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Risk factors of early spontaneous abortions among Japanese: a matched case–control study

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BACKGROUND: No epidemiological studies have examined risk factors for early spontaneous abortions among Japanese women. In this matched case–control study, we investigated the associations of reproductive, physical, and lifestyle characteristics of women and their husbands with early spontaneous abortion <12 weeks of gestation.

METHODS: Information was collected through medical records for 430 cases of early spontaneous abortion and 860 controls of term delivery. Two controls were individual-matched to one case according to maternal age (±3 years) and calendar year of events (either early spontaneous abortion or delivery). Multivariable conditional odds ratios (ORs) and 95% confidence interval (CI) were calculated with conditional logistic-regression.

RESULTS: The risk of early spontaneous abortions was higher for women with a past history of early spontaneous abortions; OR was 1.98 (95% CI: 1.35, 2.89) for one previous spontaneous abortion, 2.36 (95% CI: 1.47, 3.79) for two, and 8.73 (95% CI: 5.22, 14.62) for three or more. Other factors also influence risk; an OR of 2.39 (95% CI: 1.26, 4.25) was found for women who smoked, and 1.65 (95%CI: 1.17, 2.35) for women working outside the home.

CONCLUSIONS: Our finding suggests that for Japanese women, smoking and working may be important public health issue targets for the prevention of early spontaneous abortions.

Key words: spontaneous abortion / Japanese / case–control study / employment / smoking

Introduction

Spontaneous abortions are serious life events. The frequency of early spontaneous abortions is estimated to be 10–15% of clinically recognized pregnancies and as many as 30% of clinically unrecognized pregnancies (Wilcox *et al.*, 1988).

Chromosomal abnormalities of the fetus and increasing maternal age are the major risk factors of early spontaneous abortions (Fretts *et al.*, 1995; Cunningham *et al.*, 2010). Ogasawara *et al.* reported that 50% of recurrent spontaneous abortions and 70% of sporadic spontaneous abortions among Japanese women were attributable to chromosomal abnormalities of the fetus. However, that study focused only on the relationship between embryonic karyotype and the number of previous spontaneous abortions without any adjustment for maternal age (Ogasawara *et al.*, 2000). Some other potential risk factors that have been considered for early spontaneous abortions include a previous history of early spontaneous abortions (Regan *et al.*, 1989), underweight (Helgstrand and Andersen, 2005; Maconochie *et al.*, 2007), obesity (Lashen *et al.*, 2004), uterine defects (Cunningham *et al.*, 2010) and lifestyle factors such as maternal smoking, alcohol consumption (Armstrong *et al.*, 1992; Larsen *et al.*, 2008) and employment status (Hemminki *et al.*, 1980). Yuan *et al.* reported that the frequency of spontaneous abortion among Japanese women tended to be higher among mothers who reported and smoking during pregnancy (spontaneous abortions for non-smokers constituted 17.9% for all pregnancies and 30.8% for smokers who smoke 1–10 cigarettes per day),

but they did not adjust for maternal age (Yuan *et al.*, 1994, in Japanese). Furthermore, Sado (1995, in Japanese) indicated that women in employment had higher risk of early spontaneous abortions, but did not adjust for maternal age, either. Few epidemiological investigations have been conducted concerning risk factors for early spontaneous abortions in Japan or other Asian countries.

Therefore, we conducted a case–control study matched for maternal age to evaluate the risk factors of early spontaneous abortions among Japanese women.

Methods

Study subjects

A hospital-based matched case–control study was conducted at Osaka Medical Center and Research Institute for Maternal and Child Health in Izumi city, Osaka, Japan which provides high-quality medical care using advanced technology, but also functions as the core hospital of the local area. The cases were selected consecutively. The case patients were all women who had identified early spontaneous abortions and had been hospitalized for a medical procedure with <12 weeks of gestation from January 2001 to December 2005 at the referral hospital. A total of 430 women were enrolled in this study. For the women who experienced two or more early spontaneous abortions in this period, the information for the last pregnancy was registered. Pregnancies were confirmed by positive human chorionic gonadotropin tests.

Of the 6169 women who underwent term deliveries from January 2001 to December 2005 in the referral hospital, 860 controls (2 controls per case) were randomly selected by matching the age of women (±3 years) and calendar year of events (either early spontaneous abortion or delivery).

The study was approved by the Ethics Committee of Osaka Medical Center and Research Institute for Maternal and Child Health and it was agreed that data collected through medical records would be used for this retrospective study, based on guidelines by the Council for International Organizations of Medical Science (1991).

Measurement of risk factors

The data were collected through medical records. We collected data on maternal age, height, pre-pregnancy weight, reproductive history, lifestyles and husbands' characteristics. Reproductive history included the number of past pregnancies, deliveries, induced abortions and early spontaneous abortions, as well as age at menarche, treatment for infertility (e.g. induction of ovulation, artificial insemination by husband, *in vitro* fertilization, other and unknown). Lifestyle habits included maternal smoking and amount of daily smoking, and drinking status (current drinker or not).

Maternal and husbands' smoking status was divided into non-smokers (never and ex-smokers) and current smokers of 1–19 cigarettes per day and ≥20 cigarettes per day. We categorized women who 'quit after learning of pregnancy' as 'current smoker' in maternal smoking status, because smoking before learning of a pregnancy could already have had an effect on early spontaneous abortions. We categorized homemaker as not employed, and the others as women employed because of the small numbers of women in each employment category (clerk, sales clerk, hairdresser, medical staff, teacher, children's nurse, service industry worker, student and others).

Statistical analysis

Data were analyzed using conditional logistic-regression model for a matched case–control study. Conditional odds ratios (ORs) with 95% confidence intervals (CIs) of early spontaneous abortions were calculated to examine the contribution of potential risk factors. Missing values were included as dummy variables in the analyses. To examine the effects a past history of spontaneous abortion could have all on potential risk factors, we also calculated the risk of early spontaneous abortions in terms of a combination of variables comprising past history of spontaneous abortion and other potential risk factors. We then checked for statistically significant interactions by using cross-product terms of past history of spontaneous abortion and other potential risk factors.

All analyses were two-tailed, and $P < 0.05$ was regarded as statistically significant. SAS, version 9.13 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses.

Results

Ages of cases were normally distributed from 17 to 45 years old. The mean (standard deviation: SD) age was 32.5 (5.20). There was no difference in the distribution of age by year (Table I).

Table II presents crude and multivariable conditional ORs and 95% CIs of early spontaneous abortions associated with reproductive, physical, lifestyle and husband characteristics. These results did not alter materially when a past history of early spontaneous abortion was excluded as an adjustment variable. There was a strong trend toward increased odds of an early spontaneous abortion among women with an increasing number of past early spontaneous abortions. The multivariable ORs (95% CI) of early spontaneous abortions were 1.98 (1.35, 2.89) for one, 2.36 (1.47, 3.79) for two and 8.73 (5.22, 14.62) for three or more previous spontaneous abortions.

Of the lifestyle characteristics, smoking and being employed were associated with the risk of early spontaneous abortions. Current maternal smoking of ≥20 cigarettes per day, i.e. heavy smoking for women, was associated with a two times higher risk of early spontaneous abortions; multivariable OR was 2.39 (1.26, 4.25). Women who were employed had an ~65% higher risk of early spontaneous abortions. Husband's age ≥40 years was associated with an ~60% higher risk of early spontaneous abortions in crude analysis, but in multivariable analysis, the association was no longer statistically significant. Past history of induced abortions, age at menarche, treatment for infertility, body mass index, alcohol consumption and husband's smoking were not associated with the risk of early spontaneous abortions.

We had a large number of missing data on husband's smoking and employment. When we restricted the analysis to women without missing data, the results did not alter substantially. For example, when the subjects without husband's smoking information were excluded from the analysis, the association between risk of early spontaneous abortion and employment remained statistically significant. The multivariable OR for being employed was 2.81 (95% CI: 1.40–5.65).

To examine the effect past history of early spontaneous abortions could have on the association between potential risk factors and risk of early spontaneous abortions, we further calculated the risks by combining the variable of past history with that of employment and with that of maternal smoking (Tables III and IV). The ORs of the combined variables were additive, and no interaction was observed between history of early spontaneous abortions and these associations (P for interaction was >0.20).

Table I Distributions of women with early spontaneous abortions (cases) and controls (term deliveries) by maternal age and calendar year.

	Maternal age	Calendar year					
		2001	2002	2003	2004	2005	Total (% of total)
Case ($n = 430$)	<25	6	7	2	8	2	25 (5.8)
	25–29	26	15	19	16	17	93 (21.6)
	30–34	27	37	24	36	28	152 (35.3)
	35–39	19	19	27	22	34	121 (28.1)
	≥40	4	9	5	12	9	39 (9.1)
	Total	82	87	77	94	90	430 (100.0)
Control ($n = 860$)	<25	12	14	8	17	5	56 (6.0)
	25–29	56	44	44	39	39	222 (25.8)
	30–34	58	69	46	72	67	312 (36.3)
	35–39	30	38	51	42	59	220 (25.6)
	≥40	8	9	5	18	10	50 (5.8)
	Total	164	174	154	188	180	860 (100.0)

Discussion

In this matched case–control study of Japanese women, we show that a past history of early spontaneous abortions, smoking and being employed are associated with increased risk of early spontaneous abortions. These relationships were not substantially altered in the multivariable adjustment models.

In this study, smoking ≥20 cigarettes per day was associated with a 2-fold or higher risk of early spontaneous abortions, which is in concordant with the finding of some previous studies (Armstrong *et al.*, 1992; Risch *et al.*, 1998; Maconochie *et al.*, 2007), but not all (Windham *et al.*, 1992; Rasch, 2003; Wisborg *et al.*, 2003). Smoking and a history of early spontaneous abortions had an additive effect on the risk of early spontaneous abortions. Maternal smoking has been found to lead to smoking-associated placental insufficiency and fetal hypoxia (Cnattingius and Nordström, 1996; Metwally *et al.*, 2008a,b). In Japan, the prevalence of smoking by young women has increased during the last half century. The prevalence of smoking by women aged 20–29 increased, from 6.6% in 1965 to 18.1% in 2008, and the corresponding findings for women aged 30–39 were 13.5 and 19.3% (Ministry of Health, 2006, in Japanese). Therefore, refraining from smoking by women of reproductive age may be an emerging public health issue in Japan.

In this study, we found that women in any type of employment had a 60% higher risk of early spontaneous abortions. We also found that being employed and history of early spontaneous abortions had an additive effect on the risk of early spontaneous abortions. Maternal employment, when it involves long hours or is physically demanding, may also affect other pregnancy outcomes such as preterm birth, small for gestational age, and maternal hypertension, but, in recent investigations, this has not been demonstrated for early spontaneous abortions (Mozurkewich *et al.*, 2000; Saurel-Cubizolles *et al.*, 2004). Several studies showed that women in specific occupations had higher risk for early spontaneous abortions. For example, one investigation of Finnish women in 1980 indicated that women in several occupational groups such as construction work and agriculture were at a higher risk than women without paid occupation, maybe from the physically demanding task (Hemminki *et al.*, 1980). Another investigation of Japanese pregnant women found that the proportion of early spontaneous abortions was significantly higher for women with any type of employment compared with women who were not employed: 13.1 versus 8.9% although they did not adjust for the age of women (Sado, 1995, in Japanese). However, to our knowledge, no studies from the other countries have shown any association between employment status and early spontaneous abortions (Savitz *et al.*, 1997; Maconochie *et al.*, 2007). A possible explanation for the magnitude of the effect that being employed has on the risk of spontaneous abortion for Japanese women may be the unequal division of house work between men and women. Time devoted to childcare by Japanese women is similar to that by European women (112 min in Japan versus 193 min in the UK and 118 min in Sweden), but that by Japanese men is much shorter than that by European men (corresponding figures are 25, 90 and 70 min) (OECD, 2001; Cabinet office, 2006). This suggests that Japanese women spend much more of their time and energy on a combination of housework and child-care than women in other regions. Further, menstrual disorders, an indicator of reproductive health, have been associated with work stress among full-time working women in China. Chinese women are reported to take primary responsibilities for their families even if they are in full-time employment, so that they too may have the double burden (Zhou *et al.*, 2010).

Neither being underweight nor overweight was strongly associated with the risk of early spontaneous abortions in our study. The proportions of lean and overweight women were similar to those reported by a national study (Ministry of Health, 2006, in Japanese), but the proportion of overweight women were far lower, and that of lean women were higher than those in other developed countries. The proportions of overweight women (BMI ≥ 25.0) were 19.9% in Japan, 56.6% in UK and 61.3% in the USA in most recent years, and those of underweight women (BMI < 18.5) were 10.4, 5.9 and 3.3% in these countries (World Health Organization). A Japanese population, as in our study, may thus be suitable for examining an effect of being underweight and the risk of spontaneous abortions observed in our study, but not suitable for examining an effect of being overweight.

Table II Crude and multivariable conditional ORs of spontaneous abortions associated with reproductive, physical, lifestyle and husband's characteristics.

Characteristics	Number		Crude OR (95% CI)	Multivariable OR (95% CI)
	Cases	Controls		
Reproductive variables				
Past history of spontaneous abortion				
None	232	632	1.0	1.0
1	77	124	1.75 (1.26–2.44)‡	1.98 (1.35–2.89)†
2	45	61	2.00 (1.32–3.03)‡	2.36 (1.47–3.79)‡
3 or more	76	42	5.36 (3.46–8.29)‡	8.73 (5.22–14.6)‡
Data missing	0	1	–	–
P for trend			<0.0001	
Past history of induced abortion				
None	360	748	1.0	1.0
1 or more	68	111	1.27 (0.91–1.76)	1.26 (0.84–1.89)
Data missing	0	1	–	–
Age at menarche (years)				
≤11	139	307	1.0	1.0
>12	149	272	1.26 (0.95–1.66)	1.23 (0.89–1.72)
Data missing	142	281	–	–
Treatment for Infertility (Assisted Reproductive Technology)				
No	372	748	1.0	1.0
Yes	55	103	1.08 (0.75–1.54)	1.00 (0.67–1.49)
Data missing	3	9	–	–
Physical variables				
Body mass index (kg/m^2)				
<18.5	73	152	0.99 (0.72–1.36)	0.86 (0.59–1.25)
18.5–24.9	310	637	1.0	1.0
≥25.0	47	71	1.34 (0.91–1.96)	1.40 (0.87–2.25)
P for trend			0.31	0.23
Lifestyle variables				
Maternal smoking status				
Non-smoker	339	722	1.0	1.0
Current smoker (1–19/day)	54	101	1.24 (0.83–1.85)	1.30 (0.84–2.02)
Current smoker (≥20/day)	32	36	1.99 (1.18–3.35)†	2.39 (1.26–4.53)*
Data missing	5	1	–	–
P for trend			0.03	0.02
Current drinker				
No	346	759	1.0	1.0
Yes	75	120	1.32 (0.97–1.82)	1.04 (0.71–1.53)
Data missing	9	1	–	–
Employment				
No	174	357	1.0	1.0
Yes	146	165	1.64 (1.21–2.23)†	1.65 (1.17–2.35)†
Data missing	111	338	–	–
Husband's variables				
Husband age (year)				
<29	98	225	1.0	1.0
30–39	246	505	1.29 (0.91–1.87)	1.14 (0.75–1.74)
≥40	86	130	1.98 (1.24–3.23)†	1.65 (0.94–2.88)
P for trend			0.015	0.017

Continued

Table II *Continued*

Characteristics	Number		Crude OR (95% CI)	Multivariable OR (95% CI)
	Cases	Controls		
Husband's smoking status				
Non-smoker	114	205	1.0	1.0
Current smoker (1–19/day)	65	97	1.24 (0.83–1.85)	1.23 (0.78–1.96)
Current smoker (≥20/day)	72	103	1.30 (0.88–1.92)	1.30 (0.82–2.06)
Data missing	179	455	–	–
P for trend			0.35	0.47

95% CI, 95% confidence interval; OR, conditional odds ratio; –, valid OR was not calculated. Matched for calendar year of the event and mother age (±3 years). Multivariable conditional OR was adjusted for past spontaneous abortion history, past induced abortion history, treatment for infertility, body mass index, smoking status, drinking status, employment, husband's age and husband's smoking.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

The lack of association between being underweight and the risk of early spontaneous abortions observed in our study was consistent with the findings from two previous studies (Stein and Kline, 1991; Helgstrand and Andersen, 2005), but one case–control study reported a significant association between underweight and spontaneous abortions; the multivariable OR was 1.72 and 95% CI was 1.17, 2.53; Maconochie *et al.*, 2007).

The influence of obesity on early spontaneous abortions has been widely investigated. Obesity may increase the risk of spontaneous abortions by an adverse influence on the embryo, the endometrium or both via leptin (Metwally *et al.*, 2007, 2008a,b), but there are few studies on conception without any assisted reproductive technology. Metwally *et al.* (2008a,b) show in their meta-analysis that obesity may increase the risk of spontaneous abortions, but they included middle-term abortions until 19 weeks of gestation. On the other hand, Maconochie *et al.* (2007) showed that obesity did not increase the risk of spontaneous abortions <13 weeks in a large population-based case–control study.

This study has a number of potential limitations. First, the study subjects were sampled in a single hospital. In Japan, patients can choose the hospital in which they are treated according to their own preferences, while medical costs are uniform under the national medical insurance system. Women seeking advanced medical treatment, as well as pregnant women at normal risk or without complications, are more likely to visit our hospital. Therefore, the subjects in our study may have been biased toward high-risk pregnant women. Secondly, the case patients were defined as the women who were hospitalized for a medical procedure. A woman who had a complete spontaneous abortion without any additional treatment would not be included. We may evaluate the risk of missed abortions, but not all types of early spontaneous abortions. Thirdly, the data were collected retrospectively through medical records. Therefore, we did not measure caffeine, socio-economic status or other potential confounding variables (Cnattingius *et al.*, 2000; Luo *et al.*, 2006). However, none of these variables are established risk factors for early spontaneous abortions, so that the

Table III Crude and multivariable conditional ORs of early spontaneous abortions associated with combination of previous early spontaneous abortion and employment.

	Number		Crude OR (95% CI)	Multivariable OR (95% CI)
	Cases	Controls		
Previous abortions (−), Employment (−)	97	360	1.0	1.0
Previous abortions (−), Employment (+)	88	137	1.56 (1.08–2.26)*	1.50 (1.01 –2.24)*
Previous abortions (+), Employment (−)	77	97	2.28 (1.54 –3.39)‡	2.40 (1.56 –3.7)‡
Previous abortions (+), Employment (+)	58	29	5.16 (2.98 –8.94)‡	5.56 (3.04 –10.1)‡
Data missing	110	337	–	–

95% CI, 95% confidence interval; OR, conditional odds ratio; –, valid conditional OR was not calculated. Previous abortion (+) includes 1, 2 and 3 or more previous abortions. Matched for calendar year of the event and mother age (±3 years). Multivariable conditional OR was adjusted for past induced abortion history, treatment for infertility, body mass index, smoking status, drinking status, husband's age and husband's smoking.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

Table IV Crude and multivariable conditional ORs of early spontaneous abortions associated with combination of previous early spontaneous abortion and maternal smoking.

	Number		Crude OR (95% CI)	Multivariable OR (95% CI)
	Cases	Controls		
Previous abortion (−), Maternal smoking (−)	181	525	1.0	1.0
Previous abortion (−), Maternal smoking (+)	50	107	1.42 (0.97 −2.09)	1.39 (0.89 −2.17)
Previous abortion (+), Maternal smoking (−)	158	197	2.43 (1.83 −3.22)‡	2.95 (2.14−4.05)‡
Previous abortion (+), Maternal smoking (+)	36	30	3.53 (2.10 −5.95)‡	4.31 (2.37 −7.86)‡
Data missing	5	1	–	–

95% CI, 95% confidence interval; OR, conditional odds ratio; –, valid conditional OR was not calculated. Previous abortion (+) includes 1, 2, and 3 or more previous abortions. Maternal smoking (+) includes smoking (1−19/day) and (≥20 /day). Matched for calendar year of the event and mother age (±3 years). Multivariable conditional OR was adjusted for past induced abortion history, treatment for infertility, body mass index, smoking status, drinking status, husband's age, and husband's smoking.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

residual confounding may not be large. Fourthly, there were a large number of missing data for employment and husband's smoking, but the results did not change substantially among the subjects without these missing data.

In conclusion, our study suggested that smoking and being employed, as well as a past history of early spontaneous abortions, were associated with early spontaneous abortions among Japanese women. Our findings suggest that smoking and/or working women may be important public health targets for the prevention of early spontaneous abortions in Japan.

Authors' roles

S.B. analyzed and interpreted the data, drafted the manuscript and provided statistical expertise. H.N. and M.N. designed the study's analytic strategy. M.W. and N.M. critically revised the manuscript. H.I. conceived and designed the study, acquired and interpreted the data and critically revised the manuscript.

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Maternal death analysis from the Japanese autopsy registry for recent 16 years: significance of amniotic fluid embolism

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Abstract

Aim: To clarify the cause of maternal deaths, an autopsy is essential. However, there has been no systemic analysis of maternal death in Japan based on autopsy cases.

Material & Methods: Maternal death reports were retrieved from a large amount of registered autopsy data on maternal death in the series of 'Annual of pathological autopsy cases in Japan'. These files contain 468 015 autopsy records from 1989 to 2004. We collected 193 cases of maternal death due to direct obstetric causes. We recorded all the data into Excel files. Then we analyzed the causes of death and classified them into 11 categories.

Results: The causes of maternal death were as follows: amniotic fluid embolism (AFE), 24.3%; disseminated intravascular coagulation (DIC) related to pregnancy-induced hypertension, 21.2%; pulmonary thromboembolism, 13.0%; injury to the birth canal, 11.4%; medical and surgical complications, 9.8%; and atonic bleeding or DIC of unknown cause, 8.3%. A discrepancy between the clinical diagnosis and pathological diagnosis was frequently observed in cases of AFE, septic DIC and injury to the birth canal. AFE diagnosed by autopsy was often clinically diagnosed as atonic bleeding or DIC of unknown cause before death. Half of the cases of AFE diagnosed by autopsy were associated with DIC.

Conclusion: We found that AFE, DIC related to pregnancy-induced hypertension, pulmonary thromboembolism and injury to the birth canal were the major causes of maternal death in Japan. AFE had various clinical features such as uterine atony and DIC in addition to pulmonary cardiac collapse.

Key words: amniotic fluid embolism, DIC, Japanese autopsy registry, maternal death, pulmonary thromboembolism, uterine atony.

Introduction

Maternal death is a shocking event in obstetrical practice. In Japan, the maternal mortality rate (number of maternal deaths per 100 000 live births) was 5.6 in 2002–2006.[1] The rate has been decreasing since the 1970s and is now stable at around 5. The statistics for maternal mortality in Japan have been mainly

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derived from clinical diagnoses of death. Information provided by medical autopsies has played an important role in increasing the accuracy of cause-of-death reports and improving clinical practice. Autopsies may also provide important data on the causes of maternal death, which is essential for reducing maternal mortality and directing public health efforts. Therefore an autopsy is performed in nearly every case of maternal death.

The problem with examining maternal deaths in Japan is that an autopsy is not performed for these cases. Also, there is no information on how many cases of maternal deaths involve autopsy. To clarify the real cause of maternal deaths in Japan, an autopsy is essential. However, there has been no systemic analysis of maternal death in Japan based on autopsy cases. The aim of this study is to elucidate the cause of maternal deaths in Japan based on anatomical analysis. We found a large amount of pathological data on maternal death in the 'Annual of the pathological autopsy cases in Japan'edited by the Japanese Society of Pathology. The aim of this study was to analyze the pathological data, and to know the real cause of maternal deaths based on autopsy. Moreover, the rate of home delivery in 1990 was 0.1%, and in 2000 was 0.2%. From this point of view this analysis could reflect the real cause of maternal death in Japan.

Methods

To collect autopsy cases of maternal death, we used files from the 'Annual of pathological autopsy cases of Japan'. Data are recorded for all cases of autopsy of hospital deaths in Japan. Also, this report was based on complete autopsies with histologic examinations by authorized pathologists by the Japanese Society of Pathology. We selected cases where the women died during pregnancy or within 42 days of the completion of a pregnancy. We excluded deaths from traffic accidents and suicide. We recorded the clinical diagnosis, anatomic diagnosis and findings into Excel files (Microsoft Corporation, Redmond, WA, USA). Then we investigated the data and classified the cases into 11 categories. Three specialists in obstetrics checked the contents and confirmed the diagnosis. Some records of anatomical findings and diagnoses were not completely adjusted to current international classification of diseases (ICD) cords. We excluded DIC secondary to consumptive coagulopathy such as a massive bleeding of laceration from unknown cause of DIC category. Also, we did not include the cases of uterine atony derived from latent uterine rupture.

Results

Among the 468 015 recorded cases of autopsy from 1989 to 2004, we found 193 maternal deaths due to direct obstetric causes. In this period, the range of the Japanese population was 122 460 000 (1989) to 12 617 600 (2004). The average of the population per year during the period was 124 632 186. Live birth was 1 246 802 (1989) to 1 110 721 (2004). The average of live birth per year was 1 190 170. Total fertility rate in this period was from 1.57 (1989) to 1.29 (2004). The average of fertility rate per year was 1.42. Figure 1 shows the causes of maternal deaths detected in the autopsy records. Amniotic fluid embolism (AFE) was the most common cause with 47 cases (24.3%). DIC related to pregnancy induced hypertension (PIH) including eclampsia, HELLP syndrome, and the abruption of the placenta ranked second with 41 cases (21.2%). Third was pulmonary thromboembolism with 25 cases (13.0%) and fourth was injury to the birth canal with 22 cases (11.4%). Among these 22 cases, there were 7 cases of uterine rupture, 5 cases of cervical laceration, 3 cases of vaginal laceration, 3 cases of retroperitoneal hemorrhage of unknown cause, 3 cases of post-hemorrhage of

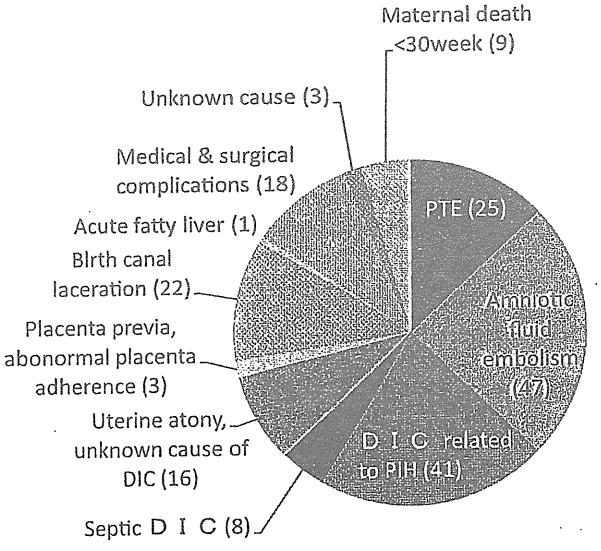

Figure 1 The cause of maternal death by autopsy in Japan from 1989 to2004. DIC, disseminated intravascular coagulation; PIH, pregnancy induced hypertension; PTE, pulmonary thromboembolism.

cesarean section, and 1 case of inversion of the uterus. The fifth leading cause of maternal death was medical and surgical complications with 18 cases (9.8%): 5 cases of rupture of an artery such as an aortic aneurysm, 4 cases of heart diseases (myopathy 3 cases, unknown cause of heat failure 1 case), 3 cases of hyperthyroidism, 2 cases of hepatitis, and 4 other cases. The sixth leading cause was uterine atony and uterine atony associated with DIC of unknown cause with 16 cases (8.3%), and the seventh cause was maternal death at less than 30 weeks of gestation with 9 cases (4.7%). Out of 9 cases of maternal death before 30 weeks of gestation, there were 4 cases of ectopic pregnancy, 3 cases of pulmonary thromboembolism, 1 case of abortion associated with DIC, and 1 case of invasive mole. The eight leading cause of maternal death was septic DIC with 8 cases (4.1%), ninth was placenta previa and placental abnormalities with 3 cases (1.6%), tenth was acute fatty liver with 1 case (0.5%), and the eleventh was unknown cause with 3 cases (1.1%).

The discrepancy between clinical and pathological diagnoses was analyzed. The rate of discrepancy was 49% for AFE, 5% for DIC related to PIH, 36% for pulmonary thromboembolism, 50% for birth canal laceration, 50% for septic DIC, 61% for medical and surgical complications and 17% for uterine atony and DIC of unknown cause. AFE, birth canal laceration, septic DIC and medical and surgical complications were frequently misdiagnosed (Fig. 2). AFE diagnosed by autopsy was often diagnosed clinically as atonic bleeding or DIC of unknown cause prior to death (Fig. 3).

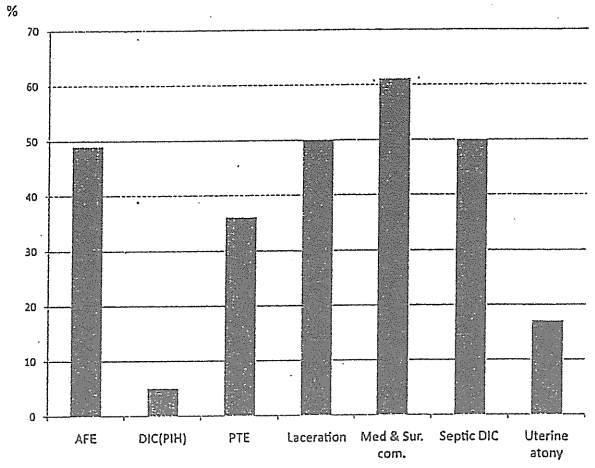

Figure 2 The discrepancy between clinical diagnosis and anatomical diagnosis. AFE, amniotic fluid embolism; DIC, disseminated intravascular coagulation; PIH, pregnancy induced hypertension; PTE, pulmonary thromboembolism; Med & Sur com, medical and surgical complication. Y-axis indicates coincidence percentage of clinical and anatomical diagnosis.

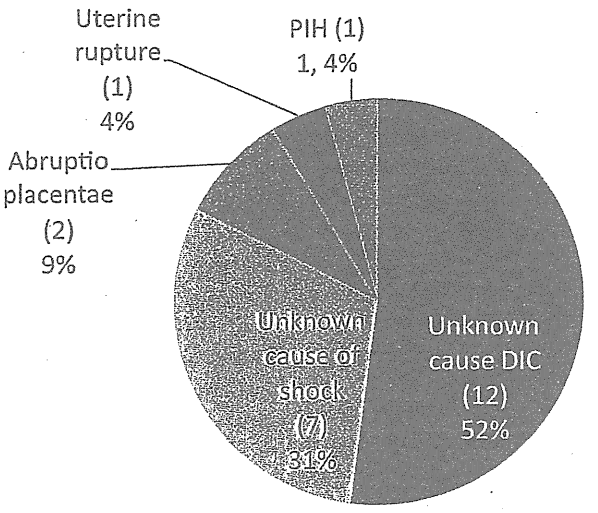

Figure 3 Distribution of clinical diagnosis of which the cases were anatomically diagnosed amniotic fluid embolism (AFE) and their clinical diagnosis were different from AFE. DIC, disseminated intravascular coagulation; PIH, pregnancy induced hypertension.

Discussion

We found that AFE, DIC related to PIH, pulmonary thromboembolism and injury to the birth canal were the major causes of maternal death in Japan. The results clearly showed that the critical diseases linked to maternal death are almost the same as in other developed nations.[2,3] To our knowledge, this is the first description of the causes of maternal death in Japan based on autopsy studies. In our study, anatomical diagnosis and clinical diagnosis were done by each hospital, and then their data was directly registered into the 'Annual of the pathological autopsy cases in Japan' report. For this reason the confidence level of diagnosis might differ in each hospital. However, specialists allowed by the Pathologic Association of Japan can perform and report the autopsy diagnosis into the report. Thus, the accuracy level of autopsy diagnosis is high.

We found that AFE was the most important cause of maternal deaths in Japan. Maternal death rate and its

causes are different from developing nations. Maternal death rate in developing nations is more than 10 times higher than that of developed nations.[4] Maternal hemorrhage, infection and abortion are the main causes of maternal death in developing nations.[5]

On the other hand, similar to our results, the leading causes of maternal death in developed nations were embolism and pregnancy induced hypertension.[6] Currently Steven L *et al.* reported that amniotic fluid embolism occupied 14% of 95 maternal deaths per 1 461 270 births in the US from 2001 to 2006.[7] This demonstrates that AFE is an important cause of maternal death in developed nations, which is also similar to our results. AFE in our results showed 24.8% of total maternal deaths, and is high compared to other reports. The definition of anatomical diagnosis of AFE is not established at present. In our study, the cases that amniotic fluid debris or fetal cells were present in several pulmonary arteries defined as anatomical AFE. From this definition we found that approximately 50% of anatomical diagnosis of AFE diagnosed clinically unknown cause of DIC or uterine atony (Figs 2,3). For this reason the incidence of AFE in our study may not compare to other reports.

Interestingly, the ranking of causes of maternal deaths based on official death certificates is different from our results. We compared our results with age matched maternal mortality data from the Japanese Mothers' and Childrens' Health & Welfare Association. The latter statistics were derived from death certificates, not from autopsies. According to the Annual report of Maternal and Child Statistics of Japan, in 1995, obstetric embolism including AFE and pulmonary thromboembolism ranked first (29.9%) among direct obstetrical causes, followed by PIH (28.4%), other direct obstetrical causes including medical and surgical complications (28.4%), post-partum hemorrhage, and placenta previa and abruptio placentae (4.5%).[1] In the year 2000, among direct obstetrical causes, obstetric embolism including amniotic embolism and pulmonary thromboembolism ranked first (22.6%), followed by placenta previa and abruption placentae (19.4%), other direct obstetrical causes including medical and surgical complications (19.4%), post-partum hemorrhage (16.4%), and PIH (12.9%). Obstetric embolism was the most frequent cause of death in both our study and the Mothers' and Childrens' Health & Welfare Association data. However, the rate of obstetric embolism including AFE and pulmonary thromboembolism from our data was 37.3%. The percentage of obstetric embolism among our autopsy data of maternal deaths was extremely high. The reason could be that half of AFE patients (23 cases) were not correctly diagnosed before death, because antemortem diagnosis of AFE is very difficult in the clinical environment. These results suggest that obstetricians should recognize that AFE has various signs and symptoms besides cardiopulmonary collapse. We would like to emphasize that DIC and uterine atony are also major symptoms of AFE. Recently, Gilbert described the complications of AFE, and reported that DIC and uterine atony are frequently associated with AFE.[8]

Our results and current findings suggest that some cases of AFE involved DIC or uterine atony rather than cardiopulmonary collapse. In fact, in spite of the presence of amniotic fluid debris in pulmonary arteries, some patients showed DIC or uterine atony mainly without cardiorespiratory symptoms. Awad *et al.* reported an AFE case in which the patient showed DIC of unknown cause and massive bleeding.[9] They found the cases of AFE presented with symptoms and signs other than the classical pattern of dyspnea, cyanosis and hypotension. They proposed that consumptive coagulopathy appears to be the 'forme frusta' of amniotic fluid embolism. Our findings support their hypothesis. We speculate that the unknown cause of uterine atony or unknown cause of post-partum DIC is attributed to the uterine type of AFE. Our hypothesis is shown in Figure 4.

Furthermore, several investigators also reported atypical AFE cases in which DIC appeared predominant.[10] Jang reported that amniotic debris in the vasculature of endocervix was found in some cases of AFE

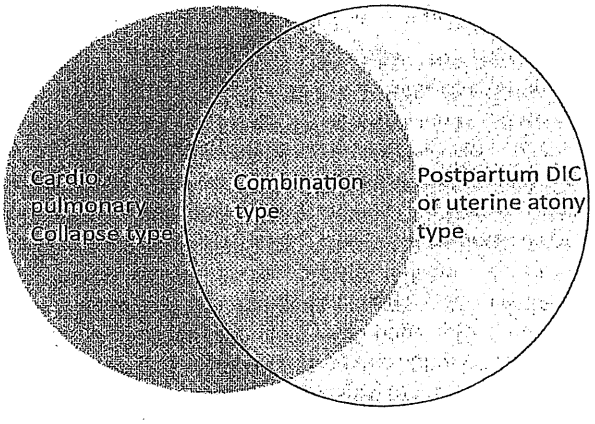

Figure 4 Hypothesis of three types of amniotic fluid embolism (AFE) based on autopsy. DIC, disseminated intravascular coagulation.

with DIC.[11] These findings may be compatible with our hypothesis. Such investigators suggest that AFE was similar to anaphylactoid shock. Serum complement levels are low in AFE.[12] Also, several reports founded that the severe vasoconstriction caused by anaphylactoid reaction was the main pathophysiology of AFE.[13,14] The aspect of anaphyractic reaction not only pulmonary arteries but also uterine vessels could be important to future investigations.

DIC related to PIH was a major cause according to our results, which was compatible with the statistics from death certificates. Improvements of management for PIH are still important in decreasing maternal mortality in Japan. Pulmonary thromboembolism is the third leading cause of maternal death. Currently, deep vein thrombosis is increasing in Japan as in other developed nations. Prophylactic guidelines were proposed by several medical societies in 2004. We hope that the incidence of deep vein thrombosis and pulmonary thromboembolism will be decreased by these guidelines.

Birth canal laceration was the fourth leading cause of maternal death. Uterine rupture, cervical laceration and vaginal laceration, in this order, were the major causes of laceration of the reproductive tract. Obstetricians must keep this in mind when they face postpartum massive bleeding.

We found that the rupture of vessels such as an aortic aneurysm, heart diseases (myopathy and heat failure), and hyperthyroidism are the major causes of medical and surgical complications. Arterial rupture induces the sudden onset of massive bleeding. It is well known to cause maternal deaths. Although it is difficult to detect such diseases during pregnancy in some cases, obstetricians should be careful regarding those diseases in antepartum care. We found four cases of heart diseases with cardiomyopathy the main cause among them. The cause of peripartum cardiomyopathy is still unknown and further investigations are needed. Hyperthyroidism is also an important disease for maternal deaths. We should recognize that hyperthyroidism is a disease linked to maternal death. The sixth cause of maternal deaths was uterine atony and uterine atony associated with DIC of unknown cause. This category of disease has not been well understood. A common pathological finding is that fibrin thrombosis is widely observed in the myometrium. We should continue to clarify the mechanism of this disease.

Maternal death at less at than 30 weeks of gestation ranked seventh. It seems to be ranked rather low; however, it might be due to a well-developed maternal check-up system especially from early stage of pregnancy in Japan. Ectopic pregnancy and thromboembolism are major causes among maternal death in early stage of pregnancy. As for the management early stage of pregnancy, obstetrician should pay attention not to misdiagnose these diseases.

In conclusion, we would like to propose several strategies to decrease the rate of maternal death in Japan. AFE has various clinical features such as uterine atony and DIC in addition to pulmonary cardiac collapse. Therefore, we should clarify the mechanism of AFE and improve the management of AFE. Notably, AFE is frequently associated with DIC. The early diagnosis and treatment of DIC will be important. Secondly, we should be aware of DIC related to PIH as a second cause of maternal death in Japan. PIH is the major cause of maternal death in all countries, but improvements in the management of DIC have been pointed for some time, and the incidence of PIH is decreasing year by year. However, our studies showed that PIH is still important in decreasing the rate of maternal deaths in Japan. When facing PIH cases, obstetricians should keep DIC in mind. Currently, prophylactic methods for pulmonary thromboembolism such as intermittent pressure pump and heparin administration are widely used. We should continue to try to reduce the incidence of thromboembolism. Birth canal laceration occurs at a constant rate in hospitals. Uterine rupture and cervical laceration should be recognized as critical diseases in obstetrical practice. Taken together, except amniotic fluid embolism, major causes of maternal deaths have been pointed out for a long time. Our results will contribute the diagnosis of obstetrical practice especially in severe maternal diseases. If we improve the management of such diseases, we believe that the maternal mortality rate in Japan will decrease in the near future.

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Circulating Levels of Soluble α-Klotho Are Markedly Elevated in Human Umbilical Cord Blood

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Context: Fetal serum levels of calcium and phosphate are higher than those in the maternal levels. Although α-Klotho is known to participate in calcium and phosphate metabolism in adults, its role in the perinatal period remains unknown.

Objective: This study aimed to determine the baseline levels of soluble α-Klotho in fetuses and compare them with those in neonates, mothers, and adults to clarify whether α-Klotho is involved in the fetal-specific regulation of calcium and phosphate metabolism.

Design and Setting: We conducted a cross-sectional evaluation of healthy babies (at birth and/or at 4 d after birth), their mothers, and adult volunteers at one hospital.

Participants: Twenty-one healthy mothers, their babies (23 in total, including two pairs of twins), and 25 adult volunteers participated in the study.

Main Outcome Measures: We measured the serum levels of soluble α-Klotho and fibroblast growth factor 23 (FGF23).

Results: In cord blood, the level of α-Klotho was markedly higher (3243 ± 1899 pg/ml) than levels in neonates at d 4 (582 ± 90 pg/ml), mothers (768 ± 261 pg/ml), and adult volunteers (681 ± 140 pg/ml) ($P < 0.001$), whereas the fetal level of FGF23 was lower than levels in the other subjects. The levels of soluble α-Klotho were negatively correlated with those of FGF23 in cord blood. Immunohistochemistry demonstrated that α-Klotho was predominantly expressed in syncytiotrophoblasts in normal term placenta.

Conclusion: Levels of soluble α-Klotho are markedly elevated in cord blood and might be useful as a biomarker for mineral metabolism in the fetus. (*J Clin Endocrinol Metab* 96: E943–E947, 2011)

Fetal mineral homeostasis is regulated differently from adult homeostasis. The levels of serum calcium and phosphate in the fetus are higher than the maternal levels during late gestation. PTH and PTHrP are known to be involved in calcium homeostasis (1, 2). On the other hand, the regulatory mechanism of the fetal phosphate level is poorly understood (3).

The *α-Klotho* gene encodes a single-pass transmembrane protein, which was originally identified as an aging-related gene (4). In adults, α-Klotho contributes to the

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Abbreviations: FGF23, Fibroblast growth factor 23; 25-OHD, 25-hydroxyvitamin D; TRPV, transient receptor potential vanilloid.

regulation of calcium and phosphate homeostasis. In the parathyroid, α-Klotho binds to Na^+/K^+-ATPase to regulate PTH secretion and is involved in transepithelial calcium transport (5). α-Klotho is also involved in the activation of transient receptor potential vanilloid (TRPV) 5 in the kidney (6), indicating its central role in the maintenance of calcium homeostasis. In addition, α-Klotho participates in phosphate homeostasis by cooperating with fibroblast growth factor 23 (FGF23) and the FGF receptor (7). FGF23 reduces the serum phosphate level both by suppressing phosphate reabsorption and activating vitamin D in the proximal tubules (8–10).

Although *α-Klotho* is predominantly expressed in the kidney, parathyroid, and choroid plexus, it is also expressed in other tissues including the placenta (4). Its expression in the placenta has led us to hypothesize that α-Klotho might play a role in fetal mineral homeostasis as well as in postnatal homeostasis.

In addition to the transmembrane form, α-Klotho also exists in a soluble form. The soluble form, which is produced by the shedding of the transmembrane protein, is detectable in serum, cerebrospinal fluid, and urine (6, 11). Although soluble α-Klotho is considered to be a humoral factor (12), its regulatory mechanisms and functions are largely unknown, and it is considered that FGF23 signaling requires the transmembrane form of the protein.

Recently, a sandwich ELISA for soluble α-Klotho has been established (13). In the present study, we used this assay to measure the serum levels of soluble α-Klotho in cord blood at birth and compared them to the levels in neonates, mothers, and adults. We found that high levels of soluble α-Klotho are present in cord blood and analyzed the relationship between those of soluble α-Klotho and FGF23 in cord blood. To the best of our knowledge, this is the first report on the measurement of soluble α-Klotho levels in perinatal blood samples.

Subjects and Methods

Study participants

We recruited healthy pregnant women, their babies, and adult volunteers and obtained informed consent from all participants or their legal guardians. The institutional review board of Osaka University Hospital approved this study. The inclusion criteria were an unremarkable medical history, physical examination, and screening laboratory test results for endocrine and metabolic function. The exclusion criteria were premature or postmature infant delivery (gestational age under 37 wk and over 42 wk, respectively), and the neonate being light or heavy for their delivery date (birth weight under −1.5 SD and over 1.5 SD, respectively).

Twenty-one mothers and their babies (n = 23, including two pairs of twins) were enrolled. For comparison, 25 healthy adult volunteers ranging in age from 27 to 48 yr (11 males and 14 females) were also enrolled.

Blood analyses

After the delivery of the neonate, we immediately obtained cord blood samples from the umbilical vein, before the placenta was delivered. We also collected blood from the neonates at d 4 after birth in the morning and fasting morning blood from their mothers and the adult volunteers. The maternal blood was obtained within 24 h of the delivery. The participants were under no dietary restrictions during the study.

After we had collected the blood samples, we separated the serum instantly and stored it at −80 C until analysis. We measured the levels of serum soluble α-Klotho and intact FGF23 in all samples. The serum soluble α-Klotho levels were measured using an ELISA kit provided from Kyowa Hakko-Kirin (Tokyo, Japan) (13). The intra- and interassay coefficients of variation ranged from 2.7 to 9.8% (13). The serum levels of intact FGF23 were determined using a commercial sandwich ELISA kit (Kainos Laboratories, Inc., Tokyo, Japan) (14). Serum calcium, phosphate, intact PTH, 25-hydroxyvitamin D (25-OHD), albumin, and creatinine levels were also measured in the samples except for those from neonates. We corrected the levels of calcium in the samples displaying hypoalbuminemia (albumin < 4.0 g/dl) as reported previously (15).

Immunohistochemistry

Normal human placenta (gestational age, 38 wk) was obtained from an uncomplicated pregnancy. The specimen was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 4-μm-thick sections. Antigen retrieval was performed using 10 mM citrate buffer (pH 5.9) for 15 min at 98 C. The sections were stained using anti-α-Klotho antibody (sc-22218; Santa Cruz Biotechnology, Santa Cruz, CA) and goat ImmunoCruz Staining System (Santa Cruz Biotechnology). The slides were counterstained with hematoxylin. Normal goat IgG was used as a negative control.

Statistical analyses

The results are expressed as the mean ± SD. We compared biochemical parameters and soluble α-Klotho and FGF23 levels among the groups by ANOVA, followed by the Tukey-Kramer method. The relationship between soluble α-Klotho and FGF23 in cord blood samples was analyzed using Pearson's correlation test.

All statistical analyses were conducted using JMP software version 8.0.1 (SAS Institute Inc., Cary, NC).

Results

Levels of soluble α-Klotho in cord blood are higher than those in neonates, mothers, and adults

The biochemical findings are shown in Table 1. Serum calcium, phosphate, intact PTH, 25-OHD, albumin, and creatinine levels were within the normal range in both the mother and adult groups (16). As previously reported, serum calcium and phosphate levels were significantly higher,

TABLE 1. Biochemical parameters

	Cord blood (n = 23)	Mother (n = 21)	Adult (n = 25)
Calcium (mg/dl)[a]	10.5 ± 0.43	9.44 ± 0.34[b]	9.42 ± 0.29[b]
Phosphate (mg/dl)	4.93 ± 0.65	3.66 ± 0.59[b]	3.62 ± 0.50[b]
Intact PTH (pg/ml)	7.11 ± 5.22	26.5 ± 12.2[b,f]	36.4 ± 9.80[b]
25-OHD (ng/ml)	8.95 ± 3.47	13.3 ± 6.45[d]	15.0 ± 4.43[c]
Albumin (g/dl)	3.53 ± 0.38	3.15 ± 0.42[c,e]	4.60 ± 0.27[b]
Creatinine (mg/dl)	0.49 ± 0.09	0.48 ± 0.08[e]	0.69 ± 0.10[b]

Data are expressed as mean ± SD and were compared among the cord blood group, mother group, and adult volunteer group using the ANOVA test.

[a] The calcium values were corrected using the following formula in cases involving hypoalbuminemia (Alb < 4.0 g/dl): corrected calcium (mg/dl) = measured calcium (mg/dl) + 4 − albumin (g/dl) (15).

[b] $P < 0.0001$ *vs.* cord blood; [c] $P < 0.01$ *vs.* cord blood; [d] $P < 0.05$ *vs.* cord blood; [e] $P < 0.0001$ *vs.* adult; [f] $P < 0.01$ *vs.* adult.

whereas serum intact PTH levels were lower in the cord blood than in the sera of the mother and adult volunteers (3).

The levels of soluble α-Klotho in the cord blood were markedly higher than those in the other groups ($P < 0.0001$) (Fig. 1A). The mean value for soluble α-Klotho in the cord blood was 3243 ± 1899 pg/ml, whereas those in the sera of the neonates at d 4, mothers, and adult volunteers were 582 ± 90, 768 ± 261, and 681 ± 140 pg/ml, respectively. There was no significant difference among the samples from neonates at d 4, mothers, and adults.

On the other hand, the levels of FGF23 in cord blood were significantly lower than those in other groups ($P < 0.0001$, *vs.* neonate and adult; $P < 0.0005$, *vs.* mother) (Fig. 1B). The mean value for FGF23 in cord blood was 8.61 ± 6.48 pg/ml, whereas those in the neonates, mothers, and adult volunteers were 28.4 ± 20.5, 26.7 ± 15.1, and 34.6 ± 7.69 pg/ml, respectively.

Negative correlation between the levels of soluble α-Klotho and FGF23 in cord blood samples

Then, using only the cord blood samples (n = 23), we examined the relationship between the levels of soluble α-Klotho and FGF23. We found that soluble α-Klotho levels were inversely correlated with FGF23 levels ($R^2 = 0.20$; $P < 0.05$) (Fig. 1C).

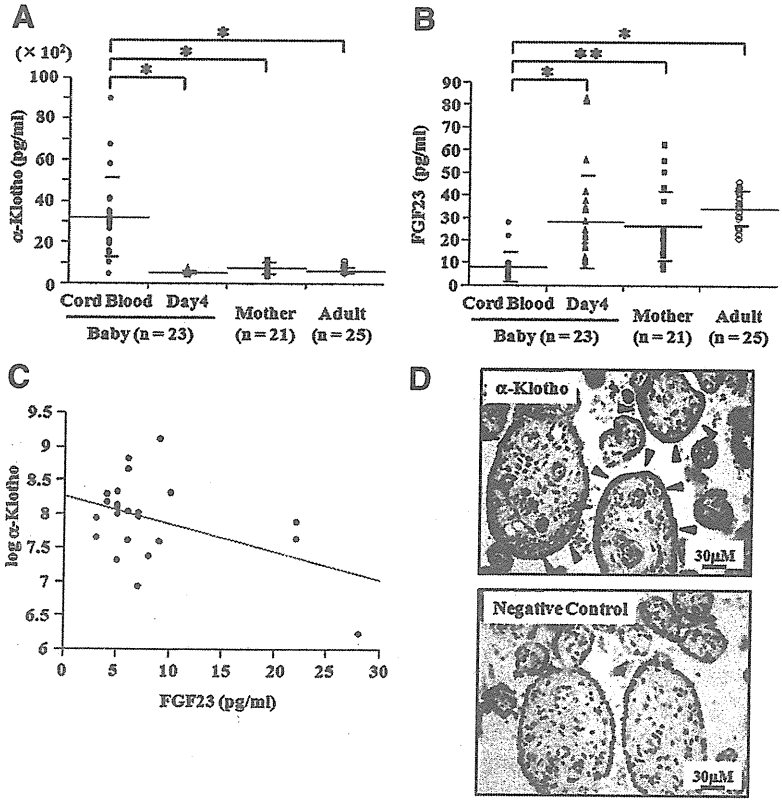

FIG. 1. A and B, Comparison of serum soluble α-Klotho (A) and FGF23 (B) levels among the cord blood, neonate at d 4, mother, and adult volunteer groups by ANOVA. *, $P < 0.0001$; **, $P < 0.0005$. *Closed circles*, *triangles*, *squares*, and *open circles* denote the values for the cord blood, neonate, mother, and adult volunteer groups, respectively. The *long and short bars* represent the mean and SD, respectively. C, Correlation of serum levels of α-Klotho with FGF23 in cord blood samples according to Pearson's correlation test. α-Klotho was log-transformed to reduce skewness. $R^2 = 0.20$; $P < 0.05$. D, Representative image of normal human term placenta stained with antibody against α-Klotho. α-Klotho was predominantly expressed in syncytiotrophoblasts (*arrowheads*). Normal goat IgG was used as a negative control.

Expression of α-Klotho in syncytiotrophoblasts in term placenta

Immunohistochemical staining demonstrated that α-Klotho was predominantly expressed in syncytiotrophoblasts with some expression in endothelium of fetal vessels and connective tissue of villi (Fig. 1D).

Discussion

In the current study, we found that the levels of soluble α-Klotho in cord vein blood that inflow to fetus after the exchange of gas and nutrients in the placenta were markedly higher than those in neonates, mothers, and adults (Fig. 1A). Immunohistochemical staining of the placenta revealed that syncytiotrophoblasts that originate from fetus predominantly expressed α-Klotho (Fig. 1D). Although we cannot exclude the possibility that other fetal tissues also contribute, it is likely that the syncytiotrophoblast is one of the major sources of the soluble α-Klotho circulating abundantly in the fetus. The lower level of soluble α-Klotho in neonates at d 4 compared with that in cord

blood supports the idea that the protein is derived from the placenta.

In contrast to the high levels of soluble α-Klotho, the levels of FGF23 in cord blood were lower than those in the other samples. This result was consistent with the findings of a previous report (17). Considering a report demonstrating that FGF23 expression in fetal rat bones was much lower than that in young adult rat bones (18), the low levels of FGF23 in cord blood might be due to the low expression of FGF23 in fetal tissues. In addition, it may suggest that the FGF23 in the mother's blood is not transferred to the fetus through the placenta.

We found a negative correlation between soluble α-Klotho and FGF23 levels in the cord blood samples. This result was also compatible with the data reported by Yamazaki *et al.* (13) in which samples from healthy children and adult volunteers were analyzed. Although the precise mechanism is unknown, the high level of soluble α-Klotho circulating in the fetus may contribute to the low level of intact FGF23 in cord blood.

We also performed multiple regression analysis and found that soluble α-Klotho was one of the determinants of the levels of phosphate (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Serum calcium level also might be associated with that of α-Klotho, although the *P* value was 0.07. It has been reported that TRPV6 is involved in maternal-fetal calcium transport in mouse models (19). Moreover, Lu *et al.* have recently reported that α-Klotho activated not only TRPV5 but also TRPV6 (20). Given these results, soluble α-Klotho may contribute to the establishment of the fetomaternal calcium gradient also. However, considering the observation that the absence of α-Klotho in mice leads to hypercalcemia and hyperphosphatemia after birth (4), it remains to be determined whether the high levels of α-Klotho is an epiphenomenon in response to the higher serum calcium and phosphate, or is causing some of the biochemical features of fetuses. Even so, we can say that the measurement of soluble α-Klotho in cord blood as a biomarker might be useful in management of some genetic neonatal conditions such as hypercalcemia and hypophosphatemia. Measurement of calcium and phosphate during the perinatal period in the α-Klotho-deficient mice and generation of syncytiotrophoblast-specific α-Klotho-knockout mice might provide further insight into the roles of Klotho in fetal mineral metabolism.

In conclusion, the levels of soluble α-Klotho in cord blood were markedly high, and syncytiotrophoblasts in placenta were likely to be one of the major sources. Soluble α-Klotho in cord blood might be useful as a biomarker for calcium and phosphate metabolism in fetus.

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ORIGINAL ARTICLE
Pediatric Cardiology and Adult Congenital Heart Disease

Evaluation of Transplacental Treatment for Fetal Congenital Bradyarrhythmia

– Nationwide Survey in Japan –

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Background: There are few large studies of fetal congenital bradyarrhythmia. The aim of the present study was to investigate the effects and risks of transplacental treatment for this condition.

Methods and Results: Using questionnaires, 128 cases of fetal bradyarrhythmia were identified at 52 Japanese institutions from 2002 to 2008. Of the 128 fetuses, 90 had structurally normal hearts. Among these 90 fetuses, 61 had complete atrioventricular block (CAVB), 16 had second-degree AVB, 8 had sinus bradycardia, and 5 had other conditions. The 61 CAVB fetuses were divided into those who did (n=38) and those who did not (n=23) receive transplacental medication. Monotherapy with β-sympathomimetics, steroid monotherapy, and combination therapy with these agents was given in 11, 5 and 22 cases, respectively. Beta-sympathomimetics improved bradycardia (P<0.001), but no medication could significantly improve the survival rate. Fetal hydrops was associated with a 14-fold increased risk of perinatal death (P=0.001), and myocardial dysfunction was a significant risk factor for poor prognosis (P=0.034). Many adverse effects were observed with steroid treatment, with fetal growth restriction increasing significantly after >10 weeks on steroids (P=0.043).

Conclusions: Treatment with β-sympathomimetics improved bradycardia, but survival rate did not differ significantly in fetuses with and without transplacental medication. It is recommended that steroid use should be limited to <10 weeks to avoid maternal and fetal adverse effects, especially fetal growth restriction and oligohydramnios. (*Circ J* 2012; **76:** 469–476)

Key Words: Anti-Ro/SSA antibody; Congenital atrioventricular block; Pregnancy; Steroids; Transplacental treatment

Fetal congenital bradyarrhythmia is an uncommon but life-threatening disease, especially in the case of complete atrioventricular block (CAVB), which has a poor prognosis because of fetal hydrops, endocardial fibroelastosis and late-onset dilated cardiomyopathy.[1–9] Predominantly untreated CAVB has a significant mortality rate of 14–34%, while congenital CAVB is irreversible and requires a pacemaker in approximately 66% of cases after birth.[10] The association of CAVB with maternal anti-Ro/Sjögren's syndrome A (SSA) antibodies is well established, but the trigger for the maternal antibody interaction with the fetal Ro particle is unknown in some cases of antibody-exposed babies.[2,7–9,11,12]

There is limited evidence for the clinical efficacy of transplacental treatment of congenital AVB.[13–19] Steroids and i.v. immunoglobulins are given as anti-inflammatory treatment, while β-sympathomimetics are used for fetal pacing.[20] A recent

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Table 1. Baseline Characteristics of CAVB Fetuses

	Medication group (n=38)	No medication group (n=23)	P value
Maternal anti-SSA antibodies	29 (76.3)	11 (47.8)	<0.05‡
Gestational age at diagnosis (weeks)	24±3.2	28±5.7	<0.005†
Fetal heart rate at diagnosis (beats/min)	58±7.9	63±14.7	NS†
Fetal hydrops	16 (42.1)	6 (26.1)	NS‡
Fetal myocardial dysfunction	13 (34.2)	7 (30.4)	NS‡
Gestational age at initiation of therapy (weeks)	26±3.6	–	
Fetal heart rate at initiation of therapy (beats/min)	56±8.4	–	
Gestational age at delivery (weeks)	34±4.0	35±4.5	NS†
Birth weight (g)	2,120±620	2,528±653	<0.001†
Delivery mode			
Vaginal	8	7	NS‡
Cesarean section	30	16	NS‡
Permanent pacemaker implantation	14 (46.7)	6 (35.3)	NS‡
Neonatal survival	30 (78.9)	17 (73.9)	NS‡

Data given as mean±SD or n (%). P<0.05, significant difference.
†Student's t-test; ‡chi-square test and Fisher's exact test.
CAVB, complete atrioventricular block; SSA, Sjögren's syndrome A.

cohort study found an improved survival rate of >90% with initiation of maternal high-dose dexamethasone at the time of CAVB detection, and maintenance of this drug during pregnancy with use of β-sympathomimetics to keep fetal heart rates at >55 beats/min.[9,21] It was also suggested that prolonged use of dexamethasone might render fetuses with congenital CAVB less likely to develop the additional manifestations of myocarditis, cardiomyopathy, and hydrops fetalis, thus improving the overall outcome. Use of steroids, however, is controversial because of the potential risks for the fetus, including problems with neurological development, growth retardation, and oligohydramnios.[22–25]

Few large studies of fetal congenital bradyarrhythmia have been performed in Japan. The aims of the present study were to determine the features of fetal congenital bradyarrhythmia in Japan, and to examine the effects and risks of transplacental treatment for this condition.

Methods

Subjects

Data were collected using questionnaires sent to Departments of Perinatology and Pediatric Cardiology at 750 institutions in Japan over 7 years (2002–2008). The response rate was 60.7% (455 institutions). Fetal bradyarrhythmia was defined as ventricular heart rate <100 beats/min at the time of diagnosis.[4] The following perinatal data were also collected: gestational age at diagnosis and delivery, presence or absence of a congenital heart defect (CHD), type of bradyarrhythmia, method of diagnosis, presence or absence of maternal autoantibodies such as anti-Ro/SSA antibodies, presence or absence of fetal hydrops, presence or absence of fetal myocardial dysfunction, fetal ventricular and atrial heart rate at presentation, prenatal treatment, mode of delivery, and outcome. Adverse effects related to prenatal treatment were also evaluated.

Statistical Analysis

Statistical analysis was performed using STATA 11.1 (StataCorp, College Station, TX, USA) and JMP 9 (SAS Institute, Cary, NC, USA). Data are presented as mean±SD or number of patients and were analyzed using Student's t-test. Categorical variables were evaluated on chi-square test and Fisher's exact test. Logistic regression was used to adjust for baseline variables known to be associated with fetal ventricular heart rate, fetal hydrops, fetal myocardial dysfunction, and maternal anti-Ro/SSA antibody. Time to fetal or neonatal death was analyzed using the Kaplan-Meier method with a log-rank test and a Cox proportional hazard model. P<0.05 was considered significant.

Results

Baseline Characteristics

A total of 128 cases were registered from 52 institutions during 7 years (2002–2008). All cases of fetal bradyarrhythmia were diagnosed during fetal life using echocardiography. In 8 cases, magnetocardiography was performed due to fetal bradyarrhythmia and family history of long QT syndrome (LQTS). Of the 128 fetuses, 38 (29.7%) had CHD, 15 had left atrial isomerism, 1 had right atrial isomerism, 5 had atrioventricular septal defect, 4 had corrected transposition of the great arteries, 4 had pulmonary stenosis, and 9 had other conditions. Patent ductus arteriosus and atrial septal defect were categorized as an absence of CHD. Ninety fetuses (70.3%) had a structurally normal heart, of whom 61 had CAVB, 16 had second-degree AVB, 8 had sinus bradycardia, 3 had sick sinus syndrome. Nine LQTS cases occurred in combination with another condition.

CAVB

Of the 61 fetuses with a structurally normal heart and CAVB (**Table 1**), 38 received transplacental medication. No fetus showed improvement of heart block. Monotherapy with β-sympathomimetics was given in 11 cases, steroids were given in 5 cases, and combination therapy with these agents was used in 22 cases. No transplacental medication was given in 23 cases. Ritodrine hydrochloride was used as the β-sympathomimetic agent. Steroids tended to be used in fetuses that were positive for maternal anti-Ro/SSA antibody throughout pregnancy, but the chosen steroid differed among institutions. Maternal i.v. immunoglobulin was not used. After birth, a pacemaker was implanted based on the Japanese guidelines of syncope, ventricular heart rate <50 beats/min, decreased cardiac function, LQTS, and a sudden pause longer than 2–3-fold the regular ventricular heart rate.