgi, which is a frequent consequence of Tg gene mutations (14). We have shown that Tg mutations, C1245R and C1977S, which perturb correct folding of Tg protein, caused impaired intracellular transport, leading to accumulation of abnormal Tg in endoplasmic reticulum (9, 15, 16). The mutation C1058R also caused defects in intracellular transport as shown by carbohydrate analysis of Tg protein and increased expression of molecular chaperones in the patients' thyroid (data not shown). Defective intracellular transport of Tg was also confirmed by the *in vitro* expression of mutant proteins in heterologous culture cells (data not shown). Allelic frequency of all the mutations was less than 0.01 (data not shown).

The three mutations, C1058R, C1245R, and C1977S, were frequently found in Japan. None of the mutations have been reported in other countries. In the Japanese population as a whole, the overall incidence of Tg mutations is estimated at one in 67,000 live births. This figure is comparable with the one in 66,000 occurrence of total iodide organification defects, the majority of which are caused by mutations in the TPO gene (17). Patients with the mutations C1058R and C1977S were restricted to specific locations within Japan, whereas those with the C1245R mutation were found all over the country. This observation suggests that the C1058R and C1977S mutations originated from a single person (founder effect). In particular, the small village where the C1058R mutation was found is isolated by steep mountains and sea, inhibiting movement to nearby areas. A public health survey in 1964 reported that 55 people of 379 dwellers (14.5%) in the village suffered from goiter (18). When taking into account that only homozygotes present with symptoms, 47% of the dwellers were deemed heterozygous carriers of the mutation.

To evaluate whether these mutations were inherited from a common ancestral chromosome or independently recurrent mutations in heterogeneous genetic backgrounds, we studied 18 SNPs within the coding region of the Tg gene. We (9) and others (13) previously reported 15 SNPs within the Tg gene. In this report, we identified six more SNPs. The frequencies of the minor alleles of these SNPs were relatively rare, *i.e.* up to 15%. The haplotypes of eight patients with the C1058R mutation and seven patients with the C1977S mutation were identical. When calculated from allelic frequencies of the SNPs in normal controls, the occurrence of the mutations C1058R and C1977S in the Japanese population would be 1.2×10^{-9} and 2.7×10^{-11} , respectively. This confirms the hypothesis that homozygous occurrence of C1058R and C1977S was a result of a founder effect. However, three patients with the C1245R mutation possessed different haplotypes from the other nine patients. In particular, some of the SNPs presented with both major and minor alleles in a single patient. These results suggest that the C1245R mutation is an independently recurrent mutation. However, taking into account that nine patients share a com-

mon allele, an alternative explanation is that C1245R is an old mutation with the new SNPs being created independently.

In conclusion, we report 26 different mutations of the Tg gene in 52 patients from 41 families. Among three frequently found mutations (C1058R, C1245R, and C1977S), haplotype analysis confirmed that C1245R and C1977S were caused by a founder effect. Therefore, Tg mutations should be considered as one of the genetic causes of familial euthyroid goiter.

Acknowledgments

We thank N. Ohmika and T. Namatame at Dokkyo University School of Medicine for their technical assistance in genetic analysis.

Received December 12, 2005. Accepted May 17, 2006.

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This study was partially supported by a Grant-in-Aid for Scientific Research (12672248 and 16590462) from the Japan Society for the Promotion of Science (to A.H.).

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Effects of dioxins on the quantitative levels of immune components in infants

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Dioxins (polychlorinated dibenzo-*p*-dioxin (PCDD) + polychlorinated dibenzofuran (PCDF)) and polychlorinated biphenyls (PCBs) are potentially hazardous compounds and have structural similarity with thyroid hormones. Animal studies have demonstrated that PCDDs, PCDFs and PCBs can alter immune functions. However, in humans it is not yet elucidated whether dioxins contained in breast milk have any effects on the immune functions in infants. To investigate the effects of dioxins on the immune system, we compared the quantitative levels of immune components between a breast-fed group and bottle-fed group, in which dioxin concentration is almost zero. Ratios of immune cells, such as CD4+ and CD8+ T-lymphocytes, as well as B-lymphocytes (CD19+ and/or CD20+) and NK cells (CD16+, CD56+) in peripheral blood lymphocytes, serum immunoglobulin level, and level of specific IgE antibody to allergens in the venous blood at 12 months of age were assessed in a subgroup of 281 infants. The relationship of post-natal dioxin exposure via breast feeding with the ratio of immunological markers and the level of humoral antibodies up to 12 month of age was not demonstrated. In conclusion, it would appear that the content of dioxins in breast milk in the Japanese general population is not enough to induce any change in these-examined immunological parameters during the first year of life, although long-term effects remain to be evaluated. *Toxicology and Industrial Health* 2006; 22: 131–136.

Key words: breast feeding; bottle feeding; dioxins; IgE; lymphocytes subsets

Introduction

Polychlorinated-dibenzo-*p*-dioxin (PCDD), polychlorinated-dibenzofuran (PCDF), and coplanar-polychlorinated biphenyl (Co-PCB) compounds, hereafter referred to as dioxins, are tricyclic aro-

matic compounds. They are mainly formed as byproducts of the synthesis of organochlorine chemicals and from the combustion of municipal and hazardous waste. In the late 1970s, the production and use of these compounds were banned because their adverse health effects had become evident.

Immune suppression is a common and extensively characterized sequela associated with acute 2,3,7,8,-tetrachloro-dibenzo-*p*-dioxin (TCDD)

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exposure in laboratory animals. Comprehensive cell-type fractionation-reconstitution studies have previously demonstrated the profound inhibition of B-cell function by TCDDs. According to direct addition studies utilizing primary cultured murine B cells, there is evidence suggesting that the suppression of antibody production by TCDDs may be closely associated with altered B-cell differentiation (Suh *et al.*, 1983). This finding is further supported by the observation that TCDDs only modestly inhibit B-cell proliferation.

There is a paucity of *in vivo* studies on the effects of dioxins on the immune system of humans (Tryphona *et al.*, 1998). Initial studies, showing that PCB and dioxins may be toxic to human immune function, were carried out on individuals accidentally exposed to these compounds (Patterson *et al.*, 1988). Weisglas-Kuperus *et al.* (1995) demonstrated that prenatal PCB/dioxin exposure was associated with changes in T cell subpopulations in the blood in Dutch infants.

It is not yet clearly determined, however, whether pre- and post-natal exposures to high background levels of PCDD, PCDF and PCB can alter the immune system in human infants, and whether the health of infants is adversely affected by these pollutants. In this study, we investigated the effects of dioxins contained in breast milk on the quantitative levels of various immune components in Japanese infants from birth to 12 months of age.

Subjects and methods

We collected breast milk from 415 mothers in 20 prefectures and cities in Japan at 30 days post-partum and quantified 14 isomers for PCDDs, 15 for PCDFs and 12 for coplanar PCBs (Co-PCBs). To express the toxic potency of the mixture of dioxins in breast milk samples, the toxic equivalency (TEQ) calculation, based on the new TEF re-evaluated by WHO in 1997, was used. The ages of the mothers were limited to 25–34 years, and all mothers were primiparous and resided in the same area for more than five years.

At one year of age, blood samples were obtained from 281 breast-fed infants (breast-fed group) for the evaluation of immune functions. The breast-fed group was infants who had mainly received breast feeding until one year of age. Blood samples were

also obtained from 20 infants who were bottle-fed at one year of age, as a control group (bottle-fed group).

The fat content in human milk was determined by weighing, as described by Patterson *et al.* (1988). In brief, breast milk (50 mL) was mixed with saturated potassium citrate (10 mL), ethanol (100 mL), diethylether (50 mL) and hexane (120 mL) in a 500-mL separatory funnel and shaken vigorously for 10 min. The hexane phase was then removed and washed first with 2 mol/L NaOH followed by sulfuric acid. The hexane phase was then dried and weighed. The fat content of breast milk at five days post-partum was $3.0 \pm 1.4\%$, and at 30 days post-partum was $3.8 \pm 1.2\%$, and did not change thereafter (Matsuura, 2001a,b).

PCDDs, PCDFs and Co-PCBs in human milk were identified by GC/MS conducted at the Japan Food Research Laboratory (Matsuura, 2001b). Surface markers of peripheral blood monocytes (PBMCs) were quantified by flow cytometry (SRL, Tokyo, Japan) (Ip *et al.*, 1982).

Serum IgE concentrations were determined by chemiluminescent enzyme immunoassay (Matsui *et al.*, 2000). Specific IgE antibodies for house dust, milk and egg white were quantified by fluoroenzyme assay (CAP RAST FEIA, Pharmacia & Upjohn, Sweden). All parents who participated in this study gave their written informed consent.

The ratio of CD3, CD4, CD8, CD4/8, CD19, CD20 and CD86 between the breast-fed and bottle-fed groups was analysed by Student's *t*-test. The serum immunoglobulin levels of IgG, IgA, IgM and IgE between the breast-fed and bottle-fed groups were analysed by Student's *t*-test. Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test. Probability (*P*) values <0.05 were considered to be statistically significant.

Results

Effect of dioxins in breast milk on T cell ratio in PBMCs

The ratios of CD3+, CD4+, CD8+ or CD4+/CD8+ cells in PBMCs were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated. The correlation between the concentration of dioxins in human milk and T cell ratio was also investigated

Table 1. The ratio of lymphocyte subsets in the breast-fed and bottle-fed groups.

	Breast-fed group (N=281)	Bottle-fed group (N=20)	P
CD3 (%)	73.1±7.2	69.9±6.1	0.053
CD4 (%)	50.1±8.4	48.6±6.7	0.436
CD8 (%)	24.1±5.8	24.1±7.4	0.861
CD4/CD8	2.2±0.9	2.3±1.2	0.718
CD19 (%)	14.7±5.6	15.9±6.7	0.362
CD20 (%)	14.3±5.6	15.9±5.8	0.242
CD86 (%)	0.8±0.5	1.2±0.9	0.078
IgG (mg/dL)	645.1±182.1	694.0±186.2	0.248
IgA (mg/dL)	34.2±22.6	36.6±19.1	0.644
IgM (mg/dL)	105.9±33.6	106.9±41.4	0.899
IgE (U/mL)	54.4±89.9	58.2±105.9	0.857

and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on B cell ratio in PBMCs

The ratios of CD19+, CD20+ or CD86+ cells, which are the surface markers of activated B cells, were compared between the breast-fed and bottle-fed groups (Table 1). There was no significant correlation. The correlation between the concentration of dioxin in human milk and B cell ratio was also investigated and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on NK cell ratio in PBMCs

The correlation of the ratio of NK cells (CD16+/CD56+) with the concentration of dioxins in human milk was examined and no significant correlation was found (Figure 1A).

Effect of dioxins in breast milk on the serum immunoglobulin levels

The serum immunoglobulin levels of IgG, IgA, IgM and IgE were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated between them. The correlation between the concentration of dioxins in human milk and the serum immunoglobulin levels was also investigated and no significant correlation was found between them (Figure 1B).

The specific IgE antibody to house dust, milk and egg white was quantified (Table 2). It was not

demonstrated that there was no significant correlation between the breast-fed and bottle-fed groups.

Discussion

In this study, we investigated the relationship of the concentration of dioxins contained in breast milk with the ratios of immune cells and immunoglobulin levels. It has been reported that *in vitro* dioxins suppress B cell differentiation. Furthermore, there are some *in vivo* studies suggesting an effect of dioxins on immune functions (Forawi *et al.*, 2004). Smoger *et al.* (1993) reported that for children born to mothers living in a TCDDs-contaminated environment in Time Beach (MO) during and after pregnancy, a decrease in CD4+ T cells and an increase in CD8+ T cells was detected in children from nine to 14 years of age. In one preliminary study conducted in Northern Quebec, the CD4+/CD8+ T cell ratio of Inuit infants, whose mothers have increased levels of PCB and dioxins in their breast milk, decreased at six and 12 months of age (Dewailly *et al.*, 1993).

Svensson *et al.* (1994) reported that the consumption of fatty fish species, such as salmon and herring, from the Baltic Sea is an important source of human exposure to persistent organochlorine compounds, eg, PCDDs, PCDFs and co-PCBs. The high fatty-fish consumers had lower ratios and numbers of NK cells, identified by the CD56 marker, in peripheral blood than the non-consumers. The weekly intake of fatty fish correlated negatively with the ratio of NK cells. This indicates that accumulation of persistent organochlorine compounds in high fatty-fish consumers may adversely affect NK cell ratios. Weisglas-Kuperus *et al.* (1995) demonstrated that a high post-natal PCB/dioxin exposure is associated with an increase in the number of TcRγδ+ T cells at birth and with an increase in the number of CD8+, TcRαβ+ or TcRγδ+ T cells at 18 months of age. Nagayama *et al.* (1998) reported that the ratios of CD4+ to CD8+ T cells had a significant increasing tendency with the estimated total TEQ intakes.

In our study, it was not demonstrated that the ratios of T cell subpopulation and CD16+ CD56+ cells (NK cells) in PBMCs correlated with the concentration of dioxins in human milk, although the number of CD4+ and CD8+ T cells were not

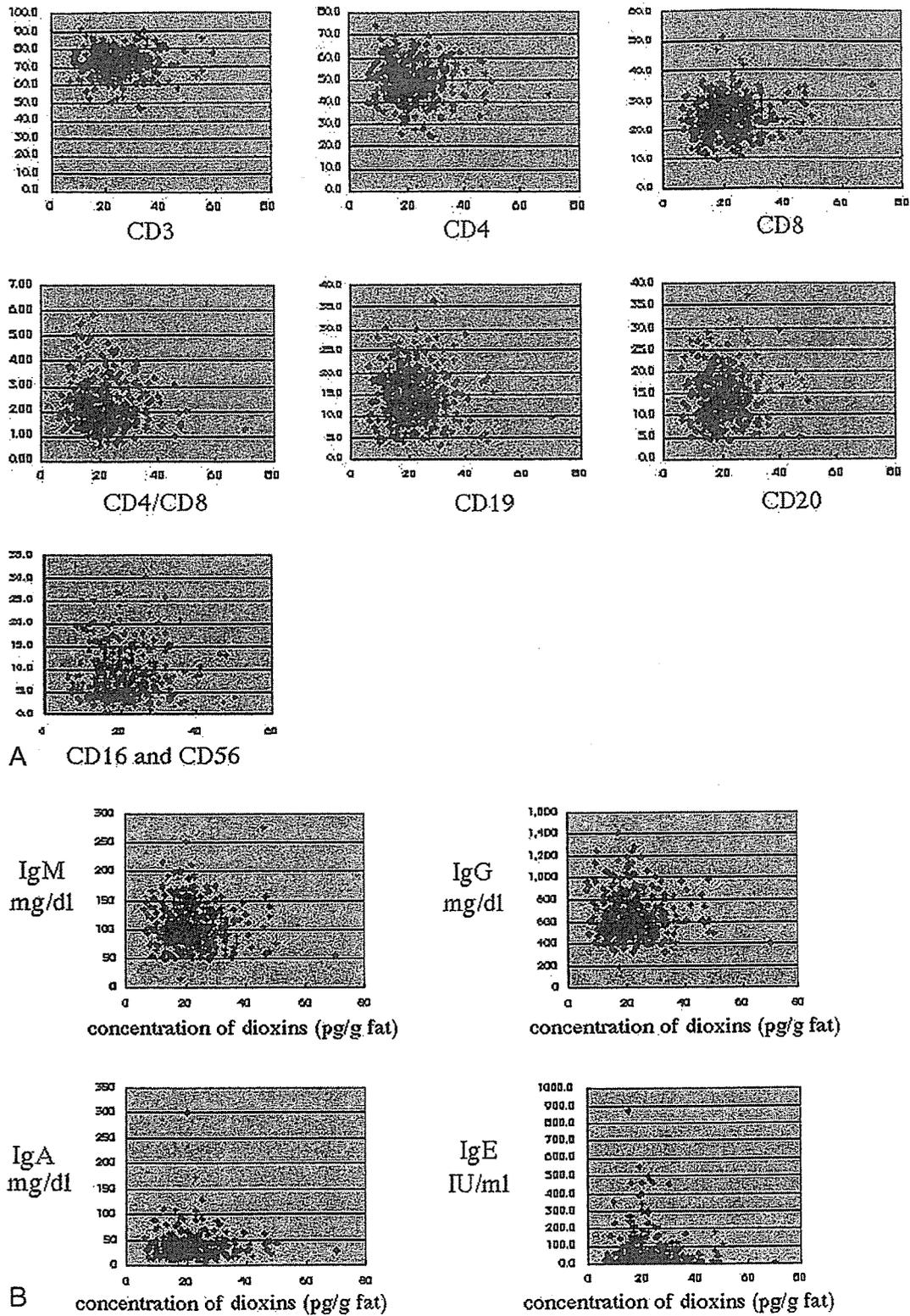


Figure 1. (A) Relationships between ratios of CD3+, CD4+, CD8+, CD4+/CD8+, CD19+, CD20+ and CD16+ CD56+ cells in PBMCs and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the concentration of dioxins (pg/g fat). Y-axis means the percent of surface marker positive cells except CD4/CD8. In CD4/CD8, Y-axis means the ratio of CD4 per CD8. (B) Relationships between ratios of serum IgM, IgG, IgA and IgE and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the estimated intake of dioxins (pg/g fat). Y-axis means the serum concentration of IgM (mg/dL), IgG (mg/dL), IgA (mg/dL) and IgE (IU/mL).

Table 2. Specific IgE antibody of breast-fed and bottle-fed groups.

	Breast-fed group							Bottle-fed group							P
	0 ^a	1	2	3	4	5	6	0	1	2	3	4	5	6	
House dust	251 ^b	8	10	5	1	1	0	19	0	1	0	0	0	0	0.568
Milk	238	12	20	5	1	0	0	17	0	2	1	0	0	0	0.558
Egg white	180	24	44	22	2	3	0	15	3	2	0	0	0	0	0.264

^aNumber (0–6) indicates CAP-RAST scores to each allergens.

^bNumber indicates the number of persons who have the CAP-RAST score.

Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test.

analysed. We could not find that the levels of serum immunoglobulins and specific IgE to allergen were significantly different between the breast-fed and bottle-fed groups. The difference between our data and those of Weisglas-Kuperus may be due to the following: the time at which immunological analysis was performed, and the amount of dioxins to which the subjects were exposed, that is, a higher concentration of dioxins in the early days after birth; the content of dioxins in breast milk was almost 2-fold higher in the Netherlands (30.75 pg TFQ/g fat) than in our study (14.8 ± 6.1 pg TFQ/g fat) (Matsuura *et al.*, 2001b).

The sample size in this study was 281 in the breast-fed group and 20 in the bottle-fed group, which is a maximum size considering the budget for this study and the co-operation of the mothers. When we consider the value between the breast-fed and bottle-fed groups (shown in Table 1 as true difference), the power of CD3 and CD86 was higher than 50%, however, CD8, CD4/8, IgA, IgM and IgE was lower than 10%.

On the basis of the results of this study, we conclude that, although the infants were exposed to some amounts of dioxins in the breast milk in Japan, we could not find that the quantitative levels of immune components at one year of age was seriously impaired. However, long-term effects remain to be evaluated.

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濾紙血 TSH、freeT4 同時測定によるクレチン症マススクリーニング ～当院における5年間の結果～

Neonatal Mass-screening for congenital hypothyroidism detected by TSH and freeT4

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【要旨】

神奈川県ではクレチン症新生児マススクリーニングにおいて、血清 TSH、free T4(以下 FT4) 同時測定が行われている。5年間の当院で扱ったクレチン症マススクリーニング精査症例について検討した。1999年4月から2004年3月の5年間に、マススクリーニングで精密診査対象となり、当院で精査を施行した症例は62例で、このうち明らかなヨード暴露12例、母体甲状腺機能異常7例を認めた。体重別内訳は、成熟児(≥2500g、24例)、低出生体重児(1500～2499g、12例)、極低出生体重児(1000～1499g、8例)、超低出生体重児(<1000g、18例)に分け、かつ TSH 高値 FT4 低値(A群)、TSHのみ高値(B群)、FT4のみ低値(C群)に分け検討した。以下の結果を得た。(1)成熟児：A,B,C群それぞれ3,11,10例、低出生体重児：A,B,C群；1,6,5例、極低出生体重児：A,B,C群；1,1,6例、超低出生体重児：A,B,C群；3,1,14例と、精査対象となった62例中35例(約56%)がFT4のみ低値を示すC群であった。(2)ヨード暴露群ではC群が多く、母体甲状腺機能異常群ではB群が多い傾向であった。(3)死亡症例10例中9例がC群のパターンを示し、C群25例中9例(約36%)が死亡例であった。TSH、FT4同時測定マススクリーニングでは、FT4のみ低値の症例が多く発見され、重症死亡例や未熟性に伴う甲状腺機能低下の疑われる症例が多く見いだされた。

【キーワード】

クレチン症、新生児マススクリーニング、超低出生体重児

【はじめに】

本邦におけるクレチン症の新生児マススクリーニングは1979年に開始されたが、ほとんどの地域で濾紙血 TSH 測定が採用された。神奈川県では1979年10月より T4、TSH 同時測定によるマススクリーニングが開始され、1990年から T4 にかわり freeT4(FT4) を用いた TSH、FT4 同時測定が採用されて

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いる。この同時測定マススクリーニングにより、クレチン症の早期発見、治療だけではなく、TSH 測定のみでのマススクリーニングでは発見できない中枢性甲状腺機能低下症の早期発見などにも効果をあげてきた^{1),2)}。一方、近年生命予後が著しく改善している超低出生体重児を中心とした早産児、低出生体重児におけるクレチン症マススクリーニングの検査方法、結果、解釈などの問題に関する報告が散見される^{3),4),5),6)}。今回、過去5年間の北里大学病院におけるクレチン症マススクリーニングの結果を、出生体重、検査結果別に分類し、それぞれの特徴について考察した。

【対象と方法】

神奈川県では予防医学協会にて TSH、FT4(現在 ELISA 法にて実施)同時測定によるクレチン症マススクリーニングが施行されている。カットオフ値は①TSH(血清表示)30 μ U/ml(全血表示に換算すると1.6を除して18.8 μ U/ml)以上を即精密診査(即精査)、②TSH 15 μ U/ml以上30 μ U/ml未満を再採血検査(要再検)、③FT4 0.7ng/dl未満を再採血検査(要再検)としている。なお、出生体重2000g以下の児の日齢5~7での初回検査は、結果のいかんにかかわらず参考値とみなし、全例日齢30前後もしくは体重が2500gに達した時点で2回目検査を施行している。また、再採血検査対象症例は再度濾紙血検査を施行し、結果が初回と同様に再び異常を示した場合(TSH 15 μ U/ml以上30 μ U/ml未満、FT4 0.7ng/dl未満)全て精密診査の扱いとなる。1999年4月から2004年3月までの5年間で、北里大学病院で出生あるいは入院となった児のマススクリーニング検査にて即精密診査対象となり、精査した62例を対象とした。これらを出生体重、出生検査結果のパターン別に分類して検討した。出生体重は成熟児(\geq 2500g)、低出生体重児(=LBW: 1500~2499g)、極低出生体重児(=VLBW: 1000~1499g)、超低出生体重

児(=ELBW: <1000g)の4グループに分けた。検査結果別にTSH高値かつFT4低値(A群)、TSHのみ高値(B群)、FT4のみ低値(C群)の3グループに分類した。

【結果】

#1 出生体重別の対象症例の割合

5年間で精査対象となった症例は62例であった。これは対象期間中に当院でマススクリーニング検査を施行した全体の約0.99%(全6290症例中62例)であった。62症例の内訳は男児32例、女児30例であった。対象期間中に当院でマススクリーニングを取り扱った全症例のうち精査対象となった症例が占める割合は、体重別内訳では成熟児で0.44%(24/5376症例)、LBWで2.18%(12/548症例)、VLBWで3.94%(8/203症例)、ELBWで11.0%(18/163症例)であった。日齢30前後に2回目検査を施行した出生体重2000g以下の児で精査対象となった症例は30例で、全62例の約48.4%を占めた。また血清TSH 30 μ U/ml以上の症例は62例中11例(17.7%)で、TSH 15~30 μ U/mlは62例中16例(25.8%)、FT4 0.7ng/dl未満(TSHは15 μ U/ml未満)は62例中35例(56.5%)であった。

#2 ヨード暴露群の解析結果

全62例の中に、児の甲状腺機能に明らかに影響を及ぼすと考えられるヨード暴露による異常を示した症例は12例であった。(表1)。これらの12例の体重別内訳は、成熟児7例、LBW2例、VLBW1例、ELBW2例であった。疾患の内訳は、横隔膜ヘルニア3例、心疾患3例(動脈管開存症2例、ファロー四徴症1例)、先天性食道閉鎖1例、CCAM(=先天性嚢胞性腺腫様奇形)1例、腸穿孔1例、緊張性気胸1例、先天性乳び腹水1例、臍カテーテル挿入1例であり、イソジン消毒によるヨード暴露が強く疑われた。検査結果パターン別内訳は、B群4例、C群8例であった。このうちB群の4例中3例は成熟児、