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Thrombosis Research 133 (2014) 914–918

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Nonsynonymous mutations in three anticoagulant genes in Japanese patients with adverse pregnancy outcomes☆

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ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 14 February 2014
Accepted 14 February 2014
Available online 21 February 2014

Keywords:
Fetal death
Fetal growth restriction
Miscarriage
Protein S
Thrombophilia

ABSTRACT

Background: Hereditary thrombophilias may associate with uteroplacental thrombosis leading to adverse pregnancy outcomes. The present study was conducted to reveal the frequency of the low-frequency thrombophilic protein S K196E mutation, as well as the frequency of very rare nonsynonymous mutations in protein S, protein C, and antithrombin genes, in patients with adverse pregnancy outcomes.
Patients and methods: We enrolled 330 Japanese patients with adverse pregnancy outcomes and divided them into 233 patients with two or more miscarriages and 114 patients with fetal growth restriction (FGR) and/or intrauterine fetal death (IUFD); 17 patients belonged to both groups. We sequenced the entire coding regions of three anticoagulant genes in all 330 patients.
Results: We found that protein S K196E mutation was identified in 4 out of 233 patients with recurrent miscarriage and in 2 out of 114 patients with FGR and/or IUFD. The frequencies of this mutation in these patient groups were not different from that in a Japanese general population. Very rare nonsynonymous mutations were identified in 3.3% (11 out of 330) of patients with adverse pregnancy outcomes.
Conclusions: Although the low-frequency protein S K196E mutation can increase the risk for venous thromboembolism, it did not increase the risk for adverse pregnancy outcomes even in Japanese.
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Introduction

Approximately 1-5% of pregnant women have a serious adverse pregnancy outcome, such as severe preeclampsia, placental abruption, intrauterine fetal death (IUFD), or severe fetal growth restriction (FGR) [1]. Recurrent miscarriage, defined as two or more miscarriages, is also a significant public health problem for women, and the prevention of these adverse pregnancy outcomes is important for women's health [2]. An estimated 5% of women of reproductive age in general experience two or more miscarriages [3]. The causes of serious pregnancy outcomes are unknown, but they may be associated with abnormal placental vasculature and disturbances of hemostasis, leading to inadequate maternal-fetal circulation [4]. Therefore, in order to prevent adverse pregnancy outcomes, it will be important to identify the inherited factors related to inadequate maternal-fetal circulation.

Substantial progress has been made in the identification and understanding of inherited hypercoagulable disorders that promote thrombosis, collectively termed inherited thrombophilia [2,5,6]. These include low-frequency mutations, the factor V Leiden mutation, the prothrombin G20210A mutation, and very rare mutations of protein S, protein C, and antithrombin genes. The factor V Leiden mutation and the prothrombin G20210A mutation are modest genetic risk factors for venous thromboembolism and are widely distributed among Caucasians [7] but are not present in the Japanese population [8,9]. Instead, other authors and we have identified a low-frequency thrombophilic mutation, the protein S K196E mutation, as a genetic risk factor for venous thromboembolism with odds ratios between 3.74 and 8.56 in several Japanese populations [10–14]. The prevalence of this mutant allele in the general Japanese population was found to be about 0.009, suggesting a substantial proportion of the Japanese population carries the protein S E-allele

Abbreviations: FRG, fetal growth restriction; IUFD, intrauterine fetal death; SD, standard deviation.

☆ This study was presented at the 59th Annual Scientific and Standardization Committee Meeting "Women's Health Issues in Thrombosis and Haemostasis" at the 26th Congress of International Society of Thrombosis and Haemostasis in Amsterdam on June 29, 2013.

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http://dx.doi.org/10.1016/j.thromres.2014.02.012
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and is at risk of developing deep vein thrombosis [13–15]. This mutation seems to be specific to Japanese, because it has not so far been identified in Chinese, Koreans, or Caucasians [13,15–18].

Hereditary thrombophilia can be explained by low-frequency mutations including factor V Leiden mutation, prothrombin G20210A mutation, and protein S K196E mutation, as well as by very rare mutations causing deficiencies of protein S, protein C, and antithrombin. The former (the low-frequency mutations) have weak effects and the latter have large effects on thrombosis. It is controversial whether there is an association between low-frequency mutations, such as the factor V Leiden mutation and the prothrombin G20210A mutation, and adverse pregnancy outcomes, such as miscarriage, preeclampsia, FGR, and placental abruption [4,19–37].

The present study was conducted to reveal the hypothesis that the mutations predisposing patients to thrombosis in Japanese may be important risk factors for inadequate maternal-fetal circulation that may explain adverse pregnancy outcomes including recurrent miscarriage, FGR, and IUFD. We therefore examined the frequency of the low-frequency protein S K196E mutation, as well as those of very rare nonsynonymous mutations in protein S, protein C, and antithrombin genes, in patients with adverse pregnancy outcomes.

Patients and Methods

Patients and Diagnostic Criteria for Adverse Pregnancy Outcomes

In this prospective observational study, 330 patients who had experienced adverse pregnancy outcomes were enrolled from four tertiary perinatal centers: the National Cerebral and Cardiovascular Center, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka Medical College, and Kaizuka City Hospital. All of these centers are located in Osaka prefecture, which has the third-largest population out of the 47 prefectures in Japan.

Patients with adverse pregnancy outcomes were classified into two groups: those with recurrent miscarriage (233 patients) and those with FGR and/or IUFD that had occurred after 22 weeks of gestation (114 patients); 17 patients belonged to both groups. We excluded patients with multiple pregnancy. Patients were excluded in a case that patients and the partners had chromosomal abnormalities. Patients with antiphospholipid syndrome, diabetes mellitus, thyroid dysfunction, infectious diseases, and uterine deformity were also excluded. Antiphospholipid syndrome was diagnosed according to the revised classification criteria, requiring at least one of the clinical criteria (vascular thrombosis or pregnancy morbidity) and at least one of the following laboratory criteria: lupus anticoagulant, anticardiolipin antibody, and anti-β2-glycoprotein-I antibody [38]. Previous miscarriage was defined as pregnancy loss at a gestational age of 22 weeks or less. The definition of miscarriage included documentation of pregnancy by a positive pregnancy test and clinical manifestations of miscarriage (e.g., abdominal pain, cramps, and vaginal bleeding). Recurrent miscarriage was defined as at least two miscarriages. We diagnosed FGR if an ultrasound examination showed a fetus with an estimated fetal weight of less than −1.5 standard deviation in Japanese at more than 22 weeks of gestation. We also diagnosed IUFD as fetal death at more than 22 weeks of gestation by ultrasound examination. The plasma samples were obtained at least 3 months' postpartum and after at least 3 months without the use of warfarin or oral contraceptives. Protein S anticoagulant activity, free protein S antigen, protein C amidolytic activity, and heparin-dependent antithrombin activity were measured as previously described [39].

The protocol of this study was approved by the Ethics Review Committee of National Cerebral and Cardiovascular Center, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka Medical College, and Kaizuka City Hospital. Only those who had given written informed consent for genetic analysis were included in the genetic analysis.

DNA Sequencing of Protein S, Protein C, and Antithrombin Genes

We sequenced the entire coding regions of protein S, protein C, and antithrombin genes in 330 patients with adverse pregnancy outcomes. The method of direct sequencing using the 96-capillary 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) was described previously [39]. We have adopted the numbering standards of the Human Genome Variation Society, wherein the A of the ATG of the initiator Met codon is denoted as nucleotide +1 and the initial Met residue is denoted as amino acid +1 [40]. The potential impact of a missense mutation on the structure and function was examined using PolyPhen-2 (URL: http://genetics.bwh.harvard.edu/pph2/), a program to predict the functional significance of missense mutations [41].

Statistical Analysis

Statistical analysis was performed using JMP 10 (SAS Institute, Cary, NC, USA). Data are presented as mean ± standard deviation (SD) or the number of patients. Comparisons between groups were analyzed

Table 1
Baseline characteristics of 233 patients with recurrent miscarriage.

	Patients with genetic mutation (n = 13)	Patients without genetic mutation (n = 220)	P value
Age			
years, mean ± SD (range)	35 ± 5 (27–43)	34 ± 4 (25–44)	0.50†
No. ≧35 years old (%)	7 (53.8)	97 (43.9)	0.51‡
Body mass index, mean ± SD (range)	19.9 ± 1.8 (17.2-22.5)	21.1 ± 2.9 (16.6-30.5)	0.13†
Miscarriage			
No. of times, mean ± SD (range)	2.9 ± 0.5 (2–4)	3.3 ± 1.0 (2–9)	0.13†
≧3 miscarriages, no. of patients (%)	11 (84.6)	202 (91.4)	0.41‡
Number of mutation carriers			
Protein S mutation	6*	0	
(protein S K196E mutation)	(4*)	(0)	
Protein C mutation	4	0	
Antithrombin mutation	3	0	
Other complications of pregnancy or infant, no.			
FGR and/or IUFD	1	16	
Preeclampsia	0	1	
Eclampsia	0	1	
HELLP syndrome	0	2	
Placental abruption	0	1	

SD, Standard deviation; FGR, fetal growth restriction; IUFD, intrauterine fetal death; HELLP, hemolysis, elevated liver enzymes, and low platelets.
*One patient was a compound heterozygote with protein S K196E and T630I mutations. †t-test. ‡chi-square test.

Table 2
Baseline characteristics of 114 patients with FGR and/or IUFD.

	Patients with genetic mutation (n = 4)	Patients without genetic mutation (n = 110)	P value
Age			
years, mean ± SD (range)	34 ± 7 (28–43)	33 ± 5 (18–48)	0.87*
no. ≧35 years old (%)	1 (25)	35 (31)	0.78†
Body mass index, mean ± SD (range)	22.7 ± 4.1 (19.8-28.6)	21.0 ± 3.0 (13.7-29.6)	0.27*
FGR and/or IUFD			
No. of times, mean ± SD (range)	1.5 ± 0.6 (1–2)	1.4 ± 0.7 (1–5)	0.77*
≧3 FGR and/or IUFD, no. of patients (%)	0	6 (5.4%)	0.63†
Number of mutation carriers			
Protein S mutation	3	0	
(protein S K196E mutation)	(2)	(0)	
Protein C mutation	1	0	
Antithrombin mutation	0	0	
Other complications of pregnancy or infant, no.			
Recurrent miscarriage	1	16	
Gestational hypertension	1	10	
Preeclampsia	0	2	
HELLP syndrome	0	3	
Placental abruption	0	5	
Deep vein thrombosis	0	1	
Sudden infant death syndrome	0	1	

SD, Standard deviation; FGR, fetal growth restriction; IUFD, intrauterine fetal death; HELLP, hemolysis, elevated liver enzymes, and low platelets. *t-test. †chi-square test.

by Student's t-test. Categorical variables were evaluated by the chi-square test. P-values <0.05 were considered significant.

Results

We enrolled 330 patients with adverse pregnancy outcomes, including 233 patients with recurrent miscarriage and 114 patients with FGR and/or IUFD; 17 patients belonged to both groups (Tables 1, 2). The patients with more than three miscarriages were 213 patients out of 233 (Table 1).

The sequencing of the coding regions of the protein S, protein C, and antithrombin genes in 233 patients with recurrent miscarriage identified three missense mutations in the protein S gene in 6 cases, three missense mutations in the protein C gene in 4 cases, and two missense mutations in 2 cases and one amino acid deletion in 1 case in the antithrombin gene (Table 3). One patient had two mutations (K196E and T630I) in the protein S gene. Among the mutations, there were three

Table 3
Nonsynonymous mutations identified in protein S, protein C, and antithrombin genes in 233 patients with recurrent miscarriage.

Patient	cDNA*	Region	Amino acid change	Domain	Prediction of possible impact of an amino acid substitution†	Protein S‡, Protein C§, or Antithrombin¶ activity, %	Protein S‡, Protein C§, or Antithrombin¶ activity, during pregnancy, %	Free protein S antigen, %	Age	No. of miscarriages	No. previous live births	Body mass index	Family history of thrombo-embolism	Other complications of pregnancy
Protein S gene														
Case 1	c.586A > G	Exon 6	K196E	EGF2	Benign	n.d.	34‡	39**	27	3	1	19.3	None	None
	c.1889C > T	Exon 15	T630I	SHBG	Probably damaging									
Case 2	c.586A > G	Exon 6	K196E	EGF2	Benign	54‡	n.d.	71	43	3	1	19.5	None	None
Case 3	c.586A > G	Exon 6	K196E	EGF2	Benign	57‡	n.d.	61	34	3	0	18.3	None	None
Case 4	c.586A > G	Exon 6	K196E	EGF2	Benign	28‡	n.d.	59	34	4	0	22.4	None	None
case 5	c.1334G > A	Exon 12	R445H	SHBG	Possibly damaging	70‡	n.d.	64	37	3	0	21.1	None	None
Case 6	c.1889C > T	Exon 15	T630I	SHBG	Probably damaging	66‡	n.d.	70	36	3	0	18.6	None	None
Protein C gene														
Case 7	c.1066C > T	Exon 9	R356C	SPD	Probably damaging	n.d.	76§	-	35	4	1	21.9	None	None
Case 8	c.983G > A	Exon 9	R328H	SPD	Probably damaging	114§	n.d.	-	43	3	0	19.8	None	FGR/IUFD††
Case 9	c.1075G > A	Exon 9	V359I	SPD	Benign	131§	n.d.	-	30	3	0	17.7	None	None
Case 10	c.983G > A	Exon 9	R328H	SPD	Probably damaging	47§	n.d.	-	33	3	0	18.8	None	None
Antithrombin gene														
Case 11	c.181_183delGAA	Exon 2	K61del		-	n.d.	104¶	-	31	3	0	21.5	None	None
Case 12	c.442 T > C	Exon 3	S148P		Benign	58¶	40¶	-	35	2	1	17.2	Farther‡‡	None
Case 13	c.1277C > T	Exon 7	S426L		Probably damaging	101¶	116¶	-	35	2	2	22.5	None	None

EGF2, epidermal growth factor-like domain 2; SHBG, sex hormone-binding globulin-like domain; SPD, serine protease domain; n.d., not determined; FGR, fetal growth restriction; IUFD, intrauterine fetal death. *position from A of initial ATG in cDNA, †Polyphen-2 prediction [41], ‡protein S anticoagulant activity, §protein C amidolytic activity, ¶antithrombin activity, **23 weeks of gestation during pregnancy, ††37 weeks of gestation, ‡‡deep vein thrombosis and pulmonary thromboembolism.

Table 4
Nonsynonymous mutations identified in proteins S and C in 114 patients with FGR and/or IUFD.

Patient	cDNA*	Region	Amino acid change	Domain	Prediction of possible impact of an amino acid substitution†	Protein S‡ or Protein C§ activity, %	Protein S activity, during pregnancy, %	Free protein S antigen, %	Age	Gravida	Parity	No. of miscarriages	No. previous live births	Body mass index	Family history of thrombo-embolism	Other complications of pregnancy
Protein S gene																
Case 1	c.586A > G	Exon 6	K196E	EGF2	Benign	57‡	n.d.	82	33	1	1	0	1	22.4	none	GH
Case 2	c.586A > G	Exon 6	K196E	EGF2	Benign	85‡	n.d.	74	30	1	1	0	1	28.6	none	none
Case 3	c.1889C > T	Exon 15	T630I	SHBG	Probably damaging	n.d.	31	36¶	28	2	2	0	2	20.1	none	none
Protein C gene																
Case 4**	c.983G > A	Exon 9	R328H	SPD	Probably damaging	114§	-	-	43	4	1	3	0	19.8	none	Recurrent miscarriage

FGR, fetal growth restriction; IUFD, intrauterine fetal death; EGF2, epidermal growth factor-like domain 2; SHBG, sex hormone-binding globulin-like domain; SPD, serine protease domain; n.d., not determined; GH, gestational hypertension.
*position from A of initial ATG in cDNA, †Polyphen-2 prediction [41], ‡protein S anticoagulant activity, §protein C amidolytic activity, ¶26 weeks of gestation during pregnancy, **same patient as case 8 in Table 3.

recurrent mutations: the K196E mutation in the protein S gene in 4 patients, the T630I mutation in the protein S gene in 2 patients, and the R328H mutation in the protein C gene in 2 patients. Among the three recurrent mutations, as previously described, the K196E mutation in the protein S gene is the most common thrombophilic mutation in the Japanese population [10–15].

The sequencing of the coding regions of three genes in 114 patients with FGR and/or IUFD identified two missense mutations in the protein S gene in 3 cases and one missense mutation in the protein C gene in 1 case (Table 4). Two patients carried the K196E mutation in the protein S gene and one had the T630I mutation in the protein S gene. One patient had the R328H mutation in the protein C gene. None of the 114 patients with FGR and/or IUFD had nonsynonymous mutations in the antithrombin gene.

Taking together the results for all 330 patients, the nonsynonymous mutations were identified in 13 out of 233 patients with recurrent miscarriage (frequency: 0.056) and in 4 out of 114 patients with FGR and/or IUFD (frequency: 0.035) (Table 5). Thus, 16 out of 330 patients with adverse pregnancy outcomes (frequency: 0.048) had nonsynonymous mutations in three natural anticoagulant genes.

As described above, the protein S K196E mutation was identified in multiple patients. The protein S K196E mutation was identified in 4 out of 233 patients with recurrent miscarriage (frequency: 0.017) and in 2 out of 114 patients with FGR and/or IUFD (frequency: 0.018). We have previously obtained a frequency of the protein S K196E mutation in a Japanese general population in Osaka prefecture where all 4 hospitals participated in the present study are located. In that study, the protein S K196E mutation was identified in 1.8% of Japanese (66 out of 3651) [11]. The frequencies of this mutation in the two disease groups, recurrent miscarriage and FGR and/or IUFD, were not different from that in the Japanese general population ($p = 0.941$, the general population vs. the recurrent miscarriage group; $p = 0.982$, the general population vs. the FGR and/or IUFD group). Once those patients with protein S K196E mutation were excluded, remaining very rare nonsynonymous mutations were identified in 10 out of 233 patients with recurrent miscarriage (frequency: 0.043) and in 2 out of 114 patients with FGR and/or IUFD (frequency: 0.018) (Table 5). One patient with protein C mutation had both recurrent miscarriage and FGR and/or IUFD. Thus, 11 out of 330 patients with adverse pregnancy outcomes (frequency: 0.033) had nonsynonymous mutations in three natural anticoagulant genes, excluding the protein S K196E mutation (Table 5).

Table 5
Number of patients with adverse pregnancy outcomes carrying genetic mutations in three anticoagulant genes.

Gene mutation	Number of patients		
	Recurrent miscarriage (n = 233)*	FGR and/or IUFD (n = 114)*	Total (n = 330)
Protein S K196E mutation	4†	2	6†
Protein S mutation excluding K196E	3†	1	4†
Protein C mutation‡	4	1	4
Antithrombin mutation	3	0	3
Total	13	4	16
Frequency	0.056 (13/233)	0.035 (4/114)	0.048 (16/330)
Frequency, excluding protein S K196E mutation	0.043 (10/233)	0.018 (2/114)	0.033 (11/330)

FGR, fetal growth restriction; IUFD, intrauterine fetal death.*Seventeen patients had both recurrent miscarriage and FGR and/or IUFD. †One patient had protein S K196E and T630I mutations. ‡One patient with recurrent miscarriage and FGR and/or IUFD had protein C mutation.

Discussion

To examine the association of hereditary thrombophilia with adverse pregnancy outcomes, we sequenced three anticoagulant genes, protein S, protein C, and antithrombin, in 330 Japanese women with adverse pregnancy outcomes including recurrent miscarriage, FGR, and IUFD. We found that the protein S K196E mutation was identified in 1.7% of patients with recurrent miscarriage and in 1.8% of patients with FGR and/or IUFD and these frequencies were not different from that obtained in the Japanese general population in Osaka area (1.8%, 66 out of 3651) [11] where all 4 hospitals participated in the present study are located. Thus, the protein S K196E mutation was not related to adverse pregnancy outcomes. The results are in contrast to that obtained in patients with deep vein thrombosis. In Japanese patients with deep vein thrombosis, the protein S K196E mutation was identified in 8.7% of the patients (15 out of 173) [42].

In the present study, we identified very rare nonsynonymous mutations in three genes in 3.3% of patients with adverse pregnancy outcomes. Thus, carriers with very rare mutations were not dominant in patients with adverse pregnancy outcomes, indicating that the contributions of the very rare mutations of these three genes to the development of adverse pregnancy outcomes were likely weak. In Japanese patients with deep vein thrombosis, 23% (40 out of 173) carried at least one very rare nonsynonymous mutation in the three genes [42].

In conclusion, we collected 330 Japanese women with adverse pregnancy outcomes, including recurrent miscarriage, FGR, and IUFD, and sequenced protein S, protein C, and antithrombin genes. This is the first effort to sequence three anticoagulant genes in patients with adverse pregnancy outcomes. We found that protein S K196E mutation was identified in patients with adverse pregnancy outcomes with the

similar frequency to that obtained in the Japanese general population. We found that very rare nonsynonymous mutations in three genes were only found in 3.3% of patients with adverse pregnancy outcomes. Our study has a limitation. The patients were collected from four tertiary perinatal centers, all located in the Osaka area. To generalize our findings, more patients should be collected from across Japan.

Conflict of Interest Statement

The authors report no conflicts.

Funding Sources

This work was supported in part by grants-in-aid from the Ministry of Health, Labor, Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Uehara Memorial Foundation.

Acknowledgments

We thank Drs. M. Ohmichi and H. Kamegai at Osaka Medical College, Dr. M. Nagamatsu at Kaizuka City Hospital, and Dr. N. Suehara, Dr. N. Mitsuda, and Mr. Y. Imayoshi at Osaka Medical Center and Research Institute for Maternal and Child Health for patient recruitment; Dr. T. Sakata at the National Cerebral and Cardiovascular Center for the activity assay; and Ms. J. Ishikawa, Ms. K. Sakashita, Ms. Y. Sato, and Dr. S. Honda at the National Cerebral and Cardiovascular Center for DNA sequencing.

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THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.12336 J. Obstet. Gynaecol. Res. Vol. 40, No. 5: 1267–1273, May 2014

Factors contributing to mortality and morbidity in pregnancy-associated intracerebral hemorrhage in Japan

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Abstract

Aim: The aim of this study was to analyze the causes and outcomes for intracerebral hemorrhage (ICH) occurring during pregnancy and postnatally.

Material and Methods: A nationwide study of pregnancy-related ICH in Japan was performed. We contacted 1582 facilities to identify women with ICH in pregnancy or postnatally between 1 January 2006 and 31 December 2006. A total of 1012 facilities (70%) responded with completed questionnaires. Risk factors for ICH, neurological features, onset to diagnosis time (O–D time), and obstetric data were recorded.

Results: Thirty-eight cases of pregnancy-associated ICH were identified, corresponding to 3.5 per 100 000 deliveries. There were seven maternal deaths, giving a case mortality rate of 18.4%. Pre-eclampsia was identified in 10 cases (26.3%) and hemolysis elevated liver enzymes and low platelet count (HELLP) syndrome was present in five. There were four cases (10.5%) with Moyamoya disease and seven (18.4%) with arteriovenous malformation. HELLP syndrome and moderately or severely disturbed consciousness at disease onset were significantly associated with a poor outcome (modified Rankin Scale ≥3). Pre-eclampsia, HELLP syndrome and O–D time >3 h were significantly associated with maternal mortality.

Conclusion: Early diagnosis may prevent maternal death, even in severe cases of pregnancy-related ICH. However, maternal–fetal care centers do not always have full-time neurosurgeons or diagnostic imaging tools suitable for diagnosis of ICH. Thus, a network should be established between maternity centers and neurosurgery departments with computed tomography or magnetic resonance imaging available at all times. We recommend transferal of pregnant women with neurological symptoms to a regional facility that is equipped to treat such patients.

Key words: cerebrovascular disease, morbidity, mortality, postnatally, pregnancy.

Introduction

Intracerebral hemorrhage (ICH) is a subtype of intracranial hemorrhage that occurs within the brain tissue itself and is a serious medical emergency because it can increase intracranial pressure. The mortality rate for ICH is 13–23% in Japan, which is the second highest among subtypes of cerebral stroke, after subarachnoid hemorrhage.[1] ICH is an infrequent but severe complication in pregnancy. The absolute risk is small, but ICH complicated with pregnancy has a significant impact on maternal and fetal outcome. Pregnancy-related ICH

Received: March 19 2012.
Accepted: October 25 2013.
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has an estimated mortality of 9–38%[2–6] and survivors may suffer profound and permanent disability. An association of ICH with pregnancy has long been recognized and many factors have been proposed to be partly responsible for the recent increase in the incidence of this condition. Nagaya *et al.*[7] examined factors contributing to maternal mortality in the Japanese population in 1991 and 1992, and identified 27 maternal deaths due to intracranial hemorrhage, which was the second most common cause of maternal mortality after obstetrical hemorrhage.

The incidence, cause, and outcome of ICH in pregnancy or postnatally are poorly understood. Furthermore, the characteristics of pregnancy-associated ICH in the Japanese population may differ from those in other countries, as the Hisayama study[8] found that the incidence of ICH in the general Japanese population was much higher than that in Western countries. A high prevalence of small-artery cerebrovascular lesions may be responsible for the high prevalence of ICH in Asians. The higher incidence of Moyamoya disease in Japan may be another reason for the high incidence of pregnancy-associated ICH.[9] Moyamoya disease is characterized by progressive stenosis of the terminal portion of the internal carotid artery and is a known cause of cerebral stroke, which is mainly caused by hemodynamic stress of pregnancy on fragile Moyamoya vessels. With this background, the aim of this study was to analyze the causes and outcomes for ICH occurring during pregnancy and postnatally.

Methods

A nationwide study of pregnancy-related ICH was performed in Japan. In December 2007, we contacted obstetricians and neurosurgeons at 1582 facilities to identify cases of ICH that occurred in association with pregnancy or postnatally (until 1 year after delivery) from 1 January to 31 December 2006. The survey sent to each facility is shown in Figure 1. To check for cases that may have eluded identification, we also contacted neurology, neurosurgery and intensive care departments. A total of 1012 facilities (70%) responded with completed questionnaires. We asked each facility to identify from its records all patients with an ICH occurring during pregnancy or postnatally. Risk factors for ICH, neurological features, onset to diagnosis time (O–D time) and obstetric data were recorded. In all patients, ICH was verified by brain computed tomography (CT) or magnetic resonance imaging (MRI). Global outcome at discharge was assessed using the modified Rankin Scale (mRS),[10] with 0–2 defined as a good outcome, 3–5 as a poor outcome, and 6 as death. The consciousness level was measured by the Japan Coma Scale (JCS).[11] In an attempt to further define the epidemiology of ICH in pregnancy, we undertook an analysis of this disease using the vital statistics database of the Ministry of Health, Labour and Welfare (MHLW), which is the largest clinical database available in Japan.

The primary aim of the study was to identify risk factors for maternal death related to ICH. Univariate logistic regression analysis was performed using six factors: maternal age ≥ 35 years old, pre-eclampsia, HELLP syndrome, moderate to severe disturbed consciousness (JCS II to III) at onset, interval between onset and diagnosis ≥3 h, and history of neurosurgery. These six factors were chosen because they frequently appear in case reports of fatal maternal ICH. Statistical procedures were performed using SPSS version 11. Univariate logistic regression analysis was performed to identify factors of maternal death related to ICH. The study was approved by the institutional review committee.

Results

Thirty-eight cases of pregnancy-associated ICH were identified out of a total of 1 092 674 deliveries in 2006. These 38 cases were reported from 31 facilities, mostly in urban areas. Seven facilities reported two cases and 27 of the facilities were tertiary referral centers. These findings correspond to 3.5 cases of pregnancy-associated ICH per 100 000 deliveries. There were seven maternal deaths, giving a case mortality rate of 18.4%. Overall maternal death in 2006 occurred at a rate of 0.8 per 100 000 deliveries (calculated using the 2006 vital statistics of the MHLW of Japan). The incidence and mortality rates of ICH at the ages of 15–19, 20–24, 25–29, 30–34, 35–39 and 40–44 years were 6.3, 0.8, 3.9, 3.9, 5.9 and 18.5, and 0.0, 0.0, 0.3, 0.7, 1.7 and 0.0 per 100 000 deliveries, respectively. The mean age of pregnant women with ICH was 31.5 years old (range: 19–43 years old). Pregnancy-associated ICH was highest in the 40–44 years age group. The odds ratio for the incidence of pregnancy-associated ICH in women aged ≥35 years old was 1.9 (95% confidence interval [CI]: 1.0–3.9). Mortality was highest in the 35–39-years age group.

Baseline demographic variables and clinical characteristics are shown in Table 1. ICH developed in twice as many primiparous women as multiparous women

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Questionnaire

Intracerebral hemorrhage during pregnancy or that occurring within one year after delivery. Cases from January to December 2006 should be included.

Years at onset	Years old
Previous delivery (over 22 weeks)	( ) times
Delivery (weeks) of current pregnancy	Abortion (<22 weeks), Preterm (22-36 weeks), Full term (37-42 weeks)
Onset time	During pregnancy, Intrapartum, Within 24 h after delivery, Postpartum 1 to 42 days, Postpartum 43 days to 1 year
Delivery mode	Vaginal, Cesarean section, No delivery
Prognosis (Neonate)	Alive, Neonatal death, Still birth
Complications	PIH, Hypertension, DM, Hyperlipidemia, Smoking, Cardiovascular disease, Af, Arrhythmia, Deep vein thrombosis, APS, Habitual abortion, Headache
Type of cerebrovascular disease	TIA, Cerebral Infarction, Intracerebral hemorrhage [Category] Intraparenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, subdural hemorrhage [Causes] Hypertension, AVM, aneurysm, Moyamoya disease, pre-eclampsia, vein sinus thrombosis
Diagnosed by	CT, MRI, Angiography, MRA
First symptoms	Headache, nausea, paralysis, seizure, consciousness disturbance, visual disturbance, speech disturbance
Place of first symptoms	Out of / In hospital
Department that first admitted the patient	Obstetrics and Gynecology, Internal Medicine, Neurosurgery, Emergency
Department that finally treated the patient	Obstetrics and Gynecology, Internal Medicine, Neurosurgery, Emergency
Onset to diagnosis time	Within 3 h, 3-24 h, More than 24 h
Patient JCS on arrival at your hospital	I-1, I-2, I-3, II-10, II-20, II-30, III-100, III-200, III-300,
At admission to hospital	Modified Rankin scale (0,1,2,3,4,5,6) Transferred to another hospital in the acute phase

Figure 1 Questionnaire. Intracerebral hemorrhage during pregnancy or that occurring within 1 year after delivery. Cases from January to December 2006 should be included. Af, atrial fibrillation; APS, antiphospholipid–antibody syndrome; AVM, arteriovenous malformation; CT, computed tomography; DM, diabetes mellitus; JCS, Japan Coma Scale; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; PIH, pregnancy-induced hypertension; TIA, transient ischemic attack.

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and occurred mostly in the antepartum period, especially in the third trimester. Cesarean section was performed in 63.2% of women with ICH in the antepartum or intrapartum period. One stillbirth following maternal death was identified. In this case, putaminal hemorrhage occurred at 40 weeks of gestation. Eight premature live deliveries were identified. The condition of these infants is unknown as long-term follow-up was not performed due to the short observation period. The clinical features included headache in 22 cases, seizures in nine, and disturbance of consciousness in 26.

Pre-existing disorders and risk factors are shown in Table 2. Pre-eclampsia was identified in 10 cases (26.3%) and hemolysis elevated liver enzymes and low platelet count (HELLP) syndrome was present in five cases. Death occurred in four of the 10 cases with pre-eclampsia and in three of the four cases in which HELLP syndrome developed among cases of pre-eclampsia. HELLP syndrome occurred without prior pre-eclampsia in two cases, which resulted in one death and one case of subsequent severe disability in which the patient was bedridden. Three of the five cases of HELLP syndrome were diagnosed later than 3 h after onset of the disease. Thus, there is a possibility that a confounding factor (O–D time) could have influenced the outcome of ICH in these cases. Moyamoya disease occurred in four cases (10.5%) and arteriovenous malformation (AVM) was found in seven (18.4%). There were no pre-existing disorders in 16 (42.1%) of the cases of pregnancy-associated ICH. Collectively, these numbers reflect three cases with both pre-eclampsia and HELLP syndrome and one with pre-eclampsia and AVM (Table 2).

The relation between the consciousness disturbance level at onset and outcome was clear, with greater consciousness disturbance leading to a poor outcome (Table 3). The O–D time was also associated with the outcome. The survival rate was >90% for patients diagnosed within 3 h from onset of bleeding, although permanent moderate or severe disability remained in 68.2% of survivors (Table 3). The majority of patients ($n = 28$) first consulted (or were transferred to) a department of obstetrics. Of these patients, 21 (75%) were subsequently transferred to a neurosurgery department. Overall, 32 of the 38 patients were finally treated in neurosurgery. The O–D time in the 21 transferred patients was similar regardless of the location of the initial examination. Fourteen of these patients underwent surgery and this tended to be more common in patients who were first examined by

Table 1 Baseline demographics and clinical characteristics of peripartum ICH patients

Item	Value
Age (years, mean ± SD)	31.5 ± 5.1
Parity (primipara/multipara)	26/12
Gestational age at onset (mean, weeks)	32.4
Timing of onset in gestation	
Antepartum	21 (55.2)
1st, 2nd, 3rd trimester	0, 7, 14
Intrapartum	7 (18.4)
Postpartum	10 (26.3)
Mode of delivery	
Cesarean section	24 (63.2)
Vaginal delivery	14 (36.8)
Prognosis of newborn	
Alive	37 (97.3)
Stillbirth	1 (2.6)
Newborn death	0
Initial symptoms	
Headaches	22 (58.0)
Nausea/vomiting	4 (10.5)
Convulsion	9 (23.7)
Disturbed consciousness	25 (65.8)
Visual disturbance	3 (7.9)
Paralysis	9 (23.7)

Values are shown as *n* (%). ICH, intracerebral hemorrhage.

Table 2 Pre-existing disorders and Modified Rankin scale†

		Modified Rankin Scale§		
	Total (%)‡	0–2	3–5	6
Pre-eclampsia	10 (26.3)	3 (30)	3 (30)	4 (40)
HELLP syndrome	5 (13.2)	0 (0)	1 (20)	4 (80)
Arteriovenous malformation	7 (18.4)	2 (28.6)	4 (57.1)	1 (14.3)
Moyamoya disease	4 (10.5)	1 (25)	3 (75)	0 (0)
No pre-existing disorder	16 (42.1)	9 (56.3)	5 (31.3)	2 (12.5)

†Values are shown as *n* (%). ‡Percentage of all ICH cases, including three cases with pre-eclampsia and HELLP syndrome and one case with arteriovenous malformation and pre-eclampsia. §Modified Rankin Scale 0–2: good outcome, 3–5: poor outcome, 6: death. HELLP, hemolysis elevated liver enzymes and low platelet count.

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Table 3 Outcome based on consciousness disturbance at the onset of ICH or O–D time in patients who did and did not undergo neurosurgery†

		Neurosurgery		Modified Rankin Scale‡		
				0–2	3–5	6
Consciousness disturbance	None to mild	Yes	17	2 (11.8)	11 (64.7)	4 (23.5)
		No	2	1 (50.0)	0 (64.7)	1 (50.0)
	Moderate to severe	Yes	7	5 (71.4)	2 (28.5)	0 (0)
		No	12	9 (75.0)	1 (8.3)	2 (16.7)
O–D time	<3 h	Yes	17	3 (17.6)	14 (82.4)	0 (0)
		No	7	4 (57.1)	1 (14.3)	2 (28.6)
	≥3 h	Yes	7	2 (28.6)	1 (14.3)	4 (57.1)
		No	5	4 (80.0)	0 (0)	1 (20.0)

†Values are shown as *n* (%). ‡Modified Rankin scale 0–2: good outcome, 3–5: poor outcome, 6: death. ICH, intracerebral hemorrhage; O–D time, interval between onset and diagnosis.

Table 4 Patient characteristics that are associated with mortality in pregnancy-associated ICH

Item	Odds ratio (95%CI)	
	For poor outcome†	For death
Age ≥35 years old	0.8 (0.2–3.4)	2.2 (0.4–11.8)
Pre-eclampsia	2.0 (0.4–9.5)	5.6 (1.0–31.7)
HELLP syndrome	21.5 (1.1–424.4)	40.0 (3.3–483.7)
Moderate to severe disturbed consciousness‡	3.6 (1.7–7.8)	0.8 (0.6–1.1)
O–D time ≥3 h	0.4 (0.1–1.6)	6.1 (1.0–37.5)
Neurosurgery	0.8 (0.2–3.0)	0.4 (0.1–1.9)

Univariate logistic regression analysis was performed. †Poor outcome was defined as a modified Rankin scale score ≥3 (including 6, death). ‡Moderate to severe disturbed consciousness defined as JCS II-III. CI, confidence interval; HELLP, hemolysis elevated liver enzymes and low platelet count; ICH, intracerebral hemorrhage; O–D time, interval between onset and diagnosis.

a neurosurgeon. The outcome was poorer in women who were initially examined in a department of obstetrics. Neurosurgeries were performed in 26 cases, including four with pre-eclampsia, one with HELLP syndrome, seven with AVM, three with Moyamoya disease, and 14 with no pre-existing disorders. In most of these cases, the operation was either removal of the hematoma or drainage from the lateral ventricles. In AVM cases, the operation included removal of the AVM vessels. Treatment at a department of neurosurgery also tended to improve survival in severe cases of ICH based on classification by the consciousness level at the onset of the disease. However, severe disability persisted in survivors. Treatment at a department of neurosurgery also improved survival in cases with an earlier diagnosis, although still resulted in severe disability, but had no effect in those with a delayed diagnosis.

The odds ratios for each risk factor for poor outcome and mortality are shown in Table 4. HELLP syndrome and moderately or severely disturbed consciousness at onset of the disease was significantly associated with a poor outcome (mRS ≥3). Pre-eclampsia, HELLP syndrome and an O–D time >3 h were significantly associated with maternal mortality.

Discussion

There are few data that specifically address pregnancy-associated ICH in Japan and this is the first nationwide study to examine the current status of this condition. The study covered all regions in Japan and more than 70% of facilities responded to our survey, which supports the reliability of the findings. The reported incidence of pregnancy-associated ICH is 3.8 to 18.1 per 100 000 deliveries[3,4,12–16] and the mortality rate is 9–38%,[2–6] both of which are consistent with the results of the current study. The mortality rate of ICH in pregnancy was higher than that in an age-matched

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population of women who were not pregnant, based on vital statistics of the MHLW in Japan, but the difference was not significant. Ronsman *et al.*[17] described a 'healthy pregnant women effect' in a report on maternal death in the United Kingdom,[18] based on the observation that mortality during pregnancy or within 1 year after birth was four to five times lower than mortality in women without a recent pregnancy. Using the 2006 MHLW vital statistics, the mortalities of women with and without pregnancy were 5.1 and 47.7 per 100 000 in 2006. Thus, the 'healthy pregnant women effect' was even more evident in Japan. The finding that mortality from ICH was statistically equal in pregnant and non-pregnant women indicates that this effect does not apply in ICH.

The current study showed some differences in the cause of pregnancy-associated ICH compared with previous reports. We found that more primiparous women had ICH in Japan, in contrast to previous studies,[3,6] showing that the majority of pregnancy-associated ICH occurred in multiparous women. Another difference with these studies was the timing of ICH. In the current study, the antepartum rate of ICH was higher than the postpartum rate, but these rates were similar or higher than postpartum rates in other studies of risk factors for ICH.[3,4,12]

The rates of eclampsia or pre-eclampsia reported in patients with ICH have ranged from 14% to 50%.[3,4,14,15] We found a similar rate of 26.3% in patients with pregnancy-associated ICH. The mortality of ICH with pre-eclampsia followed by HELLP syndrome was higher than that of ICH without pre-eclampsia or HELLP syndrome. Horton *et al.*[19] found that 45% of maternal deaths due to HELLP syndrome were associated with cerebral hemorrhage. A JCS of III-300 was present at the onset of disease in three women with HELLP syndrome. Thus, the current findings confirm that HELLP syndrome complicated by ICH is associated with a poor maternal outcome. We speculate that in addition to hypertension, a decrease in platelet count or coagulation factors and endothelial dysfunction of the cerebral vasculature contribute to the high mortality of ICH with HELLP syndrome. However, as mentioned above, it should be noted that an influence of delayed diagnosis on the outcome of ICH with HELLP syndrome could not be eliminated.

It remains uncertain whether pregnancy increases the risk of rupture in pre-existing AVM.[12,19] In the current study, the incidence of AVM in pregnancy-associated ICH was lower than that of 20–67% found in other reports.[12] However, this was still the second most frequent risk cause and was also associated with a poor morbidity. Thus, AVM is an important risk factor in pregnancy-associated ICH. We also expected that a higher incidence of Moyamoya disease would influence the incidence of pregnancy-associated ICH, which is mainly caused by hemodynamic stress on fragile Moyamoya vessels. We found that 10.5% of cases with pregnancy-associated ICH had Moyamoya disease, which confirms that Moyamoya disease modifies the characteristics of pregnancy-associated ICH in Japan.

Consciousness at the onset of disease was found to be an important factor in the outcome of ICH. This factor did not influence mortality, but did affect morbidity in our study. O–D time was another important factor, with an increased mortality rate associated with a diagnosis made later than 3 h after onset of ICH. However, the functional outcome was also poor even if the diagnosis was made within 3 h after onset. In the current study, the majority of women with pregnancy-associated ICH initially presented to a department of obstetrics. In particular, all patients with pre-eclampsia were initially examined by obstetricians, and a substantial percentage of all the patients with ICH had pre-eclampsia. Pre-eclampsia can be followed by eclampsia, which shows similar clinical features to those in ICH: convulsion, headaches, and disturbed consciousness. Eclampsia is also more common than ICH and greater familiarity with the disease may have caused many physicians to consult with obstetricians for cases with pre-eclampsia. Many maternity centers in Japan do not have CT or MRI facilities and this may be a limitation in an initial examination made by an obstetrician.

One limitation in the current study was that multivariate regression analysis could not be performed because of the small number of subjects. We classified the subgroups of risk factors into two (consciousness level at onset of ICH and O–D time) that significantly influenced the outcome of patients. However, the influence of confounding factors could not be eliminated.

In summary, we found a mortality rate of 18.4% in 38 patients with pregnancy-associated ICH. This rate was higher than our initial expectations and we did not observe a 'healthy pregnant effect' in women with pregnancy-associated ICH. Early diagnosis may prevent maternal death, even in severe cases of ICH, but survivors still had neurological disabilities. For diagnosis of ICH, imaging such as CT or MRI is the most prompt and accurate diagnostic tool. In this decade, regional maternal–fetal care centers have been established in Japan and the perinatal care network has

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become more sophisticated by placing the regional maternal–fetal care center at the core of the network. However, these centers do not always have full-time neurosurgeons or imaging tools for ICH diagnosis. Therefore, another network is required to link maternity centers with departments of neurosurgery that have CT or MRI available at all times. Pregnant women with neurological symptoms could then be transferred to regional facilities that are suitable for management of such patients.

Acknowledgments

This work was supported by the Ministry of Health, Labor and Welfare of Japan, H18-children-general-006. We would like to thank Dr Akiko Kada for statistical support.

Disclosure

None of the authors has a financial or other conflict of interest regarding the contents of this study.

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Original Paper

Neonatology

Neonatology 2014;106:81–86
DOI: 10.1159/000358189

Received: August 14, 2013
Accepted after revision: December 23, 2013
Published online: May 10, 2014

Association of Antenatal Corticosteroids and the Mode of Delivery with the Mortality and Morbidity of Infants Weighing Less than 1,500 g at Birth in Japan

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Key Words

Antenatal corticosteroids · Very low birth weight · Cesarean section · Vaginal delivery

Abstract

Objective: This study aimed to re-evaluate the effectiveness of antenatal corticosteroids (ACS) and to analyze the association between ACS and the mode of delivery in the context of perinatal morbidity and mortality in very-low-birth-weight (VLBW) infants. ***Study Design:*** This retrospective cohort study involved 15,765 VLBW infants born between 2003 and 2008 at less than 34 weeks of gestation and weighing less than 1,500 g at birth. Data were obtained from the Japanese neonatal research network database. Univariate and multivariate logistic regression analyses were performed to evaluate the impact of ACS and mode of delivery on the risk of infant mortality and morbidity. ***Results:*** Administration of ACS was associated with decreases in mortality rate, intraventricular hemorrhage (IVH) and retinopathy of prematurity (ROP), and was not associated with the incidence of respiratory distress syndrome (RDS), periventricular leukomalacia or necrotizing enterocolitis (NEC). When the administration of ACS was analyzed in the context of different modes of delivery, the incidence of IVH and ROP tended to decrease with cesarean section deliveries, whereas the incidence of RDS tended to decrease and the incidence of NEC tended to increase for infants delivered vaginally. The incidence of chronic lung disease tended to increase in association with both delivery methods. ***Conclusions:*** This large cohort study reconfirms that ACS treatment is associated with decreases in infant mortality and severe morbidity. Furthermore, the delivery method may be associated with severe morbidity in VLBW infants exposed to ACS.

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Introduction

A neonatal research network (NRN) database was established in Japan in 2003 with a grant from the Japanese Ministry of Health, Labour and Welfare. Recommendations regarding the use of antenatal corticosteroids (ACS) have been published, and they indicate that all fetuses at

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risk of delivery between 24 and 34 weeks of gestation should be considered as candidates for ACS [1]. Recent studies have confirmed that the use of ACS is associated with decreases in infant mortality and reduced incidence of respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC) and intraventricular hemorrhage (IVH) in preterm infants [2, 3]. Our group has previously reported the differences in morbidity and mortality in very-low-birth-weight (VLBW) infants and the effects of ACS on the survival of these infants at tertiary centers registered with the NRN database in Japan [4, 5].

The optimal delivery method for preterm infants is controversial. Some studies report lower mortalities or morbidities in VLBW infants following deliveries by cesarean section (CS) compared with vaginal delivery (VD) [6–9], whereas other investigators have found no improvements in perinatal outcomes on the basis of the delivery method [10–12].

The present study aimed to re-evaluate the effectiveness of ACS and to analyze the association between ACS and the mode of delivery in the context of perinatal morbidity and mortality in VLBW infants using the large volume of population data available on the NRN database. The results from this study should encourage ACS use in Japan and should help determine the appropriate mode of delivery for VLBW infants.

Materials and Methods

For this retrospective cohort study, patient data were obtained from the Japanese NRN database that contains maternal and neonatal data collected in accordance with common database definitions (http://plaza.umin.ac.jp/nrndata/hyo1.pdf). All government-designated tertiary neonatal units in Japan contribute to this database. The NRN database contains information on the morbidity and mortality of infants weighing less than 1,500 g at birth and born in or admitted to participating hospitals within 28 days of birth. Data on infants who were born alive but died in the delivery room were also included in this study, but data on infants born with congenital anomalies or chromosomal aberrations were excluded. All information about the infants was collected anonymously and independently from the original data. The infants were categorized according to whether or not their mothers had received ACS and whether they were delivered by CS or VD. Data about the administration of full and partial courses of ACS were collected, but data about the timings of ACS administration and the doses given were not available. Central internal review board approval of this study was obtained from Tokyo Women's Medical University, where all data were collected and stored.

Definitions

We studied the effects of ACS on the risk of infant death or the risk of infants being born with RDS, IVH, periventricular leukomalacia (PVL), chronic lung disease (CLD), NEC or retinopathy of prematurity (ROP) when ACS were administered to mothers who were at risk of experiencing preterm births at 22–34 weeks of gestation. Infant death was defined as the death of an infant before discharge and included death in the delivery room. RDS was diagnosed on the basis of clinical and radiographic findings. The IVH grade was determined using cranial echography and the classification system developed by Papile. CLD was defined as a persistent need for supplemental oxygen for the first 28 days after birth or at 36 weeks postmenstrual age. NEC was defined as Bell's stage II or higher. The stage of ROP was determined in accordance with the classification endorsed by the Japanese Ministry of Health, Labour and Welfare, which directly correlates with ICROP (International Classification of ROP). In this study, the presence of ROP was defined as ophthalmoscopic findings consistent with ICROP stages 2, 3, 4, or 5.

Statistical Analysis

To investigate the effects of exposure to ACS, all infants on the Japanese NRN database who died or were born with RDS, IVH, PVL, CLD, NEC or ROP were compared with infants who had not died or did not have these complications. All outcomes were measured at the time of discharge from the neonatal unit. Missing data were excluded from the analyses.

For the first analysis, the demographic characteristics of the mothers treated with ACS were compared with those of the mothers who were not administered ACS, using Student's t test and the Wilcoxon rank-sum test, as appropriate. Univariate and multivariate logistic regression analyses were used to determine the correlations between ACS treatment and the risk of infant death, RDS, IVH, PVL, CLD, NEC or ROP. Maternal age, infant gender, gestational age at delivery (in weeks), birth weight, the presence of twins, intrauterine growth restriction (IUGR) occurrence, delivery by CS, and the occurrence of premature rupture of the membrane (PROM) were included as adjustments in the multivariate model.

For the second analysis, the demographic characteristics of the mothers who delivered their infants by CS were compared with those of the mothers who delivered their infants by VD, using Student's t test and the Wilcoxon rank-sum test, as appropriate. Univariate and multivariate logistic regression analyses were applied to determine correlations between ACS treatment and the risk of infant death, RDS, IVH, PVL, CLD, NEC or ROP in the CS and VD subgroups. Maternal age, infant gender, gestational age at delivery (in weeks), birth weight, the presence of twins, IUGR occurrence, and PROM occurrence were included as adjustments in the multivariate model. We also tested the interaction between the effect of ACS and mode of delivery for all outcomes.

All tests were two-tailed and differences were considered significant for $p < 0.05$. Stata statistical software, release 12 (StataCorp LP, College Station, Tex., USA) was used for all of the statistical analyses.

Results

The study population comprised 15,765 infants born between 2003 and 2008 (fig. 1); of these, 6,400 (40.6%) had been exposed to ACS. Betamethasone is generally used for ACS therapy in Japan. Table 1 shows the demographic and clinical characteristics of the study population categorized

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Table 1. Demographic and baseline clinical characteristics categorized according to ACS exposure

Variable	ACS exposure (n = 6,400)	No ACS exposure (n = 9,365)	p value
Female	48.5	50.1	0.0461
Gestational week	27.9±2.67	28.3±3.15	<0.001
Birth weight, g	994.5±293.6	1,025.6±309.2	<0.001
Birth length, cm	34.9±3.85	35.3±4.05	<0.001
Twin pregnancy	31.6	26.4	<0.001
CS	78.8	74.3	<0.001
PROM	35.4	23.6	<0.001
Mother's age, years	31.1	31.0	0.618
Death	6.1	9.0	<0.001
RDS	56.8	52.6	<0.001
IVH	12.2	14.3	0.001
IVH grade 3 or 4	3.9	5.7	<0.001
CLD	38.6	30.8	<0.001
PVL	3.5	3.7	0.652
NEC	1.6	1.3	0.229
ROP stage >II	51.1	57.8	<0.001
IUGR	33.1	38.6	<0.001

Data are presented as mean ± standard deviation or percentage.

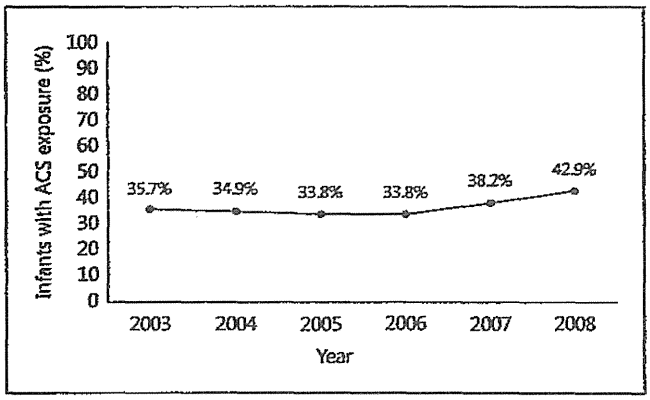

Fig. 1. Frequency of exposure to ACS by year of birth.

Table 2. Association between ACS administration and infant mortality and morbidity

Variable	OR	p value	95% CI
Death	0.632	<0.001	0.54–0.72
RDS	0.99	0.721	0.92–1.06
IVH	0.76	<0.001	0.68–0.84
IVH grade 3 or 4	0.64	<0.001	0.54–0.75
CLD	1.18	<0.001	1.08–1.30
PVL	0.87	0.117	0.73–1.04
NEC	1.15	0.309	0.88–1.52
ROP	0.74	<0.001	0.69–0.79

Data are adjusted for maternal age, infant gender, gestational age, birth weight, the presence of twins, delivery by CS, occurrence of IUGR and PROM.

Table 3. Demographic and baseline clinical characteristics classified according to delivery method

Variable	CS (n = 12,006)	VD (n = 3,759)	p value
Female	50.0	47.6	0.010
Gestational week	28.5±2.86	27.1±3.09	<0.001
Birth weight, g	1,019.3±296.8	992.9±322.5	<0.001
Birth length, cm	35.3±3.89	34.7±4.24	<0.001
PROM	25.1	39.0	<0.001
Mother's age, years	31.3	30.2	<0.001
Steroid therapy	42.0	36.0	<0.001
IUGR	42.6	16.2	<0.001
Death	6.6	11.6	<0.001
RDS	55.9	49.0	<0.001
IVH	11.7	18.9	<0.001
IVH grade 3 or 4	4.3	7.1	<0.001
CLD	33.1	37.3	<0.001
PVL	3.6	3.5	0.674
NEC	1.4	1.6	0.281
ROP stage >II	53.1	61.5	<0.001

Data are presented as mean ± standard deviation or percentage.

according to the use of ACS. Significant differences in some characteristics were observed between the group that received ACS and the group that did not receive ACS (table 1). Results from the univariate and multivariate logistic regression analyses performed to determine the correlations between ACS treatment and the risk of infant mortality, RDS, IVH, PVL, CLD, NEC or ROP are presented in table 2. The use of ACS significantly decreased the odds ratio (OR) of infant mortality (OR 0.63; 95% CI 0.54–0.72; $p < 0.001$). With respect to infant morbidity, ACS was associated with a decreased incidence of IVH (OR 0.76; 95% CI 0.68–0.84; $p < 0.001$) and ROP (OR 0.74; 95% CI 0.69–0.79; $p < 0.001$). We did not note any improvement in the risk of RDS with ACS use (OR 0.99; 95% CI 0.92–1.06; $p = 0.721$). The CLD rate increased with the use of ACS (OR 1.18; 95% CI 1.08–1.30; $p < 0.001$).

Table 3 shows the demographic and baseline characteristics of the study population categorized according to

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Table 4. Association between ACS and infant mortality and morbidity categorized according to the delivery method

Outcome	CS (n = 12,006)			VD (n = 3,759)			p value for interaction
	OR	p value	95% CI	OR	p value	95% CI	
Death	0.68	<0.001	0.57–0.80	0.53	<0.001	0.41–0.70	0.103
RDS	1.09	0.056	1.00–1.18	0.71	<0.001	0.61–0.82	<0.001
IVH	0.65	<0.001	0.58–0.74	1.03	0.770	0.85–1.24	<0.001
IVH grade 3 or 4	0.54	<0.001	0.44–0.66	0.9	0.490	0.68–1.21	0.004
CLD	1.14	0.013	1.03–1.27	1.3	0.006	1.08–1.57	0.188
PVL	0.88	0.192	0.72–1.07	0.8	0.263	0.55–1.18	0.855
NEC	0.96	0.797	0.69–1.32	1.73	0.042	1.02–2.92	0.048
ROP	0.71	<0.001	0.65–0.76	0.9	0.153	0.77–1.04	0.006

Data are adjusted for maternal age, infant gender, gestational age, birth weight, the presence of twins, the occurrence of IUGR and PROM.

delivery method. Significant differences were observed between the CS and VD groups in some characteristics. Compared with the VD group, the CS group showed significantly higher rates of ACS use and IUGR, and higher gestational age at birth. Infant mortality and the incidence of IVH, CLD and ROP were significantly lower in the CS group than in the VD group. The associations between ACS and infant mortality and morbidity in relation to the mode of delivery are shown in table 4. Regardless of the delivery method, administration of ACS was associated with lower infant mortality rates (CS: OR 0.68; 95% CI 0.57–0.80; $p < 0.001$; VD: OR 0.53; 95% CI 0.41–0.70; $p < 0.001$). The interaction term between the mode of delivery and ACS for mortality was not significant ($p = 0.103$). CS delivery was associated with decreased incidence of IVH (OR 0.65; 95% CI 0.58–0.74; $p < 0.001$) and ROP (OR 0.71; 95% CI 0.65–0.76; $p < 0.001$). VD was associated with decreased incidence of RDS (OR 0.71; 95% CI 0.61–0.82; $p < 0.001$) and increased incidence of NEC (OR 1.73; 95% CI 1.02–2.92; $p = 0.042$). The incidence of CLD tended to increase in association with both delivery methods (CS: OR 1.14; 95% CI 1.03–1.27; $p = 0.013$; VD: OR 1.30; 95% CI 1.08–1.57; $p = 0.006$). The interaction term between the mode of delivery and ACS for RDS, IVH, NEC and ROP became significant (table 4).

Discussion

This retrospective study shows that the dissemination rate of ACS was low from 2003 until 2008, and was low compared with data published by the National Institute of Child Health and Human Development Neonatal Research Network [13], which reported that ACS were used for almost 80% of VLBW infants. Socioeconomic factors may influence the administration of ACS, but we have no data on the socioeconomic statuses of the mothers investigated in this study. We have also experienced a high level of apprehension about the adverse effects to mothers of ACS and that this may also limit the administration of ACS, especially when using obstetric tocolytic agents. The administration of betamethasone as ACS therapy is now covered by the Japanese National Medical Insurance Program, so we expect that the use of ACS therapy will soon gain momentum and become more widely used in Japanese tertiary care centers.

In this study, we analyzed data from a larger study population than was previously reported by our group [5], and we demonstrated that the use of ACS significantly reduced infant mortality and the incidence of IVH and ROP. However, sources of potential bias should be considered in the results obtained from this study because the data used included infants who died in the delivery room as well as inborn and outborn patients. Some publications report that the use of ACS is associated with overall reductions in the incidence of neonatal death, RDS, IVH, NEC and the need for respiratory support [2, 3, 5, 14, 15]. In this study, we obtained similar results regarding mortality, IVH and ROP, but the results differed from those previously described regarding RDS, CLD, NEC and PVL. The benefit of ACS in reducing the incidence of RDS has been recognized, and a recent Cochrane review reported the effectiveness of ACS in accelerating fetal lung maturation in women at risk of preterm birth [2]. One reason for the differences in the results between the present study and previously published studies may be

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that the data analyzed in the present study contained information about multiple pregnancies and IUGR; several reports have indicated that ACS are less effective at reducing morbidity and mortality in patients with IUGR [16–18] and in infants from multiple pregnancies [19, 20]. Furthermore, the timings of ACS administration and the doses of ACS administered may have led to differences between the results from our study and those from previous studies [21]. The data analyzed in the current study included data on both completed and partially completed courses of ACS, but data about the timings of ACS administration and the doses of ACS administered were not available. Regarding the increase in the occurrence of CLD in this study, it might be necessary to take into account recent improvements in infant prognoses. These improvements may have influenced the respiratory status of the study population overall. In particular, improvements in neonatal mortality can affect the incidence of CLD, because the affected infants would not have survived if they had been administered conventional treatment. This result also suggests that ACS treatment is more strongly associated with reductions in infant mortality and in the incidence of IVH and ROP, rather than merely reducing the incidence of respiratory problems. We also believe that the use of ACS helps to stabilize the systemic circulation because of improvements in systematic angiogenesis and the maturation of cardiac function in premature infants. Some authors have reported that ACS administration may play a significant role in the maturation of premature hearts [22, 23] and the cerebral vasculature [24]. Further studies are needed to determine the mechanisms underlying the effects of ACS on the systemic circulation in premature infants.

The second analysis undertaken in this study anticipated that the delivery method may be associated with infant outcomes. When pregnancies are associated with complications, including IUGR, severe pregnancy-induced hypertension, clinical chorioamnionitis and preterm deliveries, CS is the likely delivery option. Furthermore, ACS is more likely to be administered in complicated pregnancies when CS deliveries are anticipated. In the current study, the gestational week of delivery was significantly later, and the rates of IUGR and ACS were significantly higher in the CS group compared with the VD group (table 3). These selection biases should be considered in the analysis. Several reports have suggested that CS deliveries are associated with improvements in mortality and morbidity in preterm infants [6–9]. The increased incidence of CLD irrespective of the delivery method may be associated with improvements in neonatal prognoses overall, as discussed previously in the context of the first analysis. With regard to the association between the mode of delivery and the incidence of RDS in our study, this result should be interpreted prudently because the study data included both complete and incomplete courses of ACS, and data concerning the timings of ACS administration and the doses administered were not available, as discussed previously. Although it is unclear how ACS affects outcomes in relation to the mode of delivery, our results suggest that obstetric intervention may influence the morbidity of VLBW infants. As pointed out in a previous report [12], CS delivery may be associated with the improved survival of preterm SGA (small-for-gestational-age) neonates, which suggests that VD is stressful for physiologically vulnerable SGA neonates. The data from this study and previous reports [6–9] suggest that CS delivery may be preferable for VLBW infants, especially when they weigh less than 800–1,300 g or have a gestational age of 24–26 weeks, or if there is IUGR and the fetus is aged less than 30 weeks. However, it remains unclear whether CS delivery is directly associated with reduced stress and trauma compared with VD, and tertiary care centers in Japan have not developed guidelines regarding selection of mode of delivery for VLBW infants. Physicians have to select the optimal delivery method while taking into account the multiple variables associated with a preterm birth, which include fetal status and vulnerability, the mother's condition, the infant's morbidity or mortality and the potential for long-term disabilities, as well as their institution, in an effort to avoid unnecessary CS. Therefore, improvements in the technologies to support very small and premature infants have to be accompanied by changes in treatment practices during premature deliveries. In this study, we reported the importance of optimizing the obstetric management of immature infants, and we hope that future advances in obstetric management will improve infant mortality and morbidity rates. More clinical evidence regarding ACS treatment will be available from the NRN database in Japan in the future.

Acknowledgements

My heartfelt appreciation goes to Kaoru Ishikawa, Yoshihiro Miyamoto, Akira Miyazaki, Akihiko Kai, Hiroshi Ishikawa, Kazutoshi Hayashi and Rintaro Mori, whose comments and suggestions were of inestimable value to my study and to the subsequent publication.

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TECHNICAL NOTE doi: 10.2176/nmc.tn.2013-0359
Neurol Med Chir (Tokyo) 54, 819–823, 2014 Online April 23, 2014

Utility of Intraoperative Fetal Heart Rate Monitoring for Cerebral Arteriovenous Malformation Surgery during Pregnancy

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Abstract

We report two methods of intraoperative fetal heart rate (FHR) monitoring in cases of cerebral arteriovenous malformation surgery during pregnancy. In one case in her third trimester, cardiotocography was used. In another case in her second trimester, ultrasound sonography was used, with a transesophageal echo probe attached to her lower abdomen. Especially, the transesophageal echo probe was useful because of the advantages of being flexible and easy to attach to the mother's lower abdomen comparing with the usual doppler ultrasound probe. In both cases, the surgery was successfully performed and FHR was monitored safely and stably. The use of intraoperative FHR monitoring provides information about the influence of induced maternal hypotension and unexpected bleeding on fetus during surgery. These monitoring techniques would be especially emphasized in cerebrovascular surgery for the safe management of both mother and fetus.

Key words: arteriovenous malformation, pregnancy, fetal heart rate monitoring

Introduction

Intracranial hemorrhage due to rupture of a cerebral arteriovenous malformation (AVM) during pregnancy, although rare, is associated with significant maternal and fetal mortality and morbidity.[1)] Several studies have reported an increased rebleeding rate during the course of pregnancy and it is considered desirable to remove the AVM, if possible.[2,3)] While performing surgery for AVM during pregnancy, monitoring the fetal heart rate (FHR) is important to avoid uterine and placental hypoperfusion and fetal asphyxia. Although many cases of neurosurgery during pregnancy have been reported, the reference of intraoperative FHR monitoring was in few reports of brain tumor.[4,5)] So, we describe the role of intraoperative FHR monitoring in two cases of maternal AVM surgery at different stages of pregnancy, and additionally in cerebrovascular surgery.

Illustrative Cases

I. Case 1

A healthy 27-year-old woman (gravida 1, para 1) presented with sudden right hemiparesis and sensory aphasia at 25th week of gestation. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed an intracerebral hemorrhage in the left parietal lobe (Fig. 1A). Given the mild neurological symptoms, emergency removal of the hematoma was not indicated. Obstetrically, there was no indication for pregnancy termination. At 27th week of gestation, cerebral angiography revealed a left parietal AVM of Spetzler and Martin grade 2 (Fig. 1B). AVM removal was judged necessary on a neurosurgical indication to avoid the risk of rebleeding during pregnancy. As the fetus was not mature enough for extra-uterine life, we performed AVM removal at 30 weeks of gestation with the patient's consent. The patient was operated on under general anesthesia in the supine position with her abdomen slightly turned to left for the prevention of supine hypotensive syndrome. Anesthesia was induced with rocuronium 50 mg i.v., propofol 100 mg i.v., fentanyl 0.2 mg i.v., and maintained with propofol 1–4 mg/kg/h, remifentanyl 0.15–0.30 μg/kg/min, and 0.5–1.2% sevoflurane in oxygen. During the operation, the fetal status was monitored using cardiotocography (CTG) (Fig. 2A). The obstetrics team was prepared for an emergency cesarean section

Received November 27, 2013; Accepted January 13, 2014