[Short Report]

A Preliminary Study on the Use of Meconium for the Assessment of Prenatal Exposure to Heavy Metals in Japan

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Abstract : In recent years, there has been increasing interest in the relevance of environmental pollution to children's health. We investigated whether the concentrations of heavy metals in meconium can be used for the assessment of prenatal exposure in Japan. Our study subjects were newborns whose mothers were hospitalized for childbirth at Tokai University Hospital between November 2010 and March 2011. The target compounds of this study were lead (Pb), cadmium (Cd) and arsenic (As). Meconium samples collected from 102 newborns were analyzed using ICP-MS. The median concentrations of Pb and As in the meconium were 0.71 ppb and 0.03 ppb, respectively. The median concentration of Cd was below the limit of quantification. These concentration values are much lower than those used in previous studies. Further study is needed to compare the concentrations of heavy metals in biological samples and to investigate the availability of meconium for the assessment of exposure to heavy metals during pregnancy.

Keywords : children's health, prenatal exposure, meconium, heavy metals, newborn.

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Introduction

In recent years, there has been increasing interest in the relevance of environmental pollution to children's health. Environmental pollutants in soil, air, water, and food accumulate in the body through various routes, and children (including infants) and the elderly are considered as vulnerable groups to pollutants. As children grow day by day, they breathe more, eat more, and drink more water per unit of body weight than adults. It should be considered that poisons and/or pollutants will have a much larger effect on their immature organs than on those of adults. The prena-

tal period in particular is a time when the central nervous system is developing, and the fetus is sensitive to chemical exposure during the fetal stage. A pregnant mother's exposure to environmental pollutants could have a significant impact on her child [1, 2]. It is important to investigate how children's development might be affected by fetal exposure to environmental pollutants.

Much research has been conducted on the health effects of environmental pollution on children, and in recent years various studies have focused on prenatal exposure. The National Children's Study (NCS) is a 21-year long cohort study being conducted on ap-

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proximately 100,000 children that is investigating the interaction between genetic factors and environmental factors and their effects on pregnancy and childbirth, the health and development of children, and precursors of adult diseases [3]. The Children's Environment and Health Action Plan for Europe (CHHAPE), issued by the EU, is a plan to reduce environmental exposure and prevent asthma, allergies, respiratory diseases, and congenital disorders [4]. In South Korea, the Mother and Children's Health and Environment (MOCHE) Study, which is a study to assess environmental exposure of children and pregnant women and their health, began in 2006 [5]. In Japan, the Ministry of the Environment launched a cohort study in 2011 (Japan Environment and Children's Study, JECS) to investigate the development of physical and mental health of children with prenatal exposure [6].

Exposure assessment should be important in the study of the relationship between exposure to chemical substances and health effects. Even though a number of exposure assessment studies have been conducted on children, there is not yet enough information about prenatal exposure. Thus far, heavy metal exposure assessments during the fetal period were carried out mainly using children's hair and cord blood. Collection, transportation and storage of hair are easy and inexpensive, and this method provides short-term and long-term exposure information. However, there are some disadvantages in that concentration is affected by hair color and ethnicity. It has also been pointed out that hair cannot be collected at birth [7]. Blood is considered an ideal biological sample for exposure assessment, but it has the disadvantage that it must be collected in an invasive way. Therefore, the use of blood as a biomarker should be restricted. There are also a few reported cases in Japan about the use of cord blood as a biomarker for environmental exposure. Although metal concentrations in cord blood can be interpreted as exposure, it is preferable to use other biomarkers when discussing health effects due to heavy metal contaminants. Exposure information for mother and child can be obtained from breast milk, and milk is often used to monitor information about exposure to fat-soluble chemicals. It is a non-invasive biological sample, but the measurement time is limited to the period of breastfeeding [8].

Meconium is the first, viscous, odorless and black feces discharged two to three days after birth. It can be used to examine cumulative prenatal exposure, because it is not excreted before birth. Meconium is usually discarded and can be collected non-invasively. Therefore, we can expect to easily get consent for sample acquisition, and to increase the number of samples as a result. While some studies to evaluate heavy metal exposure using meconium have been conducted in other countries [1, 9, 10], there are no reports in Japan.

In this study, we examined the possibility of using meconium in Japan to measure prenatal exposure to low concentrations of heavy metals, and investigated the distribution of heavy metal concentrations in meconium, although the results are not at present linked to a discussion of health effects due to heavy metals.

Heavy metal contamination during pregnancy would be affected by the lifestyle and living habits of the mother. Higher concentrations of heavy metals have been observed with higher gestational age of the mother, and metal concentrations in mothers with a higher number of births can be lower [10, 11]. In addition, occupational exposure, residential area, lifestyle during pregnancy (medication, drinking, smoking, diet, *etc.*) have to be considered as important factors in exposure levels. Therefore, we also conducted a questionnaire survey and investigated the relevance of maternal lifestyle to the concentration of heavy metals in meconium.

Methods

Subjects and data collection

Our study subjects were newborns born at Tokai University Hospital (Isehara, Japan) between November 11, 2010 and March 11, 2011 and their mothers. We explained the purpose of the survey to each mother who was admitted to the hospital for childbirth and obtained written consent if she agreed to participate in the study.

A nurse collected meconium samples when changing diapers. If twins were born, meconium was collected from only one child, according to a request from the person in charge of the survey, in order to avoid any confusion in daily work. However, it is unclear which twin the meconium sample was taken from in this study. We also asked the mothers to fill out a

questionnaire including questions such as height and weight of her newborn, daily lifestyle such as smoking and drinking, number of pregnancies, *etc.*, at the time of discharge.

The ethics committees of Tokai University School of Medicine and Tokai University School of Health Sciences approved this study.

Analysis

Each meconium sample collected was stored at -30°C in a vinyl bag with an identification code. In light of previous studies, we decided to examine the concentrations of arsenic (As), cadmium (Cd), and lead (Pb) in the meconium.

An acid decomposition method based on Ostrea *et al* [1]. was used for pretreatment. The procedure we adopted was as follows: 70% nitric acid (20 ml) was added to 0.1 g meconium, the sample was heated to dryness using a hot plate (250°C), 5% nitric acid (25 ml) was added to the sample and evaporated to 10 ml and then cooled, following which deionized water was added to make 50 ml. Finally, we analyzed the sample using ICP-MS (Agilent 7700 x). The above pretreatment method cannot dry the sample completely. Therefore, in order to determine the dry weight, the same amount of sample was dried for 2 hours in an oven at 200°C .

The limit of quantification (LOQ) and the detection limit were determined as ten times and three times the values of the standard deviation calculated from the analysis of seven blank samples, respectively. We also verified the measurement method by a recovery test in which a standard with a concentration approximately 10 times higher than the LOQ was used.

Finally, the distribution of heavy metal concentrations in the meconium with good recovery was described, and the relationships between the various factors and concentrations were examined.

Results

Meconium was collected from 108 infants during the period, but six infants were excluded from the analysis because of the small amount of their samples (less than 0.1 g). Four sets of twins were born among the final 102 subject mothers.

The average age of the mothers was 34 years old, with a range of 22–43 years old, and the mean gestation period was 37.9 weeks. Seventeen mothers had experienced more than two pregnancies, and thirty-eight were housewives. Many of the subjects were living in the city of Yokohama, which is considered to have no problem with heavy metal environmental pollution. No subjects had job experience with occupational exposure to heavy metals.

Seventy-five persons were never smokers, and twenty-seven had quit smoking before getting pregnant. Eleven mothers were exposed to secondhand tobacco smoke during pregnancy, and their exposure time was less than 30 minutes a day on average. Three of the mothers drank alcohol during pregnancy.

Seventy-four mothers (73%) consumed fish one to three times a week, sixty-eight ate shellfish one to three times a month, fifty-two had intake of dairy products daily, fifty-one had two rice meals a day, and forty-seven sometimes drank mineral water.

The mean ($\pm$ S.D) birth weight and height of the newborns was $2,864\text{ g} \pm 332\text{ g}$ and $48.0 \pm 1.9\text{ cm}$, respectively. Sixty-five babies were male (64%).

The detection limits in this study were 0.008 ppb for Pb, 0.006 ppb for Cd, and 0.007 ppb for As, and the LOQs were 0.027 ppb, 0.019 ppb and 0.022 ppb, respectively.

The average recovery for each metal was 109.5% for Pb (range: 81.5 to 145.1%), 103.2% for Cd (79.0 to 123.3%), and 100.7% for As (87.6 to 111.9%). It is thought that good recoveries were obtained for each heavy metal, and the measurements of all the metals are described below.

The average concentrations of Pb, Cd, and As ($\pm$ standard deviation) were $1.12\text{ ppb} \pm 1.37\text{ ppb}$, $0.002\text{ ppb} \pm 0.02\text{ ppb}$ and $0.03\text{ ppb} \pm 0.04\text{ ppb}$, respectively. Since more than 90% of the Cd values were below the LOQ, the average value for Cd was less than the LOQ (Table 1). The median levels were 0.71 ppb for Pb, below the LOQ for Cd, and 0.03 ppb for As. The distribution of concentration for each metal had a right-skewed shape.

The rank correlation coefficients between the concentrations of heavy metals were 0.076 between Pb and Cd, -0.06 between Pb and As, and -0.03 between Cd and As. No relationships were observed among the metals.

Table 1. Heavy metal concentrations in meconium

Metal	Mean $\pm$ S.D.*	Median (25–75%)	Range (ppb)
Pb	1.12 $\pm$ 1.37	0.71 (0.29–1.28)	< LOQ–6.91
Cd	< LOQ $\pm$ 0.02	< LOQ (<LOQ–LOQ)	< LOQ–0.15
As	0.03 $\pm$ 0.04	0.03 (<LOQ–0.05)	< LOQ–0.18

n = 102, * < LOQ: calculated as zero level

Table 2 shows the concentration of heavy metals stratified by certain factors. Since most Cd concentrations were less than the LOQ, the stratified analysis for Cd was not conducted.

The median Pb concentration was 0.72 ppb in mothers under 35 years old and 0.70 ppb in mothers at least 35 years old. The median As level was 0.02 ppb in mothers under 35 years old and 0.04 ppb in mothers at least 35 years old. There were no significant differences. There were also no significant differences in the concentration of heavy metals in the meconium by other factors such as child sex, number of pregnancies, smoking, or dietary intake of fish, selfish, dairy products or rice.

Table 2. Heavy metal concentrations stratified by maternal factors

Factor (Number)	Pb	As (ppb)
Age		
< 35 yrs (58)	0.72 (0.33–1.53)	0.02 (< LOQ–0.04)
$\geq$ 35 yrs (44)	0.70 (0.20–1.25)	0.04 (0.03–0.06)
Sex (newborn)		
Male (65)	0.74 (0.30–1.46)	0.03 (< LOQ–0.04)
Female (37)	0.62 (0.28–1.03)	0.04 (< LOQ–0.06)
No. of pregnancies		
< 3 (95)	0.71 (0.29–1.34)	0.03 (< LOQ–0.05)
$\geq$ 3 (6)	0.63 (0.41–0.79)	0.03 (0.02–0.11)
Current medication		
Yes (37)	0.68 (0.27–1.35)	0.03 (< LOQ–0.05)
No (63)	0.72 (0.30–1.29)	0.03 (< LOQ–0.06)
Medication before pregnancy		
Yes (38)	0.80 (0.33–1.36)	0.03 (< LOQ–0.05)
No (64)	0.66 (0.28–1.23)	0.03 (< LOQ–0.05)
Smoking		
Yes (27)	0.58 (0.24–0.88)	0.03 (< LOQ–0.06)
No (75)	0.74 (0.35–1.34)	0.03 (< LOQ–0.05)
Secondhand smoke		
Yes (11)	0.71 (0.42–0.88)	< LOQ (< LOQ–0.03)
No (85)	0.71 (0.29–1.54)	0.03 (< LOQ–0.06)

Subject number in parenthesis, Median (25–75%)

Discussion

The placenta cannot prevent Pb transfer to the fetus, although the underlying transfer mechanisms remain ambiguous [12]. Ostrea *et al* [13]. discussed that a fetus can be exposed to different chemicals, most of which are deposited and accumulated in the meconium, and the process occurs through bile secretion and/or fetal swallowing of amniotic fluid, starting in the 12th week of gestation.

The primary objective of this study was to consider the possibility/applicability of the assessment of exposure to heavy metals during pregnancy by measuring their concentrations in meconium, which had not previously been reported in Japan. Another purpose was to determine the concentrations of heavy metals in meconium. We found that the concentrations of heavy metals in meconium due to prenatal exposure in Japan were low, although the applicability of meconium as a prenatal exposure marker is still unclear.

Because a method to analyze meconium has not yet been established, in this study we used the method described by Ostrea *et al*. [1]. We checked the validity of the methods of analysis, such as through recovery tests, the results of which showed that measurement in meconium could be applicable. However, it may be necessary to conduct a number of experiments of blank tests, recovery tests, *etc.*, in the future to increase reliability and validity.

Not many studies using meconium to measure exposure to hazardous chemicals have been conducted. Turker *et al*. measured contaminants in the meconium of 117 newborns in Turkey using atomic absorption spectrophotometry and reported that the median concentration of Pb was 46.5 $\mu\text{g/g}$ dry weight (wt) (interquartile range (IQR): 1,399 $\mu\text{g/g}$ dry wt) and that of cadmium was 2.3 $\mu\text{g/g}$ dry wt (IQR 55.6 $\mu\text{g/g}$ dry wt) [9].

Chen measured the concentration of heavy metals in the meconium of 198 infants in Taiwan and found concentrations of 11.69 ± 2.10 ppb dry wt for Pb (mean $\pm$ standard deviation), 13.59 ± 7.65 ppb dry wt for Cd, and 23.45 ± 50.5 ppb dry wt for As [10].

Ostrea *et al*. measured contaminants in 426 meconium samples from newborns in Manila and reported a median concentration of 35.77 $\mu\text{g} / \text{ml}$ for Pb (detection rate: 26.5%) and 13.37 $\mu\text{g} / \text{ml}$ for Cd (detection

rate: 8.5%) [1]. Mercury concentration in meconium has also been measured in several studies, although it was not measured in this study [1, 7, 8, 10–11, 14].

The concentrations obtained in this study were about 1 ppb at most. Therefore, it can be said that the concentrations were much lower than those in the previous studies described above. The low concentrations may be attributable to a possible low concentration of heavy metals in the Japanese environment, the fact that the subject mothers may not live in contaminated areas, and the fact that they have not had occupational exposure. Because of the low concentration, it is difficult to consider factors that affect the metal levels. However, the contribution to metal exposure of a mother's lifestyle, such as smoking and dietary habits, *etc.*, may be small in Japan, according to the results of the questionnaire survey.

In this study we did not collect other biological samples, such as cord blood or placenta, *etc.*, because of research restrictions. Therefore, we could not discuss the usefulness of meconium in the evaluation of prenatal exposure compared to other samples. It has been pointed out that the behavior of concentrations in meconium differs from other samples [7]. There is a need for further studies to compare the concentration of heavy metals in other biological samples and to investigate the applicability of meconium for the assessment of exposure to heavy metals during pregnancy.

Conclusion

We showed the distributions of concentrations of heavy metals in meconium due to prenatal exposure in Japan, and the concentrations were low. However, it is necessary to establish a method for using meconium to evaluate prenatal exposure and to accumulate data in order to figure out the effects of heavy metal exposure on children's health.

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日本における胎便を用いた重金属曝露評価法に関する予備的検討

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要 旨：日本における胎便を用いた胎児期重金属曝露評価法に関して、予備的検討を行った。被験者は、2010年11月から2011年3月の間に東海大学病院に出産のため入院し、胎便採取に同意した母親の新生児である。本研究の対象物質は、鉛(Pb)、カドミウム(Cd)、ヒ素(As)とし、分析は、ICP-MSを用いて行った。添加回収試験などの結果から、本研究で使用した分析方法で胎便中重金属濃度測定ができることが示された。新生児102人から採取された胎便を分析したところ、PbとAsの濃度(中央値)は、それぞれ0.71 ppb, 0.03 ppbであった。一方、Cd濃度の中央値は定量限界未満であった。胎児期曝露の曝露評価に用いることができるようにするためには、今後、種々の生体試料濃度を測定し、得られた濃度間の関係を調べることなどが必要となる。

キーワード：小児保健, 胎児期環境曝露, 胎便, 重金属, 新生児.

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