CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY
an International Journal

Founding Editor
A. Onnis
Montréal (CND)

Editors-in-Chief
M. Marchetti J.H. Check
Montréal (CND) Camden, NJ (USA)

Assistant Editor
A. Sinopoli
Toronto (CND)

Editorial Board

Audet-Lapointe P., Montréal (Canada)
Axt-Fliedner R., Lübeck (Germany)
Basta A., Krakow (Poland)
Bender H.J., Dusseldorf (Germany)
Bhattacharya N., Calcutta (India)
Bonilla Musoles F., Valencia (Spain)
Cabero-Roura L., Barcelona (Spain)
Charkviani T., Tbilisi (Georgia)
Dexeus S., Barcelona (Spain)
Di Paola G., Buenos Aires (Argentina)
Eskes T.K.A.B., Nijmegen (The Netherlands)
Farghaly S.A., New York (USA)
Friedrich M., Dusseldorf (Germany)
Gomel V., Vancouver (Canada)

Gorins A., Paris (France)
Grella P.V., Padua (Italy)
Holub Z., Kladno (Czech Republic)
Kaplan B., Petach Tikva (Israel)
Markowska J., Poznan (Poland)
Marth C., Innsbruck (Austria)
Meden-Vrtovec H., Ljubljana (Slovenia)
Murta E.F.C., Uberaba (Brazil)
Papadopoulos N., Alexandroupolis (Greece)
Rakar S., Ljubljana (Slovenia)
Rigó J., Budapest (Hungary)
Sciarra J.J., Chicago (USA)
Stelmachow J., Warsaw (Poland)
Varras M.N., Athens (Greece)
Winter R., Graz (Austria)

7847050 CANADA, Inc.

Administrative Office (M. Beaucage):
4900 Côte St-Luc, Apt #212 – Montréal, Québec, H3W 2H3 (Canada)
Tel. +1-514-4893242 – Fax +1-514-4854513 – e-mail: canlux@mgroup-online.com
Website: www.irog.net

Editorial Office (M. Critelli):
Via Martiri della Libertà, 9, 35137 Padua (Italy)
Tel. +39-049-656521 – Fax +39-049-8752018 – e-mail: irog.canada@gmail.com

CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY – CEOG (ISSN 0390-6663) publishes original work, preferably brief reports, in the fields of Gynecology, Obstetrics, Fetal Medicine, Gynecological Endocrinology and related subjects, (Fertility and Sterility, Menopause, Uro-gynecology, Ultrasound in Obstetrics and Gynecology, Sexually Transmitted Diseases, and Reproductive Biological Section). The Journal is covered by ISI Journal Master List, Index Copernicus International, Science Citation Index Expanded, Current Contents - Clinical Medicine, Web of Science, Index Medicus/MEDLINE, EMBASE Excerpta Medica, PubMed, MedSci, Pubget, Genamics JournalSeek, Sciencescape, Unbound Medicine, and PubFacts.com. CEOG is issued bimonthly in one volume per year by 7847050 CANADA Inc., Montréal (Canada). Printed in Italy by “Centro Servizi Editoriali S.r.l.” - Grisignano di Zocco - 36040 Vicenza (Italy).
The prevalence of primary neck and shoulder pain, and its related factors in Japanese postpartum women
K. Koyasu, M. Kinkawa, N. Ueyama, Y. Tanikawa, K. Adachi, H. Matsuo - Kobe, JAPAN
The factors influencing postpartum neck and shoulder pain are evaluated.

The application of high definition flow imaging in fetal hemodynamics
Y.H. He, K. Liu, X.Y. Gu, Y. Zhang, J.C. Han, X.W. Liu, Z.A. Li - Beijing, CHINA
The significance of high definition flow imaging in fetal hemodynamics was assessed.

Leptin increases luteinizing hormone secretion of fasting female rats
T. Dagklis, D. Kouvelas, K. Kallaras, G. Papazisis, S. Petousis, C. Margioula-Siarkou, P. Skepastianos, B.C. Tarlatzis - Thessaloniki, GREECE
Leptin acts directly on the hypophysis, enhancing LH but not FSH, and it is influenced by nutritional state.

Effect of nitric oxide inhalation combined with high-frequency oscillatory ventilation on the prognosis of neonatal severe hypoxemia
W. Kang, H. Sun, Y. Chen, B. Xu, D. Liu, J. Jin, J. Guo, H. Xiong - Zhengzhou, CHINA
Nitric oxide inhalation combined with high-frequency oscillatory ventilation improves the early prognosis of newborns with severe hypoxia.

Troponin I and D-Dimer levels in preeclampsia and eclampsia: prospective study
M. Bozkurt, A.E. Yumru, L. Şahin, S. Salman - Kars, TURKEY
Serum cardiac troponin I and D-Dimer levels were evaluated in preeclamptic, eclamptic, and in normotensive women in the third trimester with the aim to define the diagnostic value.

The effect of maternal polycystic ovary morphology on first-trimester maternal serum biochemical markers of aneuploidy and fetal nuchal translucency thickness
S. Hacivelioglu, A. Uysal, A.N. Cakir Gungor, M. Gencer, D.U. Cakir, E. Cosar - Canakkale, TURKEY
Presence of higher serum free β-hCG levels in maternal polycystic ovary mothers may require correction in risk calculations related to first-trimester aneuploidy screening.

Effect of hypertonic sodium chloride hydroxyethyl starch 40 on ET, TXB2, 6-keto-PGF1α, and ANP of preeclampsia in caesarean section
T. Wang, L.H. Jiang, J.B. Zhu, X.Y. Wei, L. Li, B. Liu - Zhengzhou, CHINA
The effect of hypertonic sodium chloride starch 40 is evaluated as perioperative fluid therapy for preeclamptic patients.

A comparison of the molecular distribution of proangiogenic factors in endometrium of missed abortions and of voluntary first trimester termination cases
T. Özçakır, M.A. Turan, F. Şimşek, C. Atay, S. Vatansever, K. Özbilgin - Manisa, TURKEY
Angiogenesis inhibitor factors have an important impact on missed abortions.
How does early cognitive behavioural therapy reduce postpartum depression?
Cognitive behavioural therapy is a kind of brief psychotherapy useful in preventing postpartum mood disorders.

A different approach to placenta previa accreta: intrauterine gauze compress combined B-Lynch uterine compression suture
M. Kaplanoğlu, D.K. Kaplanoğlu, O. Koyuncu - Adiyaman, TURKEY
The effectiveness of intrauterine gauze compress combined with B-Lynch compression in case of placenta accreta is evaluated.

The outcome and course of pregnancies complicated with fetal neural tube defects
M. Steric, J. Dukanac Stamenkovic, L. Srbinovic, T. Janjic, S. Vrzic Petronijevic, M. Petronijevic, A. Cetkovic - Belgrade, SERBIA
An analysis of the causes, diagnosis, treatment, and the suggested methods to treat infertility.

Changes and clinical significance of peripheral blood helper T lymphocyte and natural killer (NK) cells in unexplained recurrent spontaneous abortion (URSA) patients after abortion and successful pregnancy
The abnormal number of peripheral blood cell subsets and natural killer cells were related with unexplained recurrent spontaneous abortion.

Ruptured ipsilateral ectopic pregnancies: a rare emergency case series
M. Kaplanoğlu, D. Kaplanoğlu, T. Yüce, H. Kiran - Adiyaman, TURKEY
The management of ipsilateral ectopic pregnancy is described.

Cervical ripening agent dinoprostone for delivery induction in late pregnancy mothers: experiences of 685 cases
C. Liang, D. Xu, J. He - Hangzhou, CHINA
Dinoprostone is a very useful and safe drug for delivery induction.

Essure microinsert hysteroscopic tubal sterilization: eight-years follow-up results
M. Sakinci, T. Aksu, O. Kuru, M. Ozekinci, C. Sanhal - Antalya, TURKEY
Essure microinsert is a good alternative to laparoscopic tubal sterilization and it is well-tolerated by patients.

The effect of calcium channel blockers on prevention of preeclampsia in pregnant women with chronic hypertension
N. Jiang, Q. Liu, L. Liu, W.W. Yang, Y. Zeng - Suzhou, CHINA
Calcium channel blockers can improve the outcome of pregnancy in women with chronic hypertension.

Assessment of perioperative, early, and late postoperative complications of the inside-out transobturator tape procedure in the treatment of stress urinary incontinence
M. Bozkurt, A.E. Yumru, S. Salman - Kaps, TURKEY
The complications of urinary incontinence surgery with tension-free vaginal tape are evaluated.

CASE REPORTS

Post-partum management in a patient affected by thrombotic thrombocytopenic purpura: case report and review of literature
A.S. Laganà, V. Sofo, F.M. Salmeri, B. Chiofalo, L. Ciancimino, O. Triolo - Messina, ITALY
The management of a patient affected by thrombotic thrombocytopenic purpura, after cesarean section, is described.

Investigation of short- and long-term effects of ovarian hyperstimulation syndrome on ovarian reserve: an experimental study
S. Pala, R. Atilgan, Z.S. Ozkan, N. Akpolat, N. Ilhan, E. Sapmaz - Elazig, TURKEY
This experimental ovarian hyperstimulation syndrome (OHSS) model revealed increased serum VEGF and endothelin-1 levels and decreased ovarian follicular reserve during short-term of OHSS.
Massive haemorrhage secondary to placenta percreta in the first trimester: a case report
H.A. Hamid, R. Zulida, M. Norhafizah - Serdang, MALAYSIA
A very rare case of placenta percreta in the first trimester, with relevant hemorrhage, is reported.

Late postpartum hemorrhage due to placental and fetal membrane residuals: experience of two cases
A. Luo, P. Mao - Changsha, CHINA
Repeated abortions can increase the incidence of late postpartum hemorrhage as a result of placental residuals.

Intrauterine endometriotic cyst at the site of previous cesarean scar; scar endometriosis
H. İsci, G. Gonenc, A.B. Yigiter, N. Guducu, İ. Dünder - Istanbul, TURKEY
Management of an endometriotic cyst located on a cesarean scar is described.

Successful pregnancy and breastfeeding in a woman with mucopolysaccharidosis type I while receiving laronidase enzyme replacement therapy
M. Castorina, D. Antuzzi, S.M. Richards, G.F. Cox, Y. Xue - Rome, ITALY
Patients considering laronidase treatment during pregnancy should undergo an individualized risk/benefit assessment.

Successful management of discordant alobar holoprosencephaly in monochorionic diamniotic twins with normal karyotype: a case report
J. Zhang, T. Yang, X. Wang, H. Yu - Chengdu, CHINA
Discordant alobar holoprosencephaly occurs in monochorionic diamniotic twins, for which the prenatal diagnosis is very difficult.
The prevalence of primary neck and shoulder pain, and its related factors in Japanese postpartum women

K. Koyasu1, M. Kinkawa2, N. Ueyama3, Y. Tanikawa4, K. Adachi5, H. Matsuo1

1 Kobe University Graduate School of Health Sciences, Kobe; 2 Ube Frontier University, Ube
3 Takarazuka University School of Nursing, Osaka
4 Kansai University of International Studies, Miki; 5 Tokyo Metropolitan University, Tokyo (Japan)

Summary

Purpose: This study investigated the prevalence, location, and severity of neck and shoulder pain (NSP), its disturbance of quality of life (QOL), and the factors related to NSP in Japanese postpartum women. Materials and Methods: The study involved 308 postpartum women who had a medical examination one month after delivery. The questionnaire consisted of the background and details of NSP. Mood states were evaluated using the Profile of Mood States-Brief (POMS-B), Japanese Version. Results: The prevalence of NSP was 73.1%, one-fourth of which occurred after birth. The most common area was the superior part of the trapezius muscles. Prevalence was associated with past history of premenstrual syndrome (PMS), anemia during pregnancy, time per breastfeeding, and the mean POMS-B Fatigue score. Total breastfeeding time a day, the mean POMS-B score for Fatigue, Confusion, Anger-Hostility, and Depression were significantly higher for “worse” after birth than those for “no-change/relief”. The disturbance of daily life due to NSP in postpartum women with past history of PMS and Hiesho were significant higher than that for women without those. Conclusions: The prevalence of NSP in postpartum women was very high. The factors which affect NSP were the mental states, breastfeeding, past history of PMS, and anemia during pregnancy.

Key words: Neck and shoulder pain; Postpartum women; Prevalence; Breastfeeding.

Introduction

Neck and shoulder pain (NSP) is the most common symptom for Japanese women in a Japanese comprehensive survey of living conditions conducted by the Ministry of Health, Labour, and Welfare in 2010 [1]. The prevalence of NSP in Japanese women in their 30s is 14.6%, which is double than that for men, and it rises with age. NSP is often accompanied by a pain and an unpleasant symptom and the pain reduces quality of life (QOL) [2].

NSP is classified into primary and secondary pain. Primary NSP is defined as the absence of a definite disease, while secondary NSP defined as the presence of a definite disease. It has been demonstrated that primary NSP may be associated with multiple factors, including smoking, obesity [3], women, age [4, 5], working conditions [4, 6-8], and psychological distress such as depression or anxiety [9-13].

Postpartum women constantly play many important roles and are in a physically and mentally stressful condition [14, 15], which may be related to the prevalence or severity of NSP. However, there are no reports demonstrating what kind of factors affect the prevalence and severity of NSP, and its QOL disturbance in postpartum women. Thus, the authors conducted the present study to examine the prevalence, location, and severity of NSP and its disturbance of QOL in Japanese postpartum women, and to assess the kind of factors related to NSP.

Materials and Methods

Subjects

This study was conducted at two hospitals and one obstetric clinic in Kobe city, during October 2011 and April 2012. Subjects were postpartum women with both term births and normal newborn babies. The women were excluded if they had an orthopedic disease.

Questionnaires were distributed to the postpartum women who consented to this study at their medical examination one month after delivery, and these were then deposited into a special box beside the reception desk (collection rate: 83.7%). This study was approved by the Ethical Committee at Kobe University Graduate School of Health Sciences.

Self-administered questionnaire

The questionnaire consisted of the subject’s background and details of the NSP. Background included age, height, weight, delivery history, delivery style, period after birth, anemia during pregnancy and after delivery, past history of premenstrual syn-
drome (PMS) and Hiesho, and method, position, frequency, and duration of breastfeeding.

NSP included the present history, onset (before and during pregnancy, after birth), change of NSP after birth (five levels: worse, slightly worse, no change, a little relief, relief), areas for NSP, daily living activities which made the NSP worse, and disturbance of daily life due to NSP (level 0 (none) to 10).

Profile of Mood States-Brief (POMS-B), Japanese version

Mood states were evaluated using the 30-item POMS-B, Japanese version. POM-B is able to measure temporary change in feeling according to condition. The subject answered for each item her mood over the past one week.

The answer to each question was described using a five-point scale as: not at all, a little, moderately, quite a bit or extremely. A score of 0 to 4 was then assigned to each answer. POMS-B consists of the six mood state: “Tension-Anxiety” (T-A), “Depression” (D), “Anger–Hostility” (A-H), “Fatigue” (F), “Vigor” (V), and “Confusion” (C). The scores were the sums of the items for each mood state and these were calculated as a T-score. $T$-score $= 50 + 10 \times (\text{score} - \text{mean}) / \text{SD}$.

The scores of the aforementioned items were compared between two groups: with NSP and without NSP, and worse after birth (worse, slightly worse) and no change/relief after birth (no change, a little relief, relief), for NSP, respectively.

Statistics

The differences in background, present NSP, and POMS-B score between the two groups were tested using the $t$-test and $\chi^2$ test. Statistical significance was expressed as $p$ values at 95% confidence intervals. All statistical analyses were carried out using SPSS for Windows (20J).

Results

Characteristics of subjects

Table 1 shows the subject characteristics. The mean of age was 31.9 ± 5.1 years (range 18–43). The mean of BMI was 20.4 ± 2.7 kg/m². The percentage of primipara and multipara were 48.1% (148/308) and 51.9% (160/308).

The rates of anemia during pregnancy and after birth were 47.4% (146/308) and 28.2% (87/308). Postpartum women with a past history of PMS were 20.5% (63/308) and those with a Hiesho were 64.6% (199/308). Concerning breastfeeding, breastfeeding only, breastfeeding and bottle-feeding, and bottle-feeding only, were 76.9% (237/308), 20.8% (64/308), and 2.3% (7/308), respectively.

NSP in postpartum women

1) Prevalence of NSP:

A total of 225 individuals answered “yes” to the question “Do you presently have NSP?” The prevalence of NSP was 73.1% (225/308)

Figure 1.—Present history of neck and shoulder pain. A) Onset of neck and shoulder pain in postpartum women. B) Change of neck and shoulder pain after birth in postpartum women.
The prevalence of primary neck and shoulder pain, and its related factors in Japanese postpartum women

2) Present history of NSP (onset, change after birth): Onset of NSP was categorized into four categories: before pregnancy, during pregnancy, after birth, and others, and these were 66.2% (149/225), 4.0% (9/225), 24.9% (56/225), and 4.9% (11/225), respectively (Figure 1A). The mean of onset of NSP after birth was 8.1 ± 5.9 days. Change of NSP after birth: “worse” and “slightly worse” were about 44.5% (100/225), “no change” was reported by 40.0% (90/225), “a little relief” by 12.4% (28/225), and “relief” by 3.1% (7/225) (Figure 1B).

3) The areas of NSP (Figure 2):
The most common areas were the superior part of the trapezius muscles. The strongest area was the left posterior cervical region 25.8% (58/225), and the next one was the left superior part of trapezius 25.3% (57/225) (Figure 2B).

4) Daily living activities which made NSP worse:
A total of 92.9% (209/225) of postpartum women with NSP reported that it became worse through daily living activities. The most frequently reported daily living activities were breastfeeding 69.3% (156/225), holding the baby 60.4% (136/225), and using a computer 37.8% (85/225) (multiple answer) (Figure 3).

Factors affecting NSP
1) Factors related to prevalence of NSP:
The prevalence of NSP was associated with past history of PMS ($p = 0.000$) and anemia during pregnancy ($p = 0.032$). Hiesho, anemia after delivery, smoking, age, delivery history, and delivery style did not appear to have a significant influence on NSP. There was a significant difference in the mean duration per breastfeeding (minutes) between the two groups ($p = 0.041$). However method and position did not appear to have a significant influence on the prevalence of NSP.

Regarding the T-scores of POMS-B, the mean Fatigue (F) score for postpartum women with NSP was significantly higher than for those without NSP ($p = 0.003$). The postpartum women with NSP showed higher scores in Tension-Anxiety (T-A), Depression (D), Anger-Hostility (A-H), and Confusion (C), and a lower score for Vigor (V), compared with those without neck and shoulder pain, but there were no significant differences (Table 2).

2) Factors related to change of NSP after birth:
There were no significant differences in anemia during pregnancy, anemia after delivery, past history of PMS, Hiesho, and delivery style between the two groups. In regards to breastfeeding, total time per day was significantly longer in the “worse” group than the “no change/relief” group ($p = 0.018$). The method, position, frequency per day, and breast tension did not appear to have a significant influence on causing the NSP to become worse after birth.
Regarding the T-scores of POMS-B, the mean score for D, A-H, F, and C were significantly higher for “worse” than for “no-change/relief” of NSP ($p = 0.023$, $p = 0.030$, $p = 0.000$, and $p = 0.002$, respectively). This study showed that the T-A and V scores of postpartum women who perceived worse pain did not differ significantly from those who perceived no-change/relief after birth (Table 3).

**Discussion**

This is the first instance to demonstrate that the prevalence of primary NSP in postpartum women was 73.1%, one-fourth of which was after birth-onset, and that the most common area was the superior part of the trapezius muscles, while the strongest area was the left posterior cervical re-
The prevalence of primary neck and shoulder pain, and its related factors in Japanese postpartum women

The factors which affect NSP in postpartum women were mental states, breastfeeding, anemia during pregnancy and past history of PMS. Breastfeeding was the most common daily living activity that caused NSP to become worse. The present research demonstrated that the prevalence of NSP in Japanese postpartum women was 73.1%. In Sweden, it was found to be 29.4% in four to eight weeks after childbirth [16]. Hill et al. [3] reported that 22.3% of participants who were over 18 years of age had pain, aching or stiffness in either shoulders. Hakala et al. [5] demonstrated that pain of the neck and shoulder affected 45% in 18-year-old girls. Therefore, the prevalence of NSP for Japanese postpartum women could be higher than that for postpartum women of other countries or those of other generations.

The most common areas for NSP in postpartum women in our study were the superior part of the trapezium muscles and the posterior region of the neck. This finding coincided with that for nurses reported by Iizuka et al. [11]. However, the rate of NSP in the posterior cervical region for postpartum women was higher than that for nurses. The muscle of the posterior cervical regions were extended by anteflexion posture during breastfeeding. This posture might cause NSP. Actually, the rate of after birth-onset NSP in postpartum women had a high prevalence in our study. Thus, it was suggested that breastfeeding might contribute to the increase in NSP after birth-onset.

Interestingly, NSP in postpartum women appeared outstandingly stronger on the left side compared with the right side. Fujii et al. [17] reported that the strongest areas for neck-shoulder discomfort in employees were the right upper scapula 44.4% and the right neck 29.6%. It was noted that postpartum women put the head of the baby on the side opposite their hand-edness (i.e. the left side for right handed women) for breastfeeding. It was therefore speculated that breastfeeding caused the higher rate for the left side NSP in postpartum women.

In our study, the mean POMS-B F score for postpartum women with NSP was significantly higher for “worse” than that for “no-change/relief” after birth. Several research studies have reported that NSP is related to psychological factors such as depression [10,18], confusion [19], working stress and the extent of the feeling of satisfactions [8,13]. Psychological stress such as anxiety or depression in postpartum women may be more than that for others [20]. It was suggested that psychological states could affect the onset of NSP and cause it to worsen after birth in postpartum women.

In this study, about 70% of postpartum women with NSP answered that breastfeeding made it worse in their daily life activities. In addition, breastfeeding was carried out nine times a day, taking 20 minutes each time, for a total of 190 minutes a day. In particular, the required time per day for breastfeeding was related to the worsening of NSP after birth. Muscles around the neck and the shoulder girdle support the head and the arms, which weigh more than four kg, therefore there is always a load on the muscle around the shoulder girdle from the neck [21]. The posture of postpartum women tends to become unbalanced because of not-held head of the babies, and due to keeping the anteflexion posture for feeding. It seems likely that the after birth worsening of NSP may be related to muscles strain, due to the posture adopted for breastfeeding and the length of time it takes.

There was significant difference in QOL level in the disturbance of daily life for “worse” and “no-change/relief” NSP in postpartum women. The number of involved sites of self-reported musculoskeletal pain was associated with the level of reduction in health-related quality of life (HRQOL) among young adults [2]. Respondents with shoulder pain scored lower on all domains of the SF36 [3]. Thus, NSP after birth led to the reduction in the level of QOL. The disturbance of daily life due to NSP in postpartum women with past history of PMS and Hiesho was significantly higher than those without those. PMS and Hieshou are health of obstacles related to the ovarians steroid hormones cyclist [22,23]. We speculated that the sudden hormone change after birth might contribute to the physiology of NSP.

### Table 4. — Disturbance of daily life due to NSP (n=225).

<table>
<thead>
<tr>
<th>Change of neck and shoulder pain after birth</th>
<th>Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>worse</td>
<td>5.1±2.4</td>
<td>0.001**</td>
</tr>
<tr>
<td>no-change/relief</td>
<td>4.1±2.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heisho</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>4.8±2.2</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>4.1±2.4</td>
<td>0.043*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of PMS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>5.2±2.2</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>4.4±2.3</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

Figure 4. — Disturbance of daily life due to NSP in postpartum women.
Conclusions

This study demonstrated that the prevalence of NSP in postpartum women was very high, and the rate of after birth-onset and worsening after birth were very high. In addition, NSP after birth led to a reduction in the level of QOL of postpartum women. It was revealed that NSP greatly influenced the health of postpartum women. The factors which affect NSP were related to the mental states of postpartum women, time per breastfeeding, anemia during pregnancy, and past history of PMS. Breastfeeding in particular was identified as a factor responsible for causing their NSP to become worse. It was therefore suggested that this was an important factor.

Further studies should involve the collection and analysis of objective data to clarify how these factors influence the mechanism of NSP. It is also necessary to examine care for the prevention and improvement of NSP in postpartum women.

Acknowledgments

The authors are grateful to all of the postpartum women who took part in this study and all those concerned who supported this study.

References


Address reprint requests to:
H. MATSUO, M.D.
Kobe University Graduate School of Health Sciences
7-10-2 Tomogaoka, Suma-ku,
Kobe 654-0142 (Japan)
e-mail: matsuo@tiger.kobe-u.ac.jp
The application of high definition flow imaging in fetal hemodynamics

Y.H. He, K. Liu, X.Y. Gu, Y. Zhang, J.C. Han, X.W. Liu, Z.A. Li
Department of Ultrasound, Anzhen Hospital, Capital Medical University, Beijing (China)

Summary
Objective: This work aims to investigate the application of high definition flow imaging (HD-flow) in fetal hemodynamics, and establish reference range of hemodynamic parameters in fetal with different gestational ages. Materials and Methods: A thousand of normal pregnant women were divided into five groups: 18-22, 23-27, 28-32, 33-37, and 38-40 gestational weeks. Color Doppler flow imaging (CDFI) and HD-flow were adopted to display the heart structure and measure the blood flow velocity. The pulmonary vein display results were scored. The results of HD-flow and CDFI were compared. Results: The catheter peak velocity of fetal mitral, tricuspid, aortic, pulmonary artery, aortic arch, ductal arch, the inferior vena cava, pulmonary vein, and venous catheter increased continuously with the increase of gestational age, showing a linear correlation. HD-flow was superior to CDFI on the display of pulmonary vein in 18-22, 23-27, and 28-32 weeks (p < 0.05), but was not in 33-37 and 38-40 weeks. HD-flow was an accurate positioning method for the pulmonary veins. Conclusion: HD-flow can make accurate evaluation of fetal hemodynamics and the demonstration of low blood flow, such as pulmonary venous, is better than CDFI. Pulmonary veins can be accurately positioned with HD-flow. HD-flow can demonstrate the main blood vessels of the whole fetal circulation and can display the spatial relationship of the blood vessels. It is of important clinical significance in hemodynamic study.

Key words: HD technology; Hemodynamics; Pulmonary vein; Positioning.

Introduction
Structures of heart and great vessels and hemodynamic characteristics in fetus are different from those in newborn. Due to special anatomical structures of placenta, umbilical artery, umbilical vein, venous catheter, foramen ovale, and arterial catheter, the systemic circulation and pulmonary circulation communicate with each other, with regulation of placental blood flow. This leads to intricate and complicated changes in fetal hemodynamics. In addition, due to atelectasis and small body and heart, the blood vessels are small, with low blood flow rate. Therefore, previous ultrasound technologies have defects for evaluation of fetal hemodynamics.

In the past 30 years, with the development of ultrasound, the ability of prenatal diagnosis of fetal congenital heart disease has been improved. Compared with adult echocardiography, fetal echocardiography is more meticulous and comprehensive, in which not only is the observation in detail needed to understand the variability of cardiac anatomy caused by different diseases, but also to assess fetal hemodynamics which are very important due to the blood circulation relationship between fetus and placenta. Recently, many scholars adapt various technologies of echocardiography to evaluate fetal hemodynamics, and to measure all sorts of blood flow parameters via spectral Doppler technology, which is able to describe the trend of normal and abnormal fetal hemodynamics in different gestational age more accurately. Fetal intrauterine growth retardation [1], fetal hypoxia [2], fetal arrhythmia [3], and abnormal changes in fetal hemodynamics of congenital heart disease are assessed in order to suggest clinicians to choose a reasonable mode of production for pregnant women, to strengthen labor monitoring, and guide prenatal intervention and treatment, which can reduced perinatal fetus fetal mortality in the perinatal period greatly. Because of the poor display effect of previous technology for low blood flow, new technology is needed to show low blood flow. The high definition flow imaging technology (herein referred to as HD-flow for short) is a new technology which has appeared recently, which was applied to mammary gland, liver, etc.

Materials and Methods
Patients
A thousand cases of pregnant women were selected from October 2010 to October 2011 who came to the present hospital for fetal echocardiography, aging 24–33 years (average age: 28.1 ± 3.3), and gestational aging 23–32 weeks (average age: 25.3 ± 3.0). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Anzhen Hospital of Capital Medical University. Written informed consent was obtained from all participants. According to different gestational age, they were divided into five groups: 18–22 weeks, 23–27 weeks, 28–32 weeks, 33–37 weeks, and 38–40 weeks.
Pregnant women position
Pregnant women were in supine position. If fetal malposition occurred, left or right position could be used, and when necessary pregnant women were advised to adopt appropriate activities to change the baby’s position.

Measurement of biological indicators
The conventional obstetric conditions were used to conduct the measurement of biparietal diameter, head circumference, abdominal circumference and femur length in order to clarify the overall fetal development. Abdominal cross-cutting was performed to clarify the visceral location.

Segmentation of the fetal heart
According to the segments, fetal heart condition was assessed. According to the guideline of American Society of Echocardiography (ASE) and the International Society of Ultrasound in Obstetrics & Gynecology (ISUOG), scanning sections included four-chamber, five-chamber, left ventricular outflow tract and right outflow tracts, dual-chamber short axis, main artery minor axis, aortic arch, catheter bow, three vessels and trachea, inferior vena cava long axis, etc. If there were abnormal findings, multi-slice, multi-angle continuous scanning were performed.

Data acquisition
Color Doppler imaging and high-resolution flow imaging were simultaneously used in each section above. The measurement included: fetal mitral, tricuspid, aortic, pulmonary artery, aortic arch, ductal arch, the inferior vena cava, venous catheter, and pulmonary vein.

When all of the blood flows above were measured, the angle between the acoustic beam and the blood flow were controlled within 30 degrees. All measurements are performed three times and the averages were taken. All data were analyzed by SPSS17.0 software. Measurement data were expressed as mean ± standard deviation. T-test was used in the comparison between groups. There was a statistically significant difference when \( p < 0.05 \). All images and data were then stored for further analysis.

Results
General information
The peak velocity (mean ± standard deviation) of fetal mitral, tricuspid, aortic, pulmonary artery, aortic arch, ductal arch, the inferior vena cava, venous catheter, and pulmonary vein increased with the increase of gestational age, which was positively associated with gestational age and are shown in Tables 1 and 2.

HD and CDFI rating
The display of HD technology for pulmonary vein was significantly better than CDFI. According to the display number of pulmonary vein, the display of pulmonary vein

---

### Table 1. The peak velocity (cm/s) of fetal mitral valve, tricuspid valve, aortic artery, pulmonary artery, aortic arch, and ductal arch at different gestational weeks.

<table>
<thead>
<tr>
<th>Gestational week</th>
<th>Cases</th>
<th>Aortic</th>
<th>Pulmonary</th>
<th>Aortic</th>
<th>Ductal</th>
<th>Mitral E</th>
<th>Mitral A</th>
<th>Tricuspid E</th>
<th>Tricuspid A</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-32</td>
<td>251</td>
<td>73.96±14.62</td>
<td>69.37±13.13</td>
<td>84.37±17.57</td>
<td>87.07±25.60</td>
<td>33.94±7.01</td>
<td>40.94±7.01</td>
<td>34.58±8.46</td>
<td>50.53±10.35</td>
</tr>
<tr>
<td>33-37</td>
<td>90</td>
<td>78.03±11.12</td>
<td>73.61±34.83</td>
<td>88.74±13.97</td>
<td>92.71±19.31</td>
<td>34.75±7.61</td>
<td>43.75±7.61</td>
<td>35.66±9.07</td>
<td>51.62±9.55</td>
</tr>
<tr>
<td>38-40</td>
<td>12</td>
<td>82.99±14.07</td>
<td>80.28±9.28</td>
<td>91.10±27.44</td>
<td>98.20±22.39</td>
<td>35.59±7.07</td>
<td>47.59±7.07</td>
<td>38.61±9.21</td>
<td>52.67±10.29</td>
</tr>
</tbody>
</table>

### Table 2. The peak velocity (cm/s) of fetal inferior vena cava, venous catheter, and pulmonary vein at different gestational weeks.

<table>
<thead>
<tr>
<th>Gestational week</th>
<th>Cases</th>
<th>Venous catheter</th>
<th>Inferior vena cava</th>
<th>Pulmonary vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-22</td>
<td>138</td>
<td>43.92±13.44</td>
<td>39.48±12.20</td>
<td>22.08±9.40</td>
</tr>
<tr>
<td>38-40</td>
<td>12</td>
<td>61.72±13.15</td>
<td>55.64±11.47</td>
<td>33.45±13.11</td>
</tr>
</tbody>
</table>

### Table 3. The rating of HD and CDFI for pulmonary vein at different gestational weeks.

<table>
<thead>
<tr>
<th>Pulmonary vein rate</th>
<th>18-22w</th>
<th>23-27w</th>
<th>28-32w</th>
<th>33-37w</th>
<th>38-40w</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>CDFI</td>
<td>10</td>
<td>36</td>
<td>108</td>
<td>106</td>
<td>100</td>
</tr>
<tr>
<td>D peak</td>
<td>9</td>
<td>26</td>
<td>20</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>D peak</td>
<td>53</td>
<td>54</td>
<td>25</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>A peak</td>
<td>12</td>
<td>37</td>
<td>35</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>A peak</td>
<td>18</td>
<td>46</td>
<td>46</td>
<td>39</td>
<td>32</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>11</td>
<td>82</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>67</td>
<td>77</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>91</td>
<td>281</td>
<td>281</td>
<td>133</td>
<td>133</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>68</td>
<td>68</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>106</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>1</td>
</tr>
</tbody>
</table>

---
was rated, which was divided into grades 1, 2, 3, and 4. The rate of HD and CDFI for pulmonary vein according different gestational age was shown in Table 3.

### Pulmonary vein rate

The integral situations of pulmonary vein in each group (different gestational age) were tested by the chi-square method (Table 4). Results showed that HD technology was superior to CDFI on the display of pulmonary vein in 18-22 weeks, 23-27 weeks and 28-32 weeks, of which the difference was statistically significant ($p < 0.01$). When pregnant for 33-37 weeks and 38-40 weeks, HD was not superior to CDFI, and there was no statistical significance ($p > 0.05$).

### Discussion

Generally, it is relatively comprehensive to observe fetal hearts when pregnant more than 18 weeks [4], so the earliest elections of research objects in our study were 18 weeks pregnant.

During the measurement of fetal hemodynamics, because standard views are not uniform, measurement methods are not the same, measurement sites are different as well as fetal position, the measurement reproducibility is poor, strictly according to the guideline of American Society of Echocardiography (ASE) and the International Society of Ultrasound in Obstetrics & Gynecology (ISUOG), scanning sections included four-chamber, five-chamber,
left ventricular outflow tract and right ventricular outflow tract, dual-chamber short axis, main artery minor axis, aortic arch, catheter bow, three vessels and trachea, inferior vena cava long axis, etc.

With the changes of gestational age, the fetal structures to hemodynamics varied [5]. Seeking variation is greatly helpful for the diagnosis of heart abnormal in the early stage. Hence, it was necessary to establish the normal range of different gestational age of fetal cardiac hemodynamics. The results in the present research showed that the peak velocity of fetal mitral, tricuspid, aortic, pulmonary artery, aortic arch, ductal arch, the inferior vena cava, venous catheter, and pulmonary vein increased with the increase of gestational age, which was positively associated with gestational age [6]. In the 38th-40th weeks of gestation, the peak blood flow velocity in left and right semilunar valve and atrioventricular valve were slightly decreased, but the reason has not been reported. The authors’ previous studies on anatomical structure found that the foramen ovale and arterial duct become smaller in the 38th-40th weeks of gestation, which may be the cause of hemodynamic change. The diminishing of foramen ovale can cause decreased preload on left ventricle, and the reduced arterial duct diameter will lead to increased afterload on right ventricle. Therefore blood flow velocity is decreased. However this needs to be further confirmed with larger sample studies.

Doppler waveform of the aorta and the pulmonary artery was similar (Figure 1), and aortic blood flow was slightly higher than the pulmonary blood flow. With increasing gestational age, the peak systolic velocity of aorta and pulmonary artery gradually increased. Aortic peak systolic velocity was greater than the pulmonary artery flow velocity, because the pulmonary valve ring is slightly larger, or because after cerebral circulation, the aortic afterload was reduced [7].
The application of high definition flow imaging in fetal hemodynamics

15

The flow spectrum of mitral and tricuspid are bimodal, peak E and peak A, corresponding to early and late diastolic of heart (atrial systole) (Figure 2). The different point is that blood flow of the aorta was visible in systole on mitral spectrum. Peak A is greater than the peak E, which might be related to the high level of myocardial stiffness [8]. The tricuspid peak E velocity was higher than mitral peak E velocity, which was similar to the results confirmed by previous research, the blood flow of tricuspid was greater than that of mitral, and cardiac output of right ventricular was greater than that of left ventricular. These confirmed fetal right heart system was dominant [9].

Aortic arch was similar to ductus arteriosus arch (Figure 3). They are both systolic high-speed flow spectrum and diastolic low-speed flow spectrum. The different point is that aortic arch diastolic peak was close to the front of which the flow spectrum was placed, while the ductus arteriosus arch diastolic peak was close to the back, showing crest-like pattern [10]. This might be related to the muscular structure of the arterial duct wall which has contractibleness. In addition, the systolic blood flow of artery catheter arch was higher than systolic aortic arch [11].

The spectrum on proximal end of inferior vena cava was a three-phase wave (Figure 4). Peak flow rate of the three peaks increased with gestational age, which showed linear correlation [12]. The ratio of reverse blood flow of peak A [13], which was the time integral of atrial systolic divided by the entire time integral of forward flow velocity, reflected the pressure gradient between the right atrium and right ventricle end-diastolic. The ratio of reverse blood flow in inferior vena cava depended on the ventricular compliance and right ventricular end-diastolic pressure [14]. It decreased with increasing gestational age, possibly due to the increased ventricular compliance and the decline in peripheral resistance. The spectrum of venous catheter was bimodal, which was peak S and peak D, while the trough corresponded to peak A. Different from inferior vena cava, venous catheters were forward flow throughout the cardiac cycle. Venous catheter diameter was fine and wall was thick, with sphincter in it, which could play a role as “valve” [15], making the blood flow into the right atrium more quickly and then into the left atrium through the foramen ovale. Three peak flow velocity of the venous catheter increased with gestational age, showing linear correlation. The flow velocity of the pulmonary vein reflected the cyclic changes in left atrial throughout the cardiac cycle, triphasic wave, and peak A showed in trough [16].

The technology of examination for hemodynamics developed from the earliest CDFI to later PD, e-flow, and the emerging HD-flow technology [17].

Due to the small size of fetal heart and lung, spatial position among the pulmonary vein are adjacent to each other, which makes the image of fetal pulmonary system more complicated and the display effect more difficult to guarantee. The emergence of new image technology of hemodynamics, such as HD-flow, has made certain achievements in low blood flow (such as in ovarian and breast tumors) imaging [18, 19]. In consideration of the characteristics of the fetal pulmonary venous system, HD-flow was used in the imaging of fetal pulmonary venous system, expecting to find a more intuitive and accurate method to display the pulmonary vein.

Bidirectional power Doppler technique and two-way PDI coding were used in the HD-flow technology to display the blood flow direction and the density information, which were suitable for the imaging of microvessel [20] such as endometrial blood vessels, ovarian vessels, intrahepatic vessels, and fetal cardiovascular because of the high sensitivity to the imaging of microvessel and reduction of the color overflow. The traditional color Doppler flow imaging (CDFI) had angular dependence, which could not display the blood signal perpendicular to the sound. Common power Doppler (PD) could show the subtle blood flow but could not display directionality [21]. There were overflow artifacts of blood flow in CDFI and PD, which meant blood flow signal overflow out of the vessels. Therefore a part of the two-dimensional gray-scale image was blocked. By contrast, HD-flow combined the advantages of the two previous ones, which could display the directionality without the angular dependence and greatly reduce blood flow overflow artifacts. While displaying the bloodstream, it could clearly show the edge of the two-dimensional blood vessel or tissue [22].

Figure 5. — Four pulmonary veins overall shown by STIC combined with HDF. 1) Thoracic aorta; 2) left inferior pulmonary vein; 3) left superior pulmonary vein; 4) right inferior pulmonary vein; 5) right superior pulmonary vein; 6) right middle lobe pulmonary vein; 7) right upper lobe pulmonary vein.
According to the results of the present research, HD-flow was superior to CDFI on the imaging of pulmonary vein in pregnancies of 18-22 weeks, 23-27 weeks, and 28-32 weeks, with \( p < 0.05 \). In the second and early third trimesters, due to the moderate size, the fetus could move appropriately in utero and form an appropriate fetal position, while the fetal movement was not frequent as in the first trimester, which contributed to the ultrasound probe. Simultaneously, during this period, amniotic fluid is greater, placental maturity is moderate, and skeletal ossification of fetus is not obvious, which did not produce significant acoustic shadows. Under favourable imaging conditions, based on the sensitivity of low blood flow imaging, HD-flow was better than CDFI on the display of low speed pulmonary vein. Moreover, HD-flow was not superior to CDFI with the display of pulmonary vein at 33-37 weeks and 38-40 weeks, \( p > 0.05 \), of which differences were not statistically significant. In the third trimester, fetal position rendered ultrasonic inspection difficult due to the large size. Generally, amniotic fluid gradually decreases and the maternal abdominal wall is thick, which were unfavorable factors for the display of fetal pulmonary vein by ultrasonic technology. Of course, HD-flow technology was also greatly restricted. At present, the accepted screening time of fetal cardiac malformation is 18-24 weeks when HD-flow is superior to CDFI with the display of pulmonary vein during this period. So HD-flow technology is still useful in clinical application when pregnant woman has fetal echocardiography indications at 18-24 weeks’ gestation.

Fetal pulmonary vein positioning method: compared with the adult pulmonary vein positioning method, fetal pulmonary vein positioning was much more difficult, in which the important anatomical structures were selected as mark to position the pulmonary vein by reference to the adult pulmonary vein positioning. Upper right pulmonary vein was close to side of atrial septum, lower right pulmonary vein was close to thoracic aorta side, left pulmonary vein was close to the left atrium side, and lower left pulmonary vein was close to thoracic aorta side. It was easier to be understood and be more accurate with this method to positioning pulmonary vein (Figure 5).

USES bidirectional PD coding is adopted in HD-flow technology, which can show blood flow direction without the angular dependence. It is extremely sensitive to low speed flow due to short pulse. Therefore the technology is suitable for the display of fetal pulmonary vein blood flow condition and is more accurate for pulmonary vein positioning.

**Conclusion**

HD-flow technology, which can accurately evaluate fetal hemodynamics and is superior to CDFI with the image of pulmonary vein, can be applied to accurate positioning for pulmonary vein as well and has important clinical significance in fetal cardiac hemodynamics study.

**References**


Address reprint requests to:
Y.H. HE, M.D.
Department of Ultrasound,
Beijing Anzhen Hospital,
Capital Medical University,
Beijing 100029 (China)
e-mail: yihuahecn@yeah.net
Leptin increases luteinizing hormone secretion of fasting female rats

T. Dagklis¹, D. Kouvelas¹, K. Kallaras¹, G. Papazisis¹, S. Petousis³, C. Margioulas-Siarkou³,
P. Skepastianos¹, B.C. Tarlatzis⁴

¹Department of Physiology and Pharmacology, Aristotle University of Thessaloniki
²3rd Department of Obstetrics and Gynaecology, Aristotle University of Thessaloniki
³Advanced Technological Educational Institute, Thessaloniki
⁴Unit for Human Reproduction, 1st Department of Obstetrics and Gynaecology, Papageorgiou General Hospital, Aristotle University of Thessaloniki, Thessaloniki (Greece)

Introduction

Leptin is a hormone that is mainly produced in fat tissue that influences the reproductive axis [1, 2]. In the infertile, genetically obese ob/ob mice, fertility is restored by leptin administration [3]. Leptin may prevent the effect of fasting on various aspects of reproduction as the onset of puberty [4-9], the length of the menstrual cycle [10, 11], the levels of gonadotropins and gonadal steroids [12], and the pulsatile secretion of the luteinizing hormone (LH) in rodents and primates [13, 14].

The mechanism by which leptin influences the reproductive axis is still unclear. There is evidence for an indirect action of leptin on the gonadotropin-releasing hormone (GnRH) neurons, through intermediate neurons that produce neuropeptides as cocaine- and amphetamine-regulated transcript (CART) peptide [15], galanin-like peptide [16], and melanin-concentrating hormone (MCH) [17]. Leptin administration in the arcuate nucleus of the hypothalamus, the site of action of neuropeptide Y (NPY), increases GnRH pulsatility [15]. There is also evidence for a direct action of leptin on the (GnRH) producing neurons. In vitro, leptin administration stimulates GnRH release from a GnRH-secreting neuronal cell line [18]. In vivo, leptin administration in the medial preoptic area (MPOA) and the median eminence results in an increase of LH release [19]. Leptin may act directly at the level of the anterior hypophysis as well [20]. Leptin receptors have been identified in gonadotropin-producing cells [21] and leptin was found to stimulate LH and FSH release from rat pituitary extracts in vitro [12]. In the rat, GnRH-secreting neurons are concentrated in the MPOA of the hypothalamus. Lesion of the above area eliminates GnRH release [22]. Therefore, from all the above in vitro [18] and in vivo [19] studies, it is evident that leptin increases LH release acting on MPOA and the median eminence, but only in vitro investigations [12, 20, 21] suggest that leptin augments LH release acting also directly on the anterior hypophysis.

Main objective of the present study was to investigate in vivo whether leptin, when administered in MPOA lesioned female rats can act directly on the anterior hypophysis by affecting gonadotropin secretion, since in vivo experiments have the advantage of approaching subject experimentation holistically, as the nature and properties of a chemical or humoral tool cannot be considered independently of the system it is to be tested in.
Materials and Methods

Experiments have been conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) and were approved by the Ethics Committee of the School of Medicine, Aristotle University of Thessaloniki. Attention was paid to minimize pain and discomfort to the animals.

Experimental protocols

Female Wistar rats, weighing 220-240 g, were housed in an environment controlled for temperature, humidity, and light (12:12 h light/dark cycle). Animals had free access to food and water until the beginning of the experiment. Daily vaginal smears were taken to document at least two consecutive four-day oestrus cycles.

The first day of the experiment, rats that were found to be on oestrous were selected. Under ketamine (50 mg/kg) and xylazine (10 mg/kg) anaesthesia, animals were implanted with a jugular vein catheter for frequent blood sampling according to the technique described by Thrivikraman et al. (24). The catheter is a piece of polyethylene tubing ending in a segment of silastic tubing, [15-cm long piece of Clay Adams brand PE-50 tubing (I.D. 0.023 in.3 O.D. 0.038 in., wall 0.011 in.) and a 3.5–4.0 cm long piece of Dow Corning medical grade silastic tubing (I.D. 0.0253 O.D. 0.047 in., wall 0.011 in.) The catheter was filled with heparinised saline (20 units/ml, sterile, prepared by mixing 0.0253 O.D. 0.047 in., wall 0.011 in.)

The rats were divided into eight (A to H) experimental groups. In groups A-D, rats had free access to food and water, while in groups E-H, they were fasted until the end of the experiment. After catheterization, the animals were left to recover in their home cages until the next day.

On the second experimental day, a blood sample (tx)(400 µl) was taken and replaced with an equal volume of saline through the vein catheter. Subsequently, using a stereotaxic device Horsley-Clarke type and under anaesthesia as described above, electrolytic lesion of the MPOA was performed to the rats belonging to groups C, D, G, and H while animals in groups A, B, E, and F received a similar operation with the hypothalamus remaining intact. Stereotaxic coordinates for the electrolytic lesion were taken from the atlas of Paxinos and Watson [25]. Target coordinates of the MPOA were (mm): AP -0.8, posterior to the bregma, 0.5 lateral from the midline, and 8.8 ventral to the scull surface. Following the operation, the animals were left 24 hours to recover in their home cages.

On experimental day 3, blood sampling (400 µl) was performed at 0 min (t0). In groups B, D, F, and H, leptin (one mg/kg i.v.) [26] was administered through the catheter, while the rest of the groups received saline. Blood samples (400 µl each) were subsequently taken at 30, 60, 90, 120, and 180 minutes (t30, t60, t90, t120, and t180, respectively) after leptin administration. During blood sampling, the rats were conscious, freely moving, and had free access to water, but were deprived of food. To the end of the experiment, rats were sacrificed by decapitation. The brain was removed and the localization of the lesion was verified histologically.

Blood sample analysis

Blood samples were centrifuged for 15 min at 1,040 g and the serum was stored at -20°C until assayed. Only samples from animals in which complete bilateral lesion of the medial preoptic area was verified histologically were included in the study.

For the determination of LH and FSH levels, enhanced chemiluminescence was used. Interassay variation was 5.7% and specificity was 99%.

Statistical analysis

For the comparison of hormone levels' fluctuations within each group, the Friedman test was first applied. Differences were considered significant if p-value was less than 0.05. The statistical analysis for differences in the hormone levels between groups for each sampling time was performed using the Kruskal-Wallis test. As the results were significant in all cases (p < 0.0001), further comparison between groups in pairs was performed with the use of Mann-Whitney U test. For all statistical analyses, SPSS 14.0 was used.

Results

In group A (fed, no lesion, no leptin), hormone levels were not significantly different between the different intervals from the time of administration. (p = 0.077 for LH, p = 0.450 for FSH). Similarly, in group B (fed, no lesion, leptin) no significant differences were observed (p = 0.061 for LH, p = 0.384 for FSH), while comparison with group A showed that leptin administration did not affect hormone levels. In group C (fed, lesion, no leptin), LH was not significantly affected (p = 0.054) and FSH was significantly decreased (p = 0.012) after induction of the lesion. In group D (fed, lesion, leptin) the same effect was observed (p = 0.086 for LH, p = 0.003 for FSH) (Figures 1, 2).

In the groups that were subjected to fasting (E-H), a significant reduction in both LH and FSH levels between the first two samples (tx, t0) (p < 0.05 for both LH and FSH) was observed (Figures 1, 2).

In group F (fasted, no lesion, leptin), LH levels were significantly increased after leptin administration. The increase became statistically significant 60 minutes after the infusion (p < 0.001), reached a peak at 90 minutes (p < 0.001) and continued to be significant until the end of the sampling period (p < 0.001 at 120 and 180 minutes post-leptin infusion) (Figure 1). No significant difference was observed regarding FSH levels. (Figure 2).

Comparison between groups F (fasted, no lesion, leptin) and E (fasted, no lesion, no leptin) indicated that LH levels in group F were higher 60 (p < 0.001), 90 (p < 0.001), 120 (p < 0.001), and 180 (p < 0.001) minutes after leptin infusion (Figure 1). No further reduction of hormone levels was observed when groups G (fasted, lesion, no leptin) and E (fasted, no lesion, no leptin) were compared. (Figure 1)

In group H (fasted, lesion, leptin) LH levels increased after leptin administration. The increase became statistically significant 60 minutes after the infusion (p = 0.012), reached a peak at 90 minutes (p = 0.011) and then decreased between 90 and 120 minutes (p = 0.012) and between 120 and 180 minutes (p = 0.011) (Figure 1). FSH levels remained unchanged during the sampling period (Figure 2).

Comparison between groups H (fasted, lesion, leptin) and G (fasted, lesion, no leptin), showed that LH levels were...
Leptin increases luteinizing hormone secretion of fasting female rats

20

significantly higher 60 (p < 0.001), 90 (p < 0.001), 120 (p < 0.001), and 180 minutes after leptin or saline infusion, in group H rats (Figure 1). FSH levels did not present a significant change (Figure 2). Finally, comparison between groups F (fasted, no lesion, leptin) and H (fasted, lesion, leptin) showed that the increase in LH was more prominent in the rats with intact hypothalamus (group F) 60 (p < 0.001), 90 (p < 0.001), 120 (p < 0.001), and 180 (p < 0.001) minutes after leptin administration (Figure 1).

Discussion

The results of the present study are indicative of the fact that in vivo leptin administration may enhance LH secretion in female rats that have been subjected to electrolytic destruction of the MPOA, suggesting a direct action of leptin on the anterior hypophysis. However, no effect of leptin administration on FSH secretion was observed.

The above findings are consistent with previous in vitro experimental data on isolated rat anterior hypophyses, which showed that leptin administration can enhance LH secretion [12]. The same study also revealed a significant effect on FSH release, however to a lesser extend than the one observed on LH. The present findings are in accordance with such a different response to leptin administration. Presumably the concentrations that were used in vitro differed significantly from the ones achieved in vivo in the present study.

In the present study, the evaluation of leptin’s action is not performed in the intact rat brain, as the electrolytic lesion of the hypothalamus disrupts the hypothalamic-hypophyseal axis. The objective, however, was the functional isolation of the hypophysis from the hypothalamic influence in vivo. For this purpose, it was essential to eliminate GnRH secretion, by inducing a lesion of the MPOA where the GnRH excreting neurons are situated. As expected, this lesion eventually will result in hypo-gonadotrophic hypogonadism. However, previous experimental data indicated that 24 hours after the lesion, LH levels were the same as prior to the lesion, while FSH levels were mildly decreased [22]. Furthermore, it was chosen not to use ovariectomised animals so as to limit the number of operations and imitate as much as possible the physiological conditions in the reproductive axis function.

The results of the present study show no effect on the fed animals. In contrast, in the fasted animals, leptin administration induced an increase in LH levels. These findings are consistent with a previous study on the direct effect of leptin on the MPOA (19); the author concluded that there was an increase in GnRH and in LH levels observed only in fasted rats and suggested that the stimulatory influence of leptin on the reproductive hormones is likely to be already maximal at the concentration that corresponds to the physiological plasma levels in normally fed female rats. However, other studies have reported that leptin administration induces GnRH release from hypothalamic explants of nor-

Figure 1. — Effects of leptin (one mg/kg, i.v.) or vehicle (saline) administration on plasma mean values of LH (ng/ml) levels. (n = 8 rats/group, **p < 0.01, *p < 0.05).

Figure 2. — Effects of leptin (one mg/kg, i.v.) or vehicle (saline) administration on plasma mean values of FSH (ng/ml) levels. (n = eight rats/group, **p < 0.01, *p < 0.05).
mally fed rats [15, 27]. This discrepancy might have been due to the differences between in vivo and in vitro experimental conditions [19].

In the group with intact hypothalamus that received leptin, the effect on LH was more prominent than that observed in the lesioned rats. The above finding suggests that leptin acts both on the hypothalamus and the hypophysis and that in order to exercise its maximal effect it is necessary for the hypothalamus to be intact. In conclusion, the findings of the present study suggest that leptin, in vivo, acts at the level of the hypophysis to mediate leptin-induced luteinizing hormone-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms". Endocrinology, 2000, 141, 1646.


Effect of nitric oxide inhalation combined with high-frequency oscillatory ventilation on the prognosis of neonatal severe hypoxemia

W. Kang, H. Sun, Y. Chen, B. Xu, D. Liu, J. Jin, J. Guo, H. Xiong

Neonatal Intensive Care Unit, Zhengzhou Children’s Hospital, Zhengzhou (China)

Summary

Objective: The current study aimed to analyze the short-term and long-term curative effects of nitric oxide (NO) inhalation combined with high-frequency oscillatory ventilation (HFOV) on neonatal severe hypoxemia. Materials and Methods: A total of 98 neonates meeting the inclusion criteria were retrospectively analyzed. The control group comprised of 48 neonates and the NO inhalation group consisted of 50 neonates. In the control group, conventional mechanical ventilation was replaced by HFOV. In the experimental group, NO inhalation combined with HFOV was performed. The death rates within 28 days, mechanical ventilation and oxygen therapy time, and complications in both groups were observed. The survivors in both groups were followed up for 18 months for neural development evaluation. Results: The treatment group showed a significantly lower death rate and noticeably shorter mechanical ventilation and oxygen therapy time than the control group (8% vs. 22.9% with \( t = 4.20 \) and \( p < 0.05 \); \( 5.84 \pm 3.36 \) days vs. \( 8.05 \pm 5.48 \) days with \( t = 2.42 \) and \( p < 0.05 \); and \( 8.02 \pm 4.31 \) days vs. \( 12.45 \pm 14.14 \) days with \( t = 4.63 \) and \( p < 0.001 \)). They did not show significant differences with regards to the complications and the incidences of cerebral palsy, hearing and visual impairments, and severe nervous damage (\( p > 0.05 \)). Conclusion: NO inhalation combined with HFOV significantly decreases the death rate of neonates with severe hypoxemia and reduces their mechanical ventilation and oxygen therapy time. It does not increase early adverse effects or affect long-term neurodevelopment.

Key words: prognosis; inhaled nitric oxide; high-frequency oscillatory ventilation; neonates.
Gradually downregulated. When FiO2 was ≤ 0.5, the concentration of NO was downregulated to five ppm. If SpO2 did not improve, the concentration of methemoglobin was checked. SpO2 ≥ 90% was maintained and FiO2 was ≤ 0.3 and that of NO2 < two ppm were carefully monitored. Nasal cannula was used to deliver NO gas. NO gas for treatment use was subpackaged in special aluminum alloy cylinders with a NO concentration of 1,000 ppm and a NO2 concentration ≤ ten ppm. It was introduced into the gas supply pipe of a breathing apparatus via a special mass flow controller. Sampling was performed proximal to the Y-shaped site of the pipe for monitoring the concentrations of NO and NO2 using an electrochemical NO/NO2 concentration detector. The original, also the highest, concentration was replaced by HFOV after enrollment. Fifty neonates satisfying the inclusion criteria between January 2008 and December 2009 comprised control group. Conventional mechanical ventilation was replaced by HFOV after recruitment. Fifty neonates satisfying the inclusion criteria between January 2010 and October 2011 comprised treatment group. After recruitment, they underwent HFOV combined with NO inhalation. Both groups were diagnosed and treated routinely according to the methods for primary diseases.

Treatment methods

NO gas for treatment use was subpackaged in special aluminum alloy cylinders with a NO concentration of 1,000 ppm and a NO2 concentration ≤ ten ppm. It was introduced into the gas supply pipe of a breathing apparatus via a special mass flow controller. Sampling was performed proximal to the Y-shaped site of the pipe for monitoring the concentrations of NO and NO2 using an electrochemical NO/NO2 concentration detector. The original, also the highest, concentration of NO was 20 ppm [2]. SpO2 ≥ 90% was maintained and FiO2 was gradually downregulated. When FiO2 was ≤ 0.5, the concentration of NO was downregulated to five ppm. If SpO2 did not improve, the original concentration of NO was maintained. When NO was five ppm and SpO2 was ≥ 90%, the concentration of NO was downregulated every six to 12 hours with one ppm per regulation. Normally, NO was withdrawn after 48-72 hours of inhalation [7].

Early observation indices

Cranial ultrasounds were performed before treatment and at three days after NO inhalation, and blood gas analyses were performed before treatment and four, 24, 48, and 72 hours after treatment, respectively. In the NO group, the concentration of methemoglobin < 3% and that of NO2 < two ppm were carefully monitored. Mechanical ventilation time, oxygen therapy time, the incidences of frequent hemorrhoid (pneumatothorax and pulmonary interstitial emphysema), pulmonary hemorrhage, necrotizing enterocolitis (NEC) and severe intracranial hemorrhage (IVH), and the fatality rates within 28 days in the two groups were recorded.

Follow-ups

Both groups were regularly followed up after discharge. Intervention treatment was given according to developmental conditions. The incidences of cerebral palsy (CP), hearing disorders, visual disorders, and severe CP, MDI < 70, PDI < 70, visual disorder, and hearing disorder that necessitated audio helpers [13].

Statistical analysis

Data were analyzed using SPSS 18.0 software. Measurement data were presented as means ± deviation of means (x) and t-tested. For enumeration data, chi-square, corrected chi-square, or exact probability tests were performed. A p < 0.05 was considered statistically significant.

Results

General data

A total of 98 neonates were recruited in this study, including 50 in the treatment group and 48 in the control group. The general data of the two groups were summarized in Table 1. No significant difference with regard to maternal educational levels was observed between the groups (p > 0.05).

Early outcomes

In the treatment group, 46 patients improved or healed and four died with a death rate of 8%. In the control group, 37 improved or healed and 11 died with a death rate of 22.9%. The two groups showed a significant difference in the death rates (t = 4.20, p < 0.05). Other early indices were also compared. With the exclusion of the deaths, all the patients in both groups were discharged. The mechanical ventilation time, oxygen therapy time, and the incidences of

### Table 1. — General data of both groups.

<table>
<thead>
<tr>
<th>Index</th>
<th>Treatment group (50)</th>
<th>Control group (48)</th>
<th>t/x 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (w)</td>
<td>38.3 ± 1.6</td>
<td>38.8 ± 1.8</td>
<td>1.45</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3320 ± 593</td>
<td>3395 ± 602</td>
<td>0.62</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Male</td>
<td>37 (74%)</td>
<td>36 (75%)</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>CMV time of origin (h)</td>
<td>7.2 ± 4.5</td>
<td>8.1 ± 5.2</td>
<td>0.91</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Primary disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic PPHN</td>
<td>8</td>
<td>7</td>
<td>0.04</td>
<td>0.85</td>
</tr>
<tr>
<td>Severe asphyxia</td>
<td>7</td>
<td>9</td>
<td>0.40</td>
<td>0.52</td>
</tr>
<tr>
<td>III–IV RDS</td>
<td>17</td>
<td>15</td>
<td>0.08</td>
<td>0.77</td>
</tr>
<tr>
<td>MAS</td>
<td>11</td>
<td>12</td>
<td>0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4</td>
<td>5</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>1</td>
<td>0.96</td>
<td>0.33</td>
</tr>
<tr>
<td>PS application</td>
<td>19</td>
<td>17</td>
<td>0.07</td>
<td>0.79</td>
</tr>
</tbody>
</table>

### Table 2. — Early indices of both groups.

<table>
<thead>
<tr>
<th>Index</th>
<th>Treatment group (46)</th>
<th>Control group (37)</th>
<th>t/x 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical ventilation duration (d)</td>
<td>5.84 ± 3.36</td>
<td>8.05 ± 5.48</td>
<td>2.42</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Oxygen therapy duration (d)</td>
<td>8.02 ± 4.31</td>
<td>12.45 ± 5.14</td>
<td>4.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Frequent hemorrhoid</td>
<td>5</td>
<td>7</td>
<td>1.07</td>
<td>0.29</td>
</tr>
<tr>
<td>Pulmonary hemorrhage</td>
<td>4</td>
<td>6</td>
<td>1.09</td>
<td>0.29</td>
</tr>
<tr>
<td>NEC</td>
<td>3</td>
<td>3</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Severe IVH</td>
<td>2</td>
<td>4</td>
<td>1.28</td>
<td>0.26</td>
</tr>
</tbody>
</table>

### Table 3. — Follow-up outcomes of both groups at 18 months.

<table>
<thead>
<tr>
<th>Index</th>
<th>Treatment group (39)</th>
<th>Control group (32)</th>
<th>t/x 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate and severe CP-n (%)</td>
<td>9 (23.1)</td>
<td>10 (31.2)</td>
<td>0.60</td>
<td>0.44</td>
</tr>
<tr>
<td>Hearing disorders-n (%)</td>
<td>3 (7.61)</td>
<td>2 (6.25)</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td>Visual disorders-n (%)</td>
<td>4 (10.26)</td>
<td>5 (15.6)</td>
<td>2.06</td>
<td>0.15</td>
</tr>
<tr>
<td>PDI-n (%)</td>
<td>90.7 ± 15.1</td>
<td>92.2 ± 14.8</td>
<td>0.42</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MDI-n (%)</td>
<td>80.4 ± 19.3</td>
<td>83.9 ± 18.5</td>
<td>0.77</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>NDI-n (%)</td>
<td>13 (33.3)</td>
<td>10 (31.2)</td>
<td>0.40</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Children’s Hospital. Written informed consents were obtained from the guardians of all the participants.

Retrospective analysis was performed. A total of 48 neonates satisfying the inclusion criteria between January 2008 and December 2009 comprised control group. Conventional mechanical ventilation was replaced by HFOV after enrollment. Fifty neonates satisfying the inclusion criteria between January 2010 and October 2011 comprised treatment group. After recruitment, they underwent HFOV combined with NO inhalation. Both groups were diagnosed and treated routinely according to the methods for primary diseases.

**Treatment methods**

NO gas for treatment use was subpackaged in special aluminum alloy cylinders with a NO concentration of 1,000 ppm and a NO2 concentration ≤ ten ppm. It was introduced into the gas supply pipe of a breathing apparatus via a special mass flow controller. Sampling was performed proximal to the Y-shaped site of the pipe for monitoring the concentrations of NO and NO2 using an electrochemical NO/NO2 concentration detector. The original, also the highest, concentration of NO was 20 ppm [2]. SpO2 ≥ 90% was maintained and FiO2 was gradually downregulated. When FiO2 was ≤ 0.5, the concentration of NO was downregulated to five ppm. If SpO2 did not improve, the original concentration of NO was maintained. When NO was five ppm and SpO2 was ≥ 90%, the concentration of NO was downregulated every six to 12 hours with one ppm per regulation. Normally, NO was withdrawn after 48-72 hours of inhalation [7].

**Early observation indices**

Cranial ultrasounds were performed before treatment and at three days after NO inhalation, and blood gas analyses were performed before treatment and four, 24, 48, and 72 hours after treatment, respectively. In the NO group, the concentration of methemoglobin < 3% and that of NO2 < two ppm were carefully monitored. Mechanical ventilation time, oxygen therapy time, the incidences of frequent hemorrhoid (pneumatothorax and pulmonary interstitial emphysema), pulmonary hemorrhage, necrotizing enterocolitis (NEC) and severe intracranial hemorrhage (IVH), and the fatality rates within 28 days in the two groups were recorded.

**Follow-ups**

Both groups were regularly followed up after discharge. Intervention treatment was given according to developmental conditions. The incidences of cerebral palsy (CP), hearing disorders, visual disorders, and severe CP, MDI < 70, PDI < 70, visual disorder, and hearing disorder that necessitated audio helpers [13].

**Statistical analysis**

Data were analyzed using SPSS 18.0 software. Measurement data were presented as means ± deviation of means (x) and t-tested. For enumeration data, chi-square, corrected chi-square, or exact probability tests were performed. A p < 0.05 was considered statistically significant.

**Results**

**General data**

A total of 98 neonates were recruited in this study, including 50 in the treatment group and 48 in the control group. The general data of the two groups were summarized in Table 1. No significant difference with regard to maternal educational levels was observed between the groups (p > 0.05).

**Early outcomes**

In the treatment group, 46 patients improved or healed and four died with a death rate of 8%. In the control group, 37 improved or healed and 11 died with a death rate of 22.9%. The two groups showed a significant difference in the death rates (t = 4.20, p < 0.05). Other early indices were also compared. With the exclusion of the deaths, all the patients in both groups were discharged. The mechanical ventilation time, oxygen therapy time, and the incidences of...
frequent hemorrhoid, pulmonary hemorrhage, NEC, and IVH were compared between the groups (Table 2).

**Follow-up outcomes**

In the treatment group, 40 of the 46 discharged patients completed the 18 months of follow-ups and six were lost. One patient died after discharge. In the control group, 32 of the 37 discharged patients completed the 18 months of follow-ups, and five were lost. No death occurred. The survivals of the patients completing follow-ups are summarized in Table 3.

**Discussion**

Neonatal severe hypoxemia is a neonatal critical disease which is primarily manifested by pulmonary hypertension concomitant with severe asphyxia, meconium aspiration syndrome, severe respiratory distress syndrome, and severe pneumonia. Pulmonary hypertension has an incidence of 1/500 and an approximate mortality rate of 10–50%; even among the survivors, 7–20% suffers from various degrees of sequelae [9]. Although conventional mechanical ventilation has saved numerous neonates, which can achieve a survival rate up to 70% in China [15], it requires very strict ventilation conditions for breathing machines, and tends to cause volemic and biological lung injury, leading to a series of clinical complications and pulmonary and cerebral development disorders [16, 17]. Moreover, pulmonary venous vasodilator drugs fail to decrease pulmonary vascular resistance and often cause a decrease in systemic circulation pressure, consequently affecting overall treatment effectiveness [17].

NO is subject to an endothelium-derived relaxing factor. Its inhalation selectively acts on small resistance vessels in the lungs to relax vascular smooth muscle. This effect dilates vessels, reduces pulmonary vascular resistance and arterial pressure, increases the blood flow volume of the lungs, rapidly improves the pulmonary ventilation-perfusion ratio, and increases blood oxygen concentration [10, 11]. NO has a half-life period of one to five seconds. Inhaled NO during this period binds with hemoglobin immediately after entering circulation. After renal metabolism, it is excreted in the forms of nitrate and nitrite (NO2- / NO3-) with urine, without influencing peripheral vascular tension and resulting in noticeable side effects [1, 2]. However, the vasodilatation effect of inhaled NO requires sufficient lung inflation to guarantee the gas to reach high-resistance pulmonary arteries. Or conversely, unsatisfactory lung inflation and insufficient pulmonary alveolar recruitment may affect the curative effect of inhaled NO, which is the most common reason of NO treatment failure as well [17]. HFOV rapidly improves ventilation by resorting to a very high frequency and an extremely small tidal volume (close to or lower than that in the anatomical dead space). It prevents pulmonary injury caused by conventional mechanical ventilation. Furthermore, the oscillation frequency of the high-frequency breathing machine is in accordance with the resonance frequency of the human lungs. Under this condition, the resistance of small airways drops to the minimum, thereby facilitating gas to move in and out of pulmonary alveoli. In addition, the micro pressure produced by the resonance of the pulmonary alveoli themselves allows alveolar gas to generate movements, which benefits the diffusion and exchange of the gas. The high-efficiency alveolar recruitment effect of HFOV endows HFOV in combination of NO inhalation with a more excellent effect than NO inhalation combined with conventional mechanical ventilation [17]. Compared with pressure/controlled ventilation combined with NO inhalation, HFOV combined with NO inhalation markedly reduces FiO2 and increases the PO2/FiO2 ratio eight hours after treatment [18]. NO inhalation combined with conventional mechanical ventilation has no effect on the death rate; compared with this method, its combination with HFOV shows a significant difference in the death rate (1/8 vs. 5/16) [15]. This finding was also evidenced by this study: NO inhalation combined with HFOV significantly reduced deaths, as well as mechanical ventilation and oxygen therapy time.

The side effects caused by NO inhalation are primarily reflected by its influence on the generation of NO2 and methemoglobin and the agglomeration of platelets as well as the increase in the risk of hemorrhage [1, 2, 13]. NO at a concentration of 20 ppm to 40 ppm is safe and does not lead to noticeable side effects [13, 17]. In this study, the cases were strictly selected and then closely monitored during treatment. The given inhaled NO concentration was controlled no higher than 20 ppm. The results did not show significant difference in terms of the incidence rates of frequent hemorrhoid, pulmonary hemorrhage, NEC, and severe IVH between the treatment and control groups, which is consistent with that reported by other scholars [1, 2, 17]. This finding proved that 20 ppm NO inhalation is safe.

Recent studies have found that the vasodilatation effect of inhaled NO is not limited to the lungs [19]. Animal experiments have discovered that it also dilates the arteries in the cerebral ischemic regions, whereby it increases local blood flow supply and improves reperfusion injury and neural prognosis after ischemia [19-22], rather than to affect normal cerebral blood flow [19]. It achieves curative effect on adult cerebral stroke [22]. However, whether it has an effect on neonatal neural development remains uncertain [2, 13, 14, 23]. Konduri *et al.* conducted a multi-centered study in which 234 neonates with severe hypoxemia were followed up for 18-24 months and found that compared with the control group, the NO inhalation group does not show significant differences in the incidences of neural development disability (25% vs. 27%) and hearing impairment (24% vs. 23%) and the MDI but a noticeably higher PDI [13]. However, this finding is in disagreement with that reported in another multi-centered, randomized, undoubled-blind control study by Field *et al.*: no statistical differences with regard to the death rate, growth development, movement disorders, hearing, and visual acuity of one-year-old neonates are observed between the treatment and control groups [14]. This study is single-
centered and in it, prognoses were evaluated by child health care practitioners that did not participate in early treatment. The results did not show significant differences in CP, hearing and visual impairments, and severe nerve damage between the treatment and control groups (p > 0.05). Although the control group had slightly higher PDI and MDI than the treatment group, the differences were not significant. These findings indicate that NO inhalation does not affect the neural development of neonates with severe hypoxemia at 18 months; it has no cerebral protective effect, either. They are consistent with those concluded by Field et al. but differ somewhat from those obtained by animal experiments [14, 19-22]. The underlying reasons may be as follows. First, most models supporting the cerebral injury-alleviating effect of NO were adult animal cerebral ischemia models [19-22]. The conclusions drawn in these studies may not be applicable to developing brains. Therefore, animal models corresponding to neonatal cerebral development levels should be chosen in future studies. Second, the sample sizes were still small.

This study has some limitations. First, it was single-centered. To obtain as many as possible patients’ data, retrospective analyses had to be performed. Second, the case number for long-term prognostic evaluation was small, which might influence the result judgment of this study. To overcome these limitations, multi-centered, randomized studies should be conducted and more cases should be observed to evaluate the effect of NO inhalation on long-term neonatal prognosis in the future.

To draw a conclusion, NO inhalation combined with HFOV noticeably improves the early prognosis of neonates’, reduces their death rate, and shortens their mechanical ventilation and oxygen therapy time without the support of the ECMO technique. It does not increase early complications or affect neonatal long-term neural development. Therefore, it is worth extending to clinical practice.

References


Address reprint requests to: W. KANG, M.D.
Neonatal Intensive Care Unit (NICU), Zhengzhou Children’s Hospital, Zhengzhou 450053 (China)
e-mail: wqhqcn@163.com
Troponin I and D-Dimer levels in preeclampsia and eclampsia: prospective study

M. Bozkurt¹, A.E. Yumru², L. Şahin³, S. Salman²

¹ Kafkas University School of Medicine, Department of Obstetrics and Gynecology, Kars
² Taksim Education and Research Hospital, Department of Obstetrics and Gynecology, Istanbul; ³ Park Hospital IVF Center, Malatya (Turkey)

Summary

Objective: The aim of this study was to evaluate serum cardiac troponin I and D-Dimer (D-Di) levels in preeclampsia (PE), eclampsia (E), and normotensive healthy pregnant women in third trimester in order to define their diagnostic value. Materials and Methods: The study group consisted of 42 preeclamptic patients and 16 eclamptic patient; 108 healthy normotensive pregnant women in third trimester who were chosen from outpatients clinic and examined regularly used as a control group. Serum cardiac troponin I and D-Di levels were measured using an immunoassay. Results: The average levels of troponin I were 0.0134 ± 0.0091, 0.017 ± 0.0085, 0.180 ± 0.136 in control group, preeclamptic, and eclamptic patients, respectively. The levels of troponin I and D-Di were statistically higher than the normotensive and preeclamptic group (p = 0.016, p = 0.014). There were no differences in terms of troponin I level between preeclamptic group and normotensive pregnant women in third trimester (p = 0.089). The average D-Di levels were 634 ± 228 ng/ml, 1426 ± 430 ng/ml, 2067 ± 580 ng/ml in control group, preeclamptic, and eclamptic patients, respectively. The levels of D-Di in preeclamptic and eclamptic patients were found significantly higher than the control groups (p = 0.034, p = 0.020). Conclusion: Serum troponin I levels increased in eclamptic patient because of myocardial damage. An increased level of troponin was not detected in preeclamptic patients. However; D-Di level increased in preeclamptic and eclamptic patients.

Key words: Preeclampsia; Eclampsia; D-Dimer; Troponin I.

Introduction

Preeclampsia (PE) is one of the most serious pregnancy complication. The worldwide prevalence of PE ranges from 3% to 8%, and affecting a total of 8.5 million women worldwide. PE is responsible for about 18% of maternal deaths and up to 40% of fetal mortality. Currently, there is no safe and effective therapy for PE. Also there is no reliable tool for early diagnosis or prediction of PE [1].

In the Western world, the reported incidence of eclampsia ranges from one in 2,000 to one in 3,448 pregnancies [2, 3].

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), PE can be defined as de novo hypertension with proteinuria occurring after 20 weeks of pregnancy. Hypertension is defined as a systolic blood pressure ≥ 140 and/or a diastolic blood pressure ≥ 90 mm Hg measured at two times with at least four-hours interval. Proteinuria is defined as urine containing ≥ 300 mg protein per day [4]. Proteinuria is a questionable marker for PE because its predictive value is low and it does not correlate with severity of the disease.

Eclampsia (E) is defined as the development of convulsions and/or unexplained coma during pregnancy or postpartum in patients with signs and symptoms of PE.

Many studies focused on investigating coagulation markers, fibrinolytic activation, and endothelial injury in PE and E. They concluded that this disorder is characterized by a maternal hypercoagulable state with intravascular coagulation, microthrombosis in several organs, and impairment of the uteroplacental circulation [5-7]. PE is associated with the deposition of fibrin in microvasculature which results in placental perfusion insufficiency, intrauterine fetal growth retardation, and dysfunction of some maternal organs. In the early stages of fibrin clot formation, activated thrombin cleaves fibrinogen is a soluble plasma protein. Molecular polymerization is observed due to the formation of soluble fibrin, which is subsequently stabilized by covalent cross-linking with factor XIII producing an insoluble fibrin matrix. Degradation is immediately initiated by plasmin, resulting in a variety of relatively stable dimeric fragments or fibrin degradation products. D-dimer (D-Di) that is the smallest fragment, is resistant to plasmin degradation. Therefore, D-Di specifically reflects both fibrin polymerization and breakdown [8]. Plasma D-Di is a well established clinical laboratory marker of this process in vivo.

There is a good evidence to suggest that hypertensive disease in pregnancy which superimposed upon the maternal...
cardiovascular adaptations required in normal pregnancy, has implications for myocardial function in terms of left ventricular mechanics, ultrastructure of intra-myocardial vessels, and cardiac myocyte damage. Troponin I is a constituent of the troponin complex which regulates the interaction of actin and myosin in striated muscle. Cardiac troponin I contains an immunologically distinct N-terminus amino acid chain, not expressed in skeletal isoforms [9]. Cardiac troponin I is released into the circulation in response to myocardial injury and has been shown to be one of the most sensitive and specific markers of myocardial damage both in ischemic and non-ischemic condition [10].

In spite of many studies in the literature, no reliable marker for the diagnosis of PE and patients who will have eclampsia has been found. The reason of the search of a specific marker is that reliability of blood pressure and proteinuria tests used in the diagnosis of PE are lacking. One of the main reason that reduces the reliability of the test is that blood pressure measurement can be influenced by body position, physical exercise, and emotional states such as stress and anxiety. Urine dips used in proteinuria tests reduce the reliability of the test because vaginal infection, extremely alkaline urine, contamination with quarternary ammonium, and chlorhexidine can cause false positive results. Although 24 hours urine test provides more reliable results it takes time to collect urine.

The purpose of this study was to investigate serum cardiac troponin I and D-Di in normal pregnancy, PE, and E.

Materials and Methods
Study subjects
The study enrolled 166 women attending Taksim Education and Research Hospital and Universal Hospitals Group, Obstetrics and Gynecology Clinic between June 2009 and August 2012. The subjects had similar ethnic and socioeconomic backgrounds. Ethical approval was obtained from the Local Ethical Committee, and informed consents were obtained from all patients. The study group consisted of 42 preeclamptic patients and 16 eclamptic patients. The control group consisted of 108 healthy normotensive pregnant women in third trimester who were chosen from outpatients clinic and examined regularly.

Exclusion criteria
Patients with a history of chronic renal disease, chronic hypertension, pre-existing diabetes or gestational diabetes, cardiovascular illness, premature rupture of membranes, clinical chorioamnionitis, urinary system infection, another symptomatic infection disease, autoimmune disease, malignancy, use of antibiotics, aspirin or heparin, deep venous thrombosis, and the history of failure of pregnancy were excluded. The patients with signs of labor or using labor induction agents were also excluded. Urine analysis was done in order to evaluate proteinuria and urinary tract infection. All patients were non-smokers and had singleton pregnancies. All patients were evaluated in the cardiology department. The patients who have known congenital or acquired valve disease, dilated cardiomyopathy, hypokinesia, and myocardial damage, were excluded from the study.

Diagnostic criteria for PE and E used in the study
The diagnosis of PE was made according to ISSHP criteria: increase in blood pressure to at least 140/90 mmHg after the 20th week of gestation in previously normotensive women, combined with proteinuria (protein excretion at least 0.3 g per 24 hours, spot urine protein/creatinine ratio 30 mg/mmol or at least 2+ protein by dipstick). Gestational age (GA) was calculated considering last menstrual period and confirmed first trimester or early second trimester ultrasonography findings. Generalized edema, proteinuria, persistent occipital and frontal headache, and abnormal weight gain were regarded as minor criteria in the diagnosis of eclampsia. Hypertension and convulsions were considered as major diagnostic criteria for the diagnosis of eclampsia. In the study, having both of major findings were required for the diagnosis of eclampsia whether having minor findings or not. For the diagnosis of convulsion, at least one health professional (nurse or doctor) was required to witness a convulsion.

Magnetic resonance imaging (MRI) was done in all patients, bilateral carotid Doppler ultrasonography was done in six patients and, MR angiography was done in two patients who had convulsions. The differential diagnosis was made with cerebral hemorrhage, ruptured aneurysm, arteriovenous malformation, arterial embolism or thrombosis, cerebral venous thrombosis, hypoxic ischemic encephalopathy, vasculitis, epilepsy, undiagnosed brain tumor or space occupying lesions. To rule out cardiac pathology, the authors did not perform perfusion scintigraphy and angiography that might show subendocardial injury. However, they excluded other cardiac pathologies which could raise troponin I levels by helping normal electrocardiographic and echocardiographic findings.

Laboratory methods
In the study group, samples were collected when the patients first presented for the evaluation and before initiation of any treatment such as magnesium sulfate, betamethasone, or labor induction agents. Blood was drawn in the morning after an eight-hour fasting from control group participants. Serum cardiac troponin I was measured using an immunoassay at the Department of Clinical Biochemistry of the present hospital. A lower limit of detection of 0.03 ng/ml is suggested for clinical use. Immunological method was used for D-Di analysis. Upper limit was 550 ng/ml (0.55 mg/L) for D-Di.

Statistical Analysis
Statistical analysis was done using the SPSS (Statistical Package for the Social Sciences) 13 program. Descriptive statistics were given as mean ± standard deviation for constant variables and in % for categorical variables. Comparisons of the demographic factors of the patients in the different groups were made using analysis of variance (ANOVA). Wilcoxon rank sum test was used to compare levels of cardiac troponin I and D-Di in the different groups. A p value of 0.05 was accepted as statistically significant.

Results
There was no statistically significant difference between preeclamptic patients, eclamptic patients, and control groups in terms of age, BMI, and GA at the time of blood drawn in the study group (p = 0.078, p = 0.065, p = 0.092) (Table 1). SBP, DBP, and MABP were higher in preeclamptic and eclamptic patients than control group (p = 0.0029)(Table 1). There was a statistically significant difference between E group and PE group (p = 0.038).

The pregnancy was terminated in eclamptic and preeclamptic patients earlier than the control group. Birth
weight and Apgar scores were lower in compared to control group \((p = 0.021, p = 0.042)\). The proteinuria was higher in eclamptic group than preeclamptic group, and the difference was statistically significant \((p = 0.001)\). Moreover, 24-hour proteinuria values in PE group was higher than normal pregnant women in third trimester \((p = 0.0028)\) (Table 1).

There was no statistically significant difference observed between normotensive pregnancies and PE patients in terms of troponin I levels \((p = 0.08, p > 0.05)\) (Table 2). Troponin I levels in E patients were found higher than normotensive and PE patients \((p = 0.016, p = 0.014, p < 0.05, \text{ Figure 1})\). D-Di levels in PE and E patients were found to be statistically significantly higher than normotensive patients \((p = 0.034, p = 0.020)\). D-Di levels in E patients were higher than PE \((p = 0.042, \text{ Figure 2})\).

### Discussion

It was demonstrated that there is a relationship between PE and endothelial dysfunction, hypercoagulation, and fibrinolytic system activation [11].

PE produces diffuse endothelial dysfunction as evidenced by increased levels of fibronectin, factor VIII antigen, thrombomodulin, endothelin, thromboxanes, and decreased nitric oxide production. There is impaired flow mediated vasodilation and impaired acetylcholine mediated vasorelaxation in PE when compared with normal pregnancy [12, 13]. Whether the endothelial dysfunction is part of the cause of PE or simply a manifestation of the disease is currently unknown. Regardless, the dysfunctional endothelium

---

**Table 1. — Demographic and obstetrics characteristics of the study and control groups.**

<table>
<thead>
<tr>
<th>Demographic and obstetrics characteristics</th>
<th>Normotensive</th>
<th>Preeclampsia</th>
<th>Eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27±5.1</td>
<td>28±4.3</td>
<td>29±6.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28±2.6</td>
<td>30.6±3.8</td>
<td>31.1±4.5</td>
</tr>
<tr>
<td>GA (at the time of blood drawn)</td>
<td>34±3.1</td>
<td>34±4.2</td>
<td>33±2.0</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114±18</td>
<td>162±0.9</td>
<td>175±20</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65(50-80)</td>
<td>90(90-140)</td>
<td>120(110-160)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>85±8.3</td>
<td>125.7±18.9</td>
<td>152±11</td>
</tr>
<tr>
<td>Parity</td>
<td>2.1±1.4</td>
<td>1.5±1.2</td>
<td>0.6±0.5</td>
</tr>
<tr>
<td>Delivery (weeks)</td>
<td>37±3.1</td>
<td>35±1.2</td>
<td>33±3.2</td>
</tr>
<tr>
<td>Newborn weight</td>
<td>3350</td>
<td>2730</td>
<td>1750</td>
</tr>
<tr>
<td>Apgar score</td>
<td>8.6±0.5</td>
<td>7±1.3</td>
<td>6±0.2</td>
</tr>
<tr>
<td>Proteinuria (mg/ day)</td>
<td>170±96</td>
<td>704±266</td>
<td>1624±680</td>
</tr>
</tbody>
</table>

BMI: body mass index, GA: gestational age, SBP: systolic blood pressure, DBP: diastolic blood pressure, MABP: mean arterial blood pressure.

**Table 2. — Troponin I and D-Di levels in control, PE, and E groups.**

<table>
<thead>
<tr>
<th>Normotensive groups</th>
<th>Troponin I ng/ml</th>
<th>D-Dimer ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.0134±0.0091</td>
<td>634±228</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>0.017±0.0085</td>
<td>1423±435</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>0.180±0.136</td>
<td>2067±580</td>
</tr>
</tbody>
</table>

**Figure 1. — Box plot graphic shows Troponin I levels in control group, PE, and E.**

**Figure 2. — Box plot graphic shows D-Di levels in control group, PE, and E.**
persists for years after delivery and may explain the reason of poor cardiovascular outcomes.

Maternal cardiac adaptation in normal pregnancy is well known. Endomyocardial biopsy samples obtained by cardiac catheterization from serious PE patients showed that there is a swelling in cardiac myocyte mitochondria and endothelial cell cytoplasm. It was stated that endomyocardial ultrastructural injury is a condition seen in PE [14].

In the literature, there are limited number of studies concerning troponin I level in PE. Several authors have reported that troponin I levels increase in both mild and severe PE [15, 16]. Fleming et al. have demonstrated that serum cardiac troponin I levels were elevated in pregnant patients with hypertension, and proteinuric hypertensive pregnant patients had the highest level of troponin I [17].

Yang et al. reported that there was a significant increase in serum troponin I levels in PE patients compared with normotensive patients; as well as it was reported that there was a positive correlation between the degree of PE (severe to moderate) and troponin I level [15]. This relationship was not shown in another study in the literature [18].

Atalay et al. reported that increasing troponin I levels can be used to diagnose PE patients in addition to demonstrate the efficacy of magnesium sulfate treatment in PE patients [16].

In the present study, the average troponin I levels were found 0.0134 ± 0.0091, 0.017 ± 0.0085, 0.180 ± 0.136 in normotensive, PE, and E patients, respectively.

While troponin I levels were not different in normotensive and PE patients, it was statistically significantly increased in E patients (Figure 1). In the present authors’ opinion, serum troponin I levels rise due to myocardial damage in eclamptic patients. Joyal et al. revealed that there is no relationship between troponin I levels and PE. They also stated that increased troponin I levels in PE patients are not only associated with diffuse inflammatory disease but these patients might have possible cardiac diseases. Therefore, it would be appropriate to monitor these patients closely [19]. This opinion was supported by some other researchers [16, 20]. One of these studies showed that although it was not statistically significant difference there was a tendency to increase troponin I levels in mild PE a in comparison to healthy groups. Interestingly, troponin I levels were quite similar in severe PE and healthy group. In conclusion, researchers declared that troponin I levels do not change in PE patients and it was not related to the severity of the disease [18].

In the present study, the authors could not detect any difference between PE patients and normal pregnancies in terms of troponin I levels. On the other hand, troponin I levels in E patients were significantly higher than PE and normotensive pregnancies. They thought that this situation could be the result from hypoxia which probably developing secondary to convulsions in E patients. Hypoxia increases troponin I levels by leading to cardiac damage in subendocardial myocardial cells. The present authors did not perform perfusion scintigraphy and angiography that might show subendocardial injury. However, they excluded other cardiac pathologies which could raise troponin I levels by helping normal electrocardiographic and echocardiographic findings. This case made them think that increasing troponin I levels in E patients could be due to subendocardial injury. There are some limitations in the present study. First, they could not perform processes such as perfusion scintigraphy and angiography. Second, they did not check other troponin levels which might affect cardiac troponin I levels.

Studies about D-Di levels in PE and normotensive pregnancies in the literature revealed that D-Di levels did not change [5, 21], moderately increased [22, 23] or significantly increased [24, 25] in PE patients.

In the present authors’ opinion, this situation could be resulted from different tests and kits (latex-based immunoassays, automated immunoturbidimetric assay, ELISA), heterogeneous patient selection, including pregnant women to study from different trimesters, and patient selection bias.

D-Di levels were found significantly higher in E and PE patients compared with normotensive patients in those study. Increasing levels in E patients were statistically significant in comparison to PE and normotensive pregnancies. It was thought that high levels of D-Di in both groups were secondary to hypercoagulation and fibrinolytic system activation that develop because of hypertensive disease in pregnancy. Diffuse inflammatory conditions, uteroplacental dysfunction, endothelial dysfunction, and formation of microthrombi seen in PE and E are the most important situations which can lead to fibrinolytic system activation. However, it was thought that D-Di levels were higher in E patients because hypoxia, that is secondary to epileptic seizures, contributes to coagulation and fibrinolytic system activation. The present authors think that elevated D-Di levels are a sign of increased coagulation system in PE and E. Kucukgoz et al. found that D-Di levels in PE was higher than control group and there was a positive correlation between D-Di levels and severity of the disease. These results were similar to the present study. It was shown that high D-Di levels result in about a five-fold increased risk (OR, 4.97; 95% CI, 1.22 - 20.29) in the development of PE and E [26]. Similar results were found by Trofatter et al., Paternoster et al., Neiger et al., indicating that the D-Di testing is useful to define subsets of patients with severe diseases such as, for example, HELLP syndrome [27-29].

In contrast; Koh et al., and Ho and Yang found no statistical differences between normal pregnancy and severe PE in terms of D-Di values [30, 31].

In the present study, D-Di levels in PE and E patients were higher than normotensive group. Increasing levels in E patients were higher. This study could not reveal that this situation was occurring whether the reasons of hypercoag-
ulability, fibrinolytic system activation, uteroplacental dysfunction, microthrombus, systemic inflammatory conditions such as endothelial dysfunction, which develop because of hypertensive disorders in pregnancy; or caused by the contribution of seizures in E patients. In spite of the present study, He et al. stated that even though there were increased D-Di levels in PE patients, it was not related to severity of the disease [24].

The present authors could not find any studies in the literature comparing normotensive and PE patients in terms of D-Di levels. There are serious hypertensive and HELLP syndrome cases in PE patients in the literature and these cases were described as severe PE. E patients were not included in this study. Therefore, the authors were only able to compare this study with this group.

Marcq et al. reported that patients developing HELLP syndrome had more than two times D-Di in comparison to PE patients. Moreover, the idea was advanced about D-Di levels in PE patients that may be predictive for HELLP development. Unfortunately, although the best threshold level was 2,170 ng/ml with 90% sensitivity and 40% sensitivity, it was not suitable for predicting HELLP syndrome in routine practice [32]. In the present study, D-Di levels in E patients were statistically significantly higher in control PE patients (2,067 ± 580, 1,423 ± 435 vs 0.042, Figure 2).

This study does not give information about D-Di and troponin I’s effectiveness in predicting E because PE patients did not monitored at regular basis and patients who had high troponin I and D-Di levels were not determined whether they experienced convulsion or developed E. It is interesting that troponin I levels in E patients were significantly higher than PE and normotensive pregnancies, while there were no differences between PE patients and control group. D-Di levels in E and PE patients were significantly increased in compared to control group. This may indicate that troponin I might be more effective in identifying E patients. It is clear that multicenter double-blinded randomized studies are required in order to interrogate this suspicion.

In contrast to possible hemostatic and fibrinolytic system disorders reported in PE, Higgins et al. claimed that this condition was associated with pregnancy itself. They showed that there was no difference between normotensive and PE patients in terms of not only D-Di levels but also other hemostatic markers such as thrombin-antithrombin III, plasmin-alpha 2 anti-plasmin complexes. They explained this condition by fibrinolytic and coagulation system that are activated in normal pregnancy and this activation is more clear in the uteroplacental circulation in comparison to the systemic circulation in both normotensive and PE group [33]. As can be seen in the literature, studies regarding relationship between high D-Di levels and severity of PE provide conflicting results. There are studies indicating that D-Di levels are correlated with the severity of PE as well as there are studies that show no correlation [24, 30].

There are some advantages of the present study. First, the study was designed as a prospective study. Second, there was no difference in terms of age, BMI, and gestational week when blood was drawn for biomarkers. Finally, the authors used ELISA test which is a very sensitive test.

In the present authors’ opinion, the endothelial integrity is essential for organ functions. Degradation of endothelial integrity leads to uncontrolled formation of clots. Loss of endothelial function and then organ damage in severe preeclamptic and eclamptic patients cause serious elevations of D-Di levels.

Consequently, serum troponin levels increased in E patients in related to myocardial damage. Increased troponin levels could not be detected in PE patient. However; D-Di levels increased in preeclamptic and eclamptic patients.

References


Address reprint requests to:
M. BOZKURT, M.D.
Kafkas Üniversitesi Kampüsü,
Sağlık Araştırma ve Uygulama Hastanesi,
Kadın Hastalıkları ve Doğum AnaBilim Dalı
Kars (Turkey)
e-mail: jindrmb@yahoo.com
The effect of maternal polycystic ovary morphology on first-trimester maternal serum biochemical markers of aneuploidy and fetal nuchal translucency thickness


1Department of Obstetrics and Gynecology, School of Medicine, Canakkale Onsekiz Mart University, Canakkale
2Department of Clinical Biochemistry, School of Medicine, Canakkale Onsekiz Mart University, Canakkale (Turkey)

Summary

Objective: To evaluate the effect of maternal polycystic ovary (PCO) morphology on maternal serum free beta-human chorionic gonadotropin (β-hCG), pregnancy associated plasma protein A (PAPP-A), and nuchal translucency (NT) thickness in the first-trimester.

Material and Methods: A total of 92 pregnant women in the first-trimester were included in the study. Of them, 57 had PCO morphology, and 35 women constituted the control group, with apparently normal ovaries. Maternal serum free β-hCG, PAPP-A, and NT thickness were measured and compared in all patients. Results: The multiples of median (MoM) levels of serum free β-hCG were significantly higher in the PCO morphology group compared to the normal ovary group (p = 0.024). However, the MoM levels of PAPP-A were similar in both groups (p = 0.947). No difference was found between the groups in terms of fasting glucose levels and NT measurements (p = 0.976 and 0.565, respectively). Conclusion: In pregnancies with maternal PCO morphology, the presence of higher maternal serum free β-hCG levels may require correction in the calculation of risks related to first-trimester screening for chromosomal abnormalities. Larger studies are needed to confirm our preliminary data.

Key words: Maternal serum screening test; Nuchal translucency measurement; Polycystic ovary syndrome; Pregnancy-associated plasma protein-A.

Introduction

First-trimester screening using the combined test including maternal free beta-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein A (PAPP-A), and nuchal translucency (NT) thickness at 11 weeks to 13 weeks of gestation is now a well-established program of aneuploidy screening, possessing a detection rate of about 90% with a 5% false-positive rate (FPR) [1].

Prenatal screening programs first describe the concentrations of biochemical markers as multiples of median (MoM) of the expected normal median for a pregnancy with the same gestational age. After that the MoMs are adjusted for factors considered to influence their levels, such as maternal weight [2,3], ethnicity [4], smoking [5], number of fetuses [6], and conception method [7]. If the MoMs are not adjusted by the aforementioned factors, the FPR of the test may be adversely affected. For instance, high FPR of a screening test is always associated with a rise in the rate of chorionic villus sampling (CVS) or amniocentesis.

The polycystic ovary (PCO) morphology is a common finding in the reproductive age population with a prevalence of up to 30% [8-10] and the association of this finding with a risk of cardiovascular disease has been described by some investigations [11-13]. In a previous case-control study, it was calculated that the risk for myocardial infarction was seven-fold higher in women with PCO morphology [14]. Another cohort study found a marked increase in the prevalence of cardiovascular risk factors in patients with PCO morphology, including hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, and an elevated waist-to-hip ratio [15]. On the other hand, PAPP-A which is one of the two serum markers in first-trimester screening test for aneuploidy, has also been shown to be a marker of adverse outcome in both acute coronary syndrome and stable coronary disease patients [16]. It was reported that PAPP-A may play a role in the development of atherosclerotic lesions and also constitute a marker of atheromatous plaque instability and the extent of cardiovascular disease. In this sense, it can be postulated that PAPP-A, as a component of maternal serum markers of aneuploidy, may be increased in pregnant women with PCO morphology during the first-trimester.

Moreover, serum PAPP-A levels were found to be higher in non-pregnant polycystic ovary syndrome (PCOS) patients compared to control subjects [17]. However, no previous study evaluated the influence of maternal PCO morphology, which is a common finding in reproductive-
aged women, on the first-trimester biochemical markers of aneuploidy and NT thickness.

The aim of the present study was therefore to evaluate the effect of maternal PCO morphology on maternal serum free β-hCG, PAPP-A and NT thickness from 11 weeks to 13 weeks six days of gestation in women screened prospectively for chromosomal anomalies.

Materials and Methods

In this prospective study, the relationship between PCO morphology and parameters of the combined test in the first-trimester of pregnancy was examined in women screened between October 2011 and September 2012 at a tertiary university hospital. Ethical approval was obtained from the institutional human research ethics committee. Informed consent was gained from all participants before entering the study.

A total of 92 pregnant women with a mean age of 27.3 ± 0.4 (17 to 37) years, at 11 + 0 to 13 + 6 weeks of gestation, were included in the study. All of the pregnancies were singleton. Of the 92 women, 57 women had PCO morphology at least in one ovary, and 35 age-, body mass index- (BMI), and gestational age-matched women with apparently normal ovaries constituted the control group. Women with systemic diseases, IVF pregnancy, pre-gestational diabetes mellitus, and ultrasound findings of fetal chromosomal abnormalities or structural defects during the period of the combined test were excluded from the study. Maternal age, BMI, and gestational age at the time of the ultrasound scan were recorded and fasting glucose levels and the combined test were studied for each case.

Ultrasound examination

Two-dimensional pelvic ultrasound scans were performed using devices equipped with convex transducer. Before the examination, each woman was asked to empty her bladder. The ovary was examined when the plane gave the best image quality, which was optimized by aid of high magnification, appropriate frequency, and by use of the automatic optimization feature. Follicle size was found from the mean of two perpendicular diameters of the antral follicles. Follicles were counted by scanning from one margin of the ovary to the other.

Diagnosis of PCO morphology on ultrasound was made according to the Rotterdam criteria in which PCO was defined as 12 or more follicles, two to nine mm in diameter [18]. The patients with less than 12 follicles measuring two to nine mm in diameter in both apparently normal ovaries constituted the control group. Additionally, transabdominal ultrasound examination was performed to obtain the aneuploidy screening in the first-trimester. NT and crown-rump length (CRL) measurements as well as screening for structural anomalies were performed in accordance with the technique described by Fetal Maternal Medicine [19].

Biochemical measurements

Fasting blood, taken at the same visit as the ultrasound examination, was collected from the median cubital vein in order to evaluate serum levels of free β-hCG and PAPP-A, as well as the fasting glucose level. The serum glucose level was determined by an enzymatic-UV photometry method.

Free β-hCG and PAPP-A measurements were performed for all women with an analyzer as part of the routine maternal serum aneuploidy screening in the first-trimester of pregnancy. Concentrations of biochemical markers of the double test were entered into the database of the first-trimester prenatal screening program at the institutional laboratory of the Department of Clinical Biochemistry and automatically converted to MoMs by the laboratory information management system. The biochemistry and risk estimates were calculated using specific software.

Statistical analyses

Statistical analyses were performed using the SPSS software version 20. The variables were investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov test) to evaluate normal distribution. Descriptive analyses were presented using means and standard errors of mean (SEM). The Student’s t-test and the Mann-Whitney U test, where appropriate, were used to compare the continuous variables between the study and control groups. Statistical significance was defined as \( p \leq .05 \).

Results

The study included 92 pregnant women in the first-trimester of pregnancy. The participants consisted of 57 patients with PCO morphology in at least one ovary and 35 patients with apparently normal ovaries. Demographic and clinical characteristics of both groups are presented in Table 1. There was no difference between the groups in terms of variables such as age, BMI, CRL, and gestational weeks.

PCO morphology in maternal ovaries had a significant effect on plasma free β-hCG levels while no significant effect was seen on the plasma PAPP-A levels during 11 + 0 and 13 + 6 weeks of pregnancy. The PCO morphology group showed higher free β-hCG levels in maternal plasma than the control group \((p = 0.024)\), whereas plasma PAPP-A levels were similar in both groups \((p = 0.947)\) (Table 1).

No difference was found between the groups in terms of fasting glucose levels and NT measurements \((p = 0.976\) and 0.565, respectively).

Discussion

The results of this prospective study showed that the serum free β-hCG levels from pregnant women with PCO morphology in their ovaries were significantly higher compared with pregnant women with normal ovaries during the combined screening test of the first-trimester. However, PAPP-A levels, which is the other serum marker of the screening test, as well as the NT measurements and fasting glucose levels, were similar in both groups.

To the best of the authors’ knowledge, a comparison in patterns of the markers from the combined screening test between pregnant women with PCO and normal ovaries has not been documented precisely in previous studies. The authors found that MoMs of free β-hCG were significantly higher in the PCO morphology group (Table 1). Contrary to these results, a retrospective study conducted in patients who underwent assisted reproductive technology (ART) by Kosus et al. [20] found that free β-hCG MoMs were significantly decreased in infertile PCOS patients compared to infertile patients with male factor. The authors reported that factors such as hormonal disturbances, obe-
The effect of maternal polycystic ovary morphology on first-trimester maternal serum biochemical markers of aneuploidy etc.

Table 1. — Demographic and clinical features of the study and control groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PCO (n=57)</th>
<th>Control (n=35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.2 ± 0.6</td>
<td>24.8 ± 1.0</td>
<td>0.822</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 1.0</td>
<td>26.8 ± 1.7</td>
<td>0.352</td>
</tr>
<tr>
<td>CRL (mm)</td>
<td>56.8 ± 1.7</td>
<td>57.1 ± 4.2</td>
<td>0.762</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>12.2 ± 0.1</td>
<td>12.2 ± 0.2</td>
<td>0.804</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>90.2 ± 4.4</td>
<td>85.9 ± 3.2</td>
<td>0.976</td>
</tr>
<tr>
<td>NT (mm)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>0.565</td>
</tr>
<tr>
<td>AFC in right ovary (n)</td>
<td>14.4 ± 1.3</td>
<td>7.2 ± 1.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AFC in left ovary (n)</td>
<td>15.7 ± 1.1</td>
<td>6.1 ± 0.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Right ovarian volume (cm³)</td>
<td>12.2 ± 0.6</td>
<td>9.8 ± 0.8</td>
<td>0.017*</td>
</tr>
<tr>
<td>Left ovarian volume (cm³)</td>
<td>11.9 ± 0.5</td>
<td>8.2 ± 0.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Free β-hCG (MoM)</td>
<td>1.3 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.024*</td>
</tr>
<tr>
<td>PAPP-A (MoM)</td>
<td>1.1 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.947</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM

CRL: crown-rump length; NT: nuchal translucency; AFC: antral follicle count; β-hCG: β-human chorionic gonadotropin; PAPP-A: pregnancy associated plasma protein A.

... and control groups. Table 1. — PCO morphology milieu of ART cycles and PCOS itself rather than a sole difference in the results may be explained by the hormonal male factor rather than patients with PCO morphology. The study as it was conducted in ART patients with PCOS or...
first-trimester combined screening test. Serum PAPP-A, fasting glucose levels, and NT measurements were not affected by the presence of maternal PCO morphology. As changes in serum markers of the screening test may alter the screening test results, consequently it may be necessary to adjust the first-trimester risk calculation accordingly. Further prospective studies should be conducted to confirm this preliminary data.

References


Address reprint requests to:
S. HACIVELIOGLU, M.D.
Canakkale Onsekiz Mart Universitesi,
Tip Fakultesi
Kadin Hastaliklari ve Dogum Klinigi
17100, Canakkale (Turkey)
e-mail: servetozden@comu.edu.tr
**Effect of hypertonic sodium chloride hydroxyethyl starch 40 on ET, TXB2, 6-keto-PGF1α, and ANP of preeclampsia in caesarean section**

T. Wang¹, L.H. Jiang¹, J.B. Zhu², X.Y. Wei¹, L. Li¹, B. Liu¹

¹ Department of Anesthesia, the Third Affiliated Hospital of Zhengzhou University, Zhengzhou
² The First Affiliated Hospital of Henan Chinese Medical College, Zhengzhou (China)

**Summary**

*Objective:* Preeclampsia is a unique disease of pregnancy. Delivery via caesarean section is the most important way of terminating the pregnancy and treating preeclampsia. Perioperative fluid therapy is performed to maintain the circulatory volume and reduce tissue edema. This study evaluated the effects of hypertonic sodium chloride hydroxyethyl starch 40 (HSH40) as perioperative fluid therapy for preeclampsia patients. *Materials and Methods:* Forty preeclamptic women were randomly divided into two groups: the Ringer’s solution group and the HSH40 group. Their ECG, HR, MAP, and SPO2 were monitored. Their MVP and HR were recorded at five, eight, and ten minutes after anesthesia induction and at the end of the caesarean section. The corresponding volume of infusion, blood loss, and urine output during the operation were also recorded. Venous samples were collected before HSH40 infusion and 30 min after infusion to measure the plasma concentrations of ET, TXB2, 6-keto-PGF1α and ANP via a radioimmunoassay. *Results:* HSH40 infusion significantly decreased the plasma ET levels (*p* < 0.01), significantly changed the plasma ANP and TXB2 levels (*p* < 0.05), and significantly increased the plasma 6-keto-PGF1α levels (*p* < 0.01) in the experimental group compared with those before infusion. The plasma levels of ET, ANP, TXB2, and 6-keto-PGF1α did not significantly change in the control group. Compared with T1, MAP decreased significantly at T2, T3, T4, and T5 within groups (*p* < 0.05) and between the two groups. MAP significantly changed at T2, T3, T4, and T5 (*p* < 0.05). HR did not significantly change at T1, T2, T3, T4, and T5 within or between groups. Volume of infusion and urine volume significantly differed between groups (*p* < 0.05). *Conclusion:* Low-dose HSH40 lowers the plasma levels of vasoconstrictor substances (ET and TXB2) and increases the levels of vasodilator substances (6-keto-PGF1α and ANP) during preeclampsia. It effectively maintains and stabilizes the circulating blood volume, increasing renal blood flow, which improves renal function and increases urine output.

*Key words:* Hypertonic sodium chloride hydroxyethyl starch; Preeclampsia; Endothelin; Atrial natriuretic peptide; Prostacyclin.

**Introduction**

Preeclampsia is a pregnancy-specific disease and a serious stage of hypertensive disorder during pregnancy. Preeclampsia is the main cause of maternal and perinatal morbidity and mortality. The basic pathophysiology includes systemic small artery spasm. The vascular spasms significantly increases plasmic endothelin (ET) in patients with hypertensive disorders during pregnancy than in normal pregnant women and is positively correlated with the severity of the disease [1-3]. Elevated ET prompts a massive release of atrial natriuretic peptide (ANP), which is significantly higher than that during normal pregnancy [4]. Vasospasms significantly reduce plasma prostacyclin (PGI2) and significantly increase thromboxane (TXB2), further aggravating the vasoconstriction, elevating blood pressure, and causing further coagulation disorders. The imbalance of these four vasomotor substances is the main cause of pregnancy-induced hypertension and an important reason for the maternal systemic spasms in small arteries, low blood volume and concentration, viscosity-induced maternal hypoperfusion of vital organs, tissue ischemia, hypoxia, acidosis, blood stasis, decreased deformation force of erythrocytes, significantly increases platelet aggregation, and a series of pathophysiologic changes [5]. Timely termination of pregnancy is the effective treatment for preeclampsia. The main way for terminating pregnancy is delivery via caesarean section. The type and dose of infusion during maternal preeclampsia caesarean section remain controversial. Hypertonic saline hydroxyethyl starch (HSH) is a hypertonic colloidal volume expander that actively expands the blood volume, stabilizes and maintains the effective circulating blood volume, improves the microcirculation, improves the tissue oxygen supply, reduces tissue edema, corrects acidosis, reduces blood viscosity, and exerts a diuretic effect. Hypertonic sodium chloride solution (hyperosmotic saline, HS) changes the levels of hormones and cytokines [6, 7]. Expanding the blood volume increases the cardiac filling pressure and significantly increases the effective plasma ANP levels, increases intracellular Ca²⁺, activates enzymes re-
quired for synthesis, and directly stimulates endothelial cells to elevate plasma PGII2 synthesis. HS also produces an endogenous infusion effect, improves microcirculation, promotes ANP release, influences lysosomal function, and inhibits the secretion of ET [8]. In this study, puerperants with maternal preeclampsia underwent waist-epidural anesthesia for caesarean section and were then injected with HSH. After preeclampsia, the plasma ET, TXB2, PGII2, and ANP levels, and the basic vital signs of the mothers and their infants were taken. The present authors further explored the possible mechanism of action of the intraoperative transfusion of small doses of HSH for preeclampsia.

Materials and Methods

Subjects

Severely preeclamptic patients who were admitted into the present unit from May 2007 to July 2008 were enrolled into the case cohort. According to the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [9], preeclampsia is defined as systolic blood pressure (SBP) >140 mmHg, diastolic blood pressure (DBP) >90 mmHg, or both, and proteinuria >100 mg/dL. Patients in labor, those with chronic hypertension, placental abruption, diabetes, or coagulopathy, and those given β-tocolytic drugs were excluded from the study. This study was conducted in accordance with the Declaration of Helsinki and with the approval from the Ethics Committee of the Third Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Grouping

The patients who provided their written informed consent to participate in this study were randomly assigned using a random number table for block randomization via numbered sealed envelopes. The groups received either Ringer’s solution (RS group of 20) or HSH solution (HSH group of 20). In the operating room, all patients were monitored with standard monitoring devices, including an automated blood pressure cuff, an electrocardiogram, and pulse oximetry.

Before administering combined spinal-epidural anesthesia, five ml/kg of HSH consisting of 4.2% hypertonic saline and 7.2% hypertonic saline for preeclampsia was infused for over 20 minutes, followed by 0.9% sodium chloride, nine g/l sodium chlo-roxyethyl starch was infused for over 20 minutes, followed by 1.5 ml/kg of HSH consisting of 4.2% hypertonic saline and 7.2% hypertonic saline for preeclampsia. After preeclampsia, the plasma ET, TXB2, PGII2, and ANP levels, and the basic vital signs of the mothers and their infants were taken. The present authors further explored the possible mechanism of action of the intraoperative transfusion of small doses of HSH for preeclampsia.

Materials and Methods

Subjects

Severely preeclamptic patients who were admitted into the present unit from May 2007 to July 2008 were enrolled into the case cohort. According to the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [9], preeclampsia is defined as systolic blood pressure (SBP) >140 mmHg, diastolic blood pressure (DBP) >90 mmHg, or both, and proteinuria >100 mg/dL. Patients in labor, those with chronic hypertension, placental abruption, diabetes, or coagulopathy, and those given β-tocolytic drugs were excluded from the study. This study was conducted in accordance with the Declaration of Helsinki and with the approval from the Ethics Committee of the Third Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Grouping

The patients who provided their written informed consent to participate in this study were randomly assigned using a random number table for block randomization via numbered sealed envelopes. The groups received either Ringer’s solution (RS group of 20) or HSH solution (HSH group of 20). In the operating room, all patients were monitored with standard monitoring devices, including an automated blood pressure cuff, an electrocardiogram, and pulse oximetry.

Before administering combined spinal-epidural anesthesia, five ml/kg of HSH consisting of 4.2% hypertonic saline and 7.2% hydroxyethyl starch was infused for over 20 minutes, followed by Ringer’s solution at 100 ml/h. The RS group received continuous Ringer’s solution (0.9% sodium chloride, nine g/l sodium chloride, osmolarity 309 mOsmol/L, Na+ concentration 154 mmol/l).

Combined spinal-epidural anesthesia was induced in all cases in the lateral decubitus position, at L2-L3 or L3-L4, with 1.5 ml of 0.75% hyperbaric bupivacaine via a 22-gauge spinal needle injected for 20 seconds under aseptic conditions by an anesthesiologist who was blinded to the study groups. The spinal needle was removed and an epidural catheter was inserted three to four cm into the epidural space and was secured with tape. The parturient was then immediately placed in the supine position with left uterine displacement. The patient reaching a sensory level of T8 was considered statistically significant.

Results

Demographic characteristics

The demographic characteristics of the two study groups are shown in Table 1. The groups did not significantly differ in terms of maternal age, weight, and parity or gestational weeks. The blocks were generally accomplished on the first attempt. A similar number of dermatomal segments (assessed by pinprick) were blocked in both groups.

Biochemical analyses

HSH infusion at five ml/kg into the preeclamptic patients significantly changed their ET, TXB2, 6-keto-PGF1α, and ANP levels relative to those of the control group (Table 2).
Effect of hypertonic sodium chloride hydroxyethyl starch 40 on ET, TXB2, 6-keto-PGF1α, and ANP of preeclampsia in caesarean section

38

The levels of plasma ET, ANP, TXB2, and 6-keto-PGF1α were not significantly changed in the control group. Compared with those before HSH infusion, the levels of plasma ET and TXB2 decreased significantly, whereas plasma 6-keto-PGF1α and ANP increased significantly.

Hemodynamic parameters and volume input and output data

The MAP and HR values of both groups are shown in Table 3. As illustrated in Table 3, both groups had similar MAP values measured during the preoperative period. The MAP measured during the induction until the delivery period was consistently lower in the control group than in the experimental group (Table 3). The control and experimental groups did not significantly differ in the duration of anesthesia. The volume of infusion in the experimental group was less than that of the control group, whereas the urine volume of preeclamptic patients was higher than that of the control group (Table 4). The volume of blood loss did not significantly differ between groups (Table 4). The two groups had similar newborn weights, Apgar scores, and admission rates into the neonatal intensive care unit.

Discussion

Preeclampsia is a unique disease of pregnancy. Caesarean section is the most important way of terminating pregnancy and treating preeclampsia. Perioperative fluid therapy needs to maintain the circulatory volume and reduce tissue edema. Ringer’s solution temporarily increases blood volume, and its massive infusion leads to tissue edema. A small volume of HSH40 produces more rapid volume expansion, increases cardiac output, systemic blood pressure, and microvascular perfusion, and it reduces tissue edema [10-13].

In this study, the levels of plasma ET decreased significantly compared with those before HSH infusion (Table 2). Thus, HSH may have inhibited angiotensin II (Ang II), which stimulates ET secretion from cultured bovine endothelial cells via a receptor-mediated process [14-16]. Moreover, HSH may have enhanced ANP secretion, which interacts with ET secretion from the endothelium through the renin–angiotensin system [17, 18]. Compared with Ringer-Locke liquor, HSH40, which inhibits ET secretion, may improve the symptoms of preeclampsia.

As already mentioned, 6-keto-PGF1α and TXB2 mainly reflect the plasma levels of PGI2 and TXA2. The 6-keto-PGF1α levels in the experimental group were significantly increased compared with that before HSH infusion, whereas TXB2 was significantly reduced (Table 2). The levels of both TXB2 and 6-keto-PGF1α did not significantly change in the control group.

Colloidal liquid of HSH40 maintains the effective circulating blood volume. Furthermore, the hypertonic saline in HSH40 potentiates ANP secretion [19] and strongly inhibits

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>30.05±3.48</td>
<td>29.85±3.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.95±7.56</td>
<td>78.90±7.83</td>
</tr>
<tr>
<td>Gestational weeks (w)</td>
<td>35.00±2.29</td>
<td>35.35±2.18</td>
</tr>
<tr>
<td>Skin incision to skin closure interval (min.)</td>
<td>23.65±4.42</td>
<td>22.84±4.28</td>
</tr>
</tbody>
</table>

Data are mean ±SD.

Table 2. — Effect of HSH on plasma ET, TXB2, 6-Keto-PGF1α, and ANP changes in preeclamptic patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-transfusion</th>
<th>Post-transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET</td>
<td>Experimental group</td>
<td>91.73±18.57</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>92.45±18.89</td>
</tr>
<tr>
<td>ANP</td>
<td>Experimental group</td>
<td>208.03±59.12</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>209.97±52.66</td>
</tr>
<tr>
<td>TXB2</td>
<td>Experimental group</td>
<td>275.80±46.41</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>273.29±43.21</td>
</tr>
<tr>
<td>6-Keto- PGF1α</td>
<td>Experimental group</td>
<td>81.01±12.74</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>83.84±15.65</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Before administering combined spinal-epidural anesthesia, HSH was given to preeclamptic patients over 20 minutes in experimental group, control group received Ringer’s solution. ▲ p < 0.05 or p < 0.01.

Table 3. — Hemodynamic data in the both groups.

| Group | Pre-transfusion (T1) 5 min (T2) 8 min (T3) 10 min (T4) End (T5) |
|-------|----------------------|-----------------|-----------------|-----------------|-----------------|
| MAP (mmHg) | Experimental group | 127.2±7.9 | 102.9±14.2* | 85.8±10.2** | 106.7±7.6* | 107±14.5** |
| | Control group | 127.3±8.2 | 91.4±10.6* | 74.3±8.5** | 93.5±8.2* | 96.1±11.7* |
| HR (bpm) | Experimental group | 102.2±13.3 | 103±11.4 | 96.4±8.1 | 98.6±8.6 | 98.3±8.2 |
| | Control group | 101.7±14.2 | 103±11.8 | 98.2±9.6 | 99.7±8.4 | 98.5±10.4 |

Data are mean ± SD, Unpaired Student’s t-test, * p < 0.05; Paired Student’s t-test, ** p < 0.01.
immunoreactive ET secretion in the aorta. Therefore, increased circulating ANP may affect ET secretion. The diuretic mechanism of HSH40 may involve the instantaneous mobilization of extravascular fluid into the intravascular space through the osmotic action of HSH40 [20, 21] and its subsequent rapid excretion via the action of extracellular fluid expansion and by the action of ANP. Furthermore, hypertonic saline solution may facilitate the action of ANP and help overcome an established ANP resistance, as demonstrated by increased renal blood flow. In fact, HSH administration potentiates the diuretic action of ANP and possibly helps overcome ANP resistance without requiring a higher concentration, thereby minimizing electrolyte disturbances and other side effects (e.g., hypotension).

In summary, low-dose HSH40 lowers the levels of vasoconstrictor substances (ET and TXB2) and increases the levels of vasodilator substances (6-keto-PGF1α and ANP) in ischemic ischemic tissue and other side effects (e.g., hypotension).

References


Address reprint requests to:
L. JIANG, M.D.
Department of Anesthesia,
The Third Affiliated Hospital of Zhengzhou University
Zhengzhou 450052 (China)
e-mail: taowangen@126.com
Introduction

Missed abortion is the expulsion of an immature fetus that could not have the ability to sustain life in extrauterine environment from uterus due to any reason and the termination of pregnancy. The factors such as chromosomal anomalies, malformations, immunological factors, endocrine disorders, and environmental factors could be the reasons for missed abortion [1]. In the studies on the etiology of abortion, underlying pathophysiological mechanisms of the half of the cases have remained uncertain [1, 2].

Although the etiology of missed abortion has not been completely clear, recent studies have provided strong evidences that support the close relation between angiogenesis and embryonic developments. An adequate vascularization of chorionic villus is essential for the development of an uneventful pregnancy [3, 4]. The abnormal changes in the balance of utero-placental vascular development are the underlying causes of pregnancy loss, and an adequate and appropriate angiogenesis is a necessity for the successful continuation of a pregnancy [5].

Angiogenesis, the development of new blood vessels from existing blood vessels, is a natural process of the body and may be pathological in some cases. Angiogenesis is rather limited in organism except for physiological cases as the development of placenta after fertilization in which angiogenesis is strictly controlled, embryogenesis, wound healing, and the renewal of the inner layer of the uterus after menstruation [6]. Under normal conditions, angiogenesis is regulated by a balance among many growth factors enabling the proliferation and activation of endothelial cells and anti-angiogenic factors [7].

Vascular endothelial growth factor (VEGF) is the most important one of the factors having a role in angiogenesis. Many biological functions of endothelial cells, cytokine synthesis and release, the expression of molecules in thrombolytic and coagulation pathways, and the regulation of smooth muscle cell hyperplasia are carried out by means of VEGF [8]. The biological activity of VEGF occurs basically by means of its three receptors. These receptors with their structure of the tyrosine kinase are VEGF-R1 (Flt-1), VEGF-R2 (Flk-1/KDR) and VEGF-R3 (Flt-4). While

Summary

Objective: The authors aimed to evaluate the angiogenic changes that occur in the cases with missed abortions compared with the voluntary termination of pregnancy as control group, with this controlled clinical study. Materials and Methods: The study included fifteen healthy volunteer women with unwanted pregnancy less than 10th gestational week in an academic research environment. The patients were 19 women between 6th and 11th gestational weeks diagnosed with missed abortion as the patient group. Immunohistochemistry was utilized to examine temporal and spatial expression of vascular endothelial growth factor (VEGF) and their two receptors: VEGF-R1 (Ft-1) and VEGF-R2 (Ft/1/KDR), and Trombospondin-1, eNOS, iNOS, and HIF-1α in the both decidua and placenta of the both groups. Results: This study discovered the significant difference (p < 0.005) between the groups of controlled and missed abortion in the decidual and placental cell components, and has put forward that thrombospondin and iNOS have an impact on abortion through antiangiogenic effect in cases of missed abortions. Conclusions: The potential role of molecules affecting angiogenesis in the etiology of missed abortion has been evaluated and the authors aimed for this to be a guide for studies on further treatments and on the prevention of the development of missed abortions.

Key words: Missed abortion; Angiogenesis; Immunohistochemistry.

A comparison of the molecular distribution of proangiogenic factors in endometrium of missed abortions and of voluntary first trimester termination cases

T. Özçakır¹, M.A. Turan², F. Şimşek³, C. Atay⁴, S. Vatansever⁵, K. Özbilgin⁶

¹ Department of Gynecology and Obstetrics, School of Medicine, Celal Bayar University, Manisa
² Department of Gynecology and Obstetrics, Ercis State Hospital, Van
³ Department of Histology and Embryology, School of Medicine, Katip Celebi University, Izmir
⁴ Department of Computer Technologies, Izmir University of Economics, Balçova, Izmir
⁵ Department of Histology and Embryology, School of Medicine, Celal Bayar University, Manisa (Turkey)
VEGF-R1 and R2 of them are located in endothelial cells, VEGF-R3 is on lymph vessels [9-11].

Hypoxia, described as the case of the lack of adequate oxygen in environment, has a substantial role in the pathogenesis of many diseases [12]. Oxygen is largely necessary for the aerobic metabolism of many eukaryotic organisms including the development of embryo. A software agent HIF-1α has a role of critical importance in tissue cells such as pregnancy decidua in which vascularity is intense, by stimulating the expression of genes related to angiogenesis, glucose transportation, and anaerobic metabolism in hypoxic conditions [13]. The expression of VEGF is acknowledged to be controlled with HIF-1α during hypoxia [14].

Thrombospondin (TSP) -1 is a potential angiogenesis inhibitor with the ability of intercepting the cell migration in endothelial cells in response to the different types of angiogenic stimuli, stimulating apoptosis, and preventing the formation of new blood vessels [15, 16]. That TSP-1 has an impact on the regulation of the secretion of VEGF from extracellular stores is a significant point. VEGF and the receptor of VEGFR are shown to increase in the absence of TSP-1 [17]. As a result of the increase in the level of tissue oxygen, the necessary genes have been observed to be stimulated in the adjustment of cell to hypoxia and TSP-1 has been seen to be inhibited. Consequently, the formation of new blood vessels has been observed [18].

With its role in the regulation of systemic blood pressure, nitric oxide (NO) has a critical and important role in angiogenesis and hyper permeability induced by VEGF [19]. VEGF induces the up-regulation of endothelial nitric oxide synthase (eNOS) enzyme and accordingly, the secretion of NO. Consequently, NO, which is produced as endogenous, increases the synthesis of VEGF. That eNOS undergoes a pharmacological blockade or a genetic disorder inhibits the angiogenesis and hyper permeability induced by VEGF [20].

This study has compared the distribution of VEGF and VEGF receptors which are angiogenic molecules; TSP-1 which is the inhibitor of eNOS and angiogenesis, and HIF-1α which stimulates the expression of genes related to angiogenesis in the iNOS and hypoxic conditions in the endometrium and placental tissue samples in the cases of missed abortions and voluntary termination of pregnancy. Thus, the authors have revealed the potential role of molecules affecting angiogenesis in the etiology of missed abortion, and have aimed for this to be a guide for the studies on further treatments and on the prevention of the development of missed abortions.

Materials and Methods

Fifteen unwanted pregnancies (5–10 weeks gestational age) and 19 missed abortions (6-11 weeks gestational age) endometrial tissue samples were obtained with informed consent and in accordance with the requirements of the Celal Bayar University Ethics Committee. The mean age of women was 27.53 years. The range was 21-37 years for normal pregnancy group and mean was 28.74 years old for range 18-41 years for missed abortion group.

The abortions were diagnosed by transvaginal ultrasound and confirmed by repeat ultrasound prior to the dilation and curettage procedure. Chorionic villi and maternal decidua were separated and cleaned. Placental and decidual tissues were fixed in 10% buffered formalin solution and embedded in paraffin. The blocks were cut in four to five μm thick serial sections. The first of the tissue sections were stained with primary antibodies (VEGF; VEGF-R1 (Flt-1), VEGF-R2 (Flk-1/KDR), Thrombospondin-1, eNOS, iNOS and HIF-1α) via immunohistochemical technique.

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were used for immunohistochemical staining. Tissue samples were stored at 60°C overnight and then were dewaxed with xylene for 30 minutes. After dehydration of the sections with ethanol, they were washed with distilled water. They were then treated with 2% trypsin (ab970) at 37°C for 15 minutes and incubated in 3% H2O2 solution for 15 minutes to inhibit endogenous peroxidase activity. Then, sections were incubated with Anti-VEGF Primer Antibody (251901), Anti-Fik Primer Antibody (25180), Anti-FLT Primer Antibody (25153), Anti-eNOS Primer Antibody (25078 ), Anti-iNOS Primer Antibody (25078), Anti-Thrombospondin Primer Antibody (ab93653) and Anti-HIF-1 Primer Antibody (ab463) in a 1/100 dilution for 18 hours at +4°C. They were then given an additional three 5-minutes washes in PBS, followed by incubation with biotinylated IgG and administration of streptavidin peroxidase. After washing the secondary antibody with PBS three times for five minutes, the sections were stained with DAB substrate system containing diaminobenzidine to detect the immunoreactivity, and then stained with Mayer’s hematoxylin (72804E) for counterstaining. They were covered with mounting medium (01730) and observed with light microscopy.

Immunostaining for VEGF, Flk, Flt, iNOS, eNOS, Thrombospondin, and HIF-1α were evaluated semiquantitatively using HSCORE analysis. Immunostaining intensity was categorized by the following scores: 0 (no staining), 1 (weak, but detectable, staining), 2 (moderate staining), and 3 (intense staining). An HSCORE value was derived for each specimen by calculating the sum of the percentage of cells for fibroblast and decidual cells in uterine decidual stroma; and fibroblasts and mesenchymal cells in placental villous stroma that stained at each intensity category multiplied by its respective score, using the formula H-score=∑Pi (i+1), where i= intensity of staining with a value of 1, 2 or 3 (weak, moderate or strong, respectively) and Pi is the percentage of stained decidual tissues (decidual cell, endothelial cells and endometrial glad cells) and placental tissues (cytotrophoblast, syncytiotrophoblast, stromal cells and endothelial cells) for each intensity, varying from 0 to 100%. For each slide, five different fields were evaluated microscopically at X200 magnification. HSCORE evaluation was performed independently by at least two investigators (KO, FS) blinded to the source of the samples as well as to each other’s results; the average score of both was then used.

Results

Histochemistry

In the examination of the preparations stained with Hema-toxylin-eosin of the normal pregnancy placentas, the chori-onic villi, where mesenchymal tissue was located, were surrounded by trophoblastic cells. There were fusiform-shaped mesenchymal cells and dark-colored macrophages (Hofbauer cells) in these regions and various veins- the extensions of the umbilical arteries and veins inside the mes-
enchymal tissue (Figure 1a). The stromal cells in normal pregnancy endometrium were observed to turn into decidual cells with a quite large cytoplasm where there were various granules. Among the decidual cells, numerous uterine gland and blood vessels were determined (Figure 1b).

In the same examination, the enclosure of chorionic villi by trophoblastic cells and a thinner thickness of their syncytiotrophoblast and cytotrophoblast than the normal pregnancy were observed (Figure 1c). A decrease in the number in blood vessels in the chorionic and decidual regions of the missed abortion cases than the normal pregnancy was found remarkable while the endometrial glands had a slight increase (Figure 1d).

**Immunohistochemistry**

In the examination of placenta samples of the control group stained by means of immunohistochemical technique, it has been observed that VEGF, Flk, eNOS, and TSP immunoreactivities of syncytiotrophoblasts have been stained significantly stronger (Figures 2a, 3a, 5a, 7a) than the missed abortion group (Figures 2b, 3b, 5b 7b), while the immunoreactivities of Flt, iNOS, and HIF-1α (Figures 3a, 6a, 8a) have been significantly slightly stained in the control group than the missed abortion group (Figures 3b, 6b, 8b). The VEGF, eNOS and TSP-1 immunoreactivities of cytotrophoblast cells of the control group (Figures 2a, 5a, 7a) have been found to be stained stronger than the missed abortion group (Figures 2b, 5b, 7b); the Flt and HIF-1α antibodies have been stained slightly in the control group (Figures 3a, 6a, 8a) compared to the missed abortion group (Figures 3b, 6b, 8b). There was no significant difference observed in the Flk and HIF-1α immunoreactivity of cytotrophoblast cells of the both groups (Figures 3ab, 8ab). The placental stromal cells of the control group stained stronger (Figures 2a, 5a, 7a) than the missed abortion group (Figures 2b, 5b, 7b); the Flk and HIF-1α antibodies have been stained weakly in the control group (Figures 4a, 8a) compared to the missed abortion group (Figures 4b, 8b). There was no significant difference observed in the Flk and HIF-1α immunoreactivity of cytotrophoblast cells of the both groups (Figures 3ab, 8ab).
Figure 3. — Immunohistochemical analysis of Flk: control group (a, b); missed abortion (c, d); placental villi (a, c); decidua (b, d). Placental syncytiotrophoblast (Sy) and vascular endothelial cells (Ve) of the control group (a) stained strongly in control group (a) than missed abortion group (c). Decidual cells (Dc), vascular endothelial cells (Ve), and endometrial glands (Eg) of control group mildly stained (b) but strongly in missed abortion group (d). Original magnification (x200).

Figure 4. — Immunohistochemical analysis of Flt: control group (a, b); missed abortion (c, d); Placental villi (a, c); decidua (b, d). Placental syncytiotrophoblast (Sy), cytotrophoblasts (arrow), and vascular endothelial cells (Ve) of the control group was observed to be mildly stained (a) than missed abortion group (c). Decidual cells (Dc) and vascular endothelial cells (Ve) stained strongly and the endometrial glands (Eg) of control group (b) compared to missed abortion cases (d). Original magnification (x200).

Figure 5. — Immunohistochemical analysis of eNOS: Control group (a, b), Missed abortion (c, d); Placental villi (a,c). Decidua (b, d). Placental syncytiotrophoblast (Sy), cytotrophoblasts (Arrow), and stromal cells (Str) was observed strong in control group (a) than the missed abortion group (c). We examined that the decidual cells (Dc) and endometrial glands (Eg) of control group were stained strongly (b) than missed abortion cases (d). Original magnification (x200).
A comparison of the molecular distribution of proangiogenic factors in endometrium of missed abortions and of voluntary etc.

Figure 6. — Immunohistochemical analysis of iNOS: control group (a, b); missed abortion (c, d); placental villi (a, c); decidua (b, d). Placental syncytiotrophoblast (Sy), cytotrophoblasts (Arrow), stromal cells (Str), and vascular endothelial cells (Ve) weakly stained in number (a) in control group (a) than the missed abortion group (c). The decidual cells (Dc) and endometrial glands (Eg) of control group stained strongly (b) compared to missed abortion cases (d). Original magnification (x200).

Figure 7. — Immunohistochemical analysis of Thrombospondin-1: control group (a, b); missed abortion (c, d); placental villi (a, c); decidua (b, d). Placental syncytiotrophoblast (Sy), cytotrophoblasts (arrow), stromal cells (Str), and vascular endothelial cells (Ve) were observed to stain rather slightly (a) compared to missed abortion group (c). The authors examined that the decidual cells (Dc) and endometrial glands (Eg) and vascular endothelial cells (Ve) of control group stained quite slightly (b), whereas the staining of missed abortion cases were increased (d). Original magnification (x200).

Figure 8: — Immunohistochemical analysis of HIF-1α: control group (a, b); missed abortion (c, d); placental villi (a, c); decidua (b, d). Placental syncytiotrophoblast (Sy), cytotrophoblasts (arrow), stromal cells (Str), and vascular endothelial cells (Ve) were observed to stain quite slightly in control group (a), whereas the immunoreactivity of syncytiotrophoblast (Sy) in missed abortion was increased (c). Decidual cells (Dc), endometrial glands (Eg), and vascular endothelial cells (Ve) with slight staining of both two groups (b, d). Original magnification (x200).
stronger with eNOS antibody (Figure 5a) than missed abortion group (Figure 5b); Flt and iNOS antibodies stained significantly slighter in the missed abortion group (Figures 4b, 6b) than control group (Figures 4a, 6a). There was no significant immunoreactivity determined between VEGF, Flk, eNOS and HIF-1α of both groups (Figures 2ab, 3ab, 5ab, 8ab). Placental vascular endothelial, Flk, Flt, iNOS and TSP-1 antibodies showed a higher immunoreactivity in the control group (Figures 3a, 4a, 6a, 7a) than the missed abortion group (Figures 3b, 4b, 6b, 7b). There was no significant difference determined between the staining properties and placental endothelial cells, VEGF and HIF-1α antibody (Figures 2ab, 8ab; Table 1).

In the examination of decidual tissues, the immunoreactivities of VEGF, Flt, eNOS and TSP were found higher in the control group (Figures 2c, 4c, 5c, 7c) than in the missed abortion group (Figures 2d, 4d, 5d, 7d), while the immunoreactivity of Flk was determined higher in the missed abortion group (Figure 3d) than the control group (Figure 3c). There was no significant difference between the iNOS and HIF-1α immunoreactivities of decidual cells of the both group (Figures 6cd, 8cd). The VEGF, eNOS and TSP immunoreactivities of endothelial cells of the control group was found higher (Figures 2c, 5c, 7c) than in the missed abortion group (Figures 2d, 5d, 7d), while the Flk, Flt and iNOS activities were found significantly higher in the missed abortion group (Figures 3d).

Table 1. — Angiogenic factors expression in placenta.

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy / mean (max - min)</th>
<th>Missed abortion / mean (max - min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syncytiotrophoblasts</td>
<td>Cytotrophoblast</td>
</tr>
<tr>
<td>VEGF</td>
<td>90 (80-115)</td>
<td>57.5 (45-76)</td>
</tr>
<tr>
<td>FLK</td>
<td>40 (30-76)</td>
<td>30 (20-50)</td>
</tr>
<tr>
<td>FLT</td>
<td>85 (70-100)</td>
<td>30 (30-50)</td>
</tr>
<tr>
<td>eNOS</td>
<td>55 (20-90)</td>
<td>35 (20-75)</td>
</tr>
<tr>
<td>iNOS</td>
<td>105 (75-140)</td>
<td>102.5 (75-135)</td>
</tr>
<tr>
<td>TSP</td>
<td>20 (10-40)</td>
<td>20 (10-30)</td>
</tr>
<tr>
<td>TIA</td>
<td>10 (2-20)</td>
<td>4 (2-10)</td>
</tr>
<tr>
<td>HIF</td>
<td>8 (4-12)</td>
<td>6 (4-10)</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.005

Table 2. — Angiogenic Factors expression in decidua.

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy</th>
<th>Missed abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>VEGF</td>
<td>75 (60-115)</td>
<td>42 (20-60)</td>
</tr>
<tr>
<td>FLK</td>
<td>80 (30-115)</td>
<td>35 (20-50)</td>
</tr>
<tr>
<td>FLT</td>
<td>115 (95-130)</td>
<td>85.5 (55-120)</td>
</tr>
<tr>
<td>eNOS</td>
<td>62.5 (20-90)</td>
<td>55 (20-80)</td>
</tr>
<tr>
<td>iNOS</td>
<td>117.5 (85-145)</td>
<td>102.5 (85-145)</td>
</tr>
<tr>
<td>TSP</td>
<td>20 (10-40)</td>
<td>30 (20-40)</td>
</tr>
<tr>
<td>TIA</td>
<td>10 (6-20)</td>
<td>6 (4-10)</td>
</tr>
<tr>
<td>HIF</td>
<td>10 (6-24)</td>
<td>11 (4-30)</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.005
Discussion

The aim of this study was to compare the distribution of factors having a role in angiogenesis in deciduas of missed abortion and voluntary termination cases, and to study the role of angiogenesis in the etiology of missed abortion.

Although the etiology of missed abortion has not been completely clear, recent studies have provided strong evidence that support the close relation between angiogenesis and embryonic developments [3, 5]. The authors have determined a low VEGF immunoreactivity in all decidual cell samples including decidual vascular endothelial, decidual stromal cells, and endometrial glands of missed abortion group. They have also found a relatively lower VEGF immunoreactivity than the control group in placental syncytiotrophoblast and cytotrophoblast cells. In the study of Coulam et al. in which the VEGF gene polymorphism was researched in the endometrium of fertile but non-pregnant women and women with recurrent pregnancy loss, abnormal gene polymorphism in VEGF could correlate with recurrent pregnancy losses was stated [21]. Nardo examined VEGF expression in the tissues of the normal endometrium, of endometrium in implantation window and of endometrium in early pregnancy period during the menstrual cycle [22]. He determined increased VEGF expression in the cells of endometrial glandular epithelium and endometrial stromal during the late secretory phase and peri-implantation period, and has emphasized the importance of angiogenesis in the development of endometrial tissue, as well as its importance for implantation and the continuation of the pregnancy. When assessing the findings of earlier studies, low VEGF expression in decidual and placental tissues results in insufficient angiogenesis, revealing the role of VEGF in pregnancy loss.

In the histochemical examination conducted, the thickness of trophoblast in the group of missed abortion is thinner than the control group has been observed, whereas the number of blood vessels both in chorionic and in decidual regions has been reduced compared to control group, has been found to be remarkable. Meegdes et al. compared the cases of intrauterine embryonic deaths to the cases of legal pregnancy terminations with normal villous architecture, weaker vessel development, and insufficient angiogenesis have been found in chorionic villi of the cases of intrauterine death [23].

VEGF-R1 (Flt-1) and VEGF-R2 (Flk-1) are the receptors where VEGF with its role in angiogenesis basically carries out its biological activity. Many studies have shown that both receptors (Flt-1, Flk-1) have an obvious role in capillary formation and vascular development during embryogenesis [24-26]. When comparing the immunoreactivity of Flt-1 receptor with the control group, the present authors detected a lower immunoreactivity of Flt-1 in placental vascular endothelial cells, decidual vascular endothelial cells, and the missed abortion group. Muttukrishna et al. researched maternal serum levels in pregnant women with a threatened miscarriage of soluble endothelial growth factor-1 (sFlt-1) and placental growth factor (PIGF) which are angiogenic factors, in asymptomatic pregnant women and non-pregnant women with luteal phase [27]. The samples were reanalyzed on the basis of pregnancy outcome. The pregnancy of 19 women in lower threat group resulted in complete abortion, and relatively lower levels of sFlt-1 compared to pregnancies not resulting in abortion were determined. In the comparison between non-pregnant women and the group of asymptomatic pregnancy, higher levels of sFlt-1 were determined in the group of asymptomatic pregnancy, and the levels of sFlt-1 were also found to rise in early pregnancy. They also pointed out that these proteins could be more accurate projections in predicting subsequent pregnancy loss [27]. In the study of Fong et al. in which the mutation in VEGF-R1 locus was researched in early embryo development in mice, the decidua disorganization of endothelial and abnormal blood vessel formation was observed in cases of mutated VEGF-R1 [28].

When analyzing the immunoreactivity of Flk-1 which is another receptor of VEGF, the authors observed that the immunoreactivity in syncytiotrophoblast and placental vascular endothelial cells was rather low in the group of missed abortion compared to the control group in this study. Moreover, they have determined that the immunoreactivity of Flk-1 in the samples of decidua was higher in the group of missed abortion. In the study of Vuorela et al., they found that there was no significant difference when comparing the immunoreactivity of flt-1 and flk-1 in placental vascular endothelial cells of the cases of missed abortion with the control group. However, they observed a much lower immunoreactivity in the group of missed abortion in decidual vascular endothelial cells, and supposed that the expression changes of vascular endothelial VEGF-R1 and VEGF-R2 in recurrent abortions could be associated with maternal decidua rather than placenta [29]. The present authors have found that the immunoreactivity of flt-1 and flk-1 in placental vascular endothelial cells of the group of missed abortion was lower when compared to healthy controls in this study. The immunoreactivity of flt-1 in decidual vascular endothelial cells was determined to be much higher in the group of control whereas the immunoreactiv-
ity of flk-1 was found to be higher in the group of missed abortion. These results have shown that VEGF has effect over both two receptors in the placental vascular development whereas they also suggested that the different expressions of two receptors in decidua vascular endothelium show an impact over the receptor of flt-1 during the decidual angiogenesis of VEGF.

During the first trimester, placenta develops in a hypoxic environment with the occlusion of extravillous trophoblast of uterine spiral arteries. This physiological hypoxic environment in early pregnancy protects fetus from the harmful and teratogenic effects of oxygen radicals. All cells respond to hypoxia with a number of gene modifications. HIF-1α is an important mediator in this process. In the present study, the cytotrophoblast, syncytiotrophoblast, and stromal cells in the group of missed abortion hardly stained in the analysis of monoclonal antibody to HIF in decidua and placenta tissues whereas there was a mild staining in the placenta samples of the control group. The present authors did not observe any significant difference between the groups of missed abortion and control in terms of HIF immunoreactivity in the study of decidua cells. In the study of Patel et al., the importance of HIF in the differentiation and development of the placenta was addressed [30]. Several studies have indicated that the existing physiological hypoxic environment during the development of the placenta in early pregnancy leads to induce HIF-1α, and HIF-1α expressed in the environment contributes to the increase of placental vascularization by enabling angiogenesis to be stimulated through angiogenic factors, such as the growth factors of VEGF, receptors of VEGF or insulin-like receptors [30-32]. Sun et al. stated that the abnormal level of HIF-1α in placenta may play a role in the pathogenesis of preeclampsia by affecting the cytotrophoblast invasion and placental vascular reconstruction through the modulation of VEGF and transcription of sFlt-1 [33].

NO has an important role in angiogenesis and hyperpermeability induced by VEGF. VEGF induces the up-regulation of endothelial nitric oxide synthase (eNOS) enzyme and accordingly, the secretion of NO. Consequently, NO which is produced endogenously increases the synthesis of VEGF. The expression of NO isoforms in human endometrium have not yet been completely clarified. Recent immunohistochemical studies have revealed the presence of eNOS protein in the endometrial epithelial and endothelial cells [34-36]. The expression of eNOS mRNA in endometrium has been proved by in-situ hybridization [37]. In the examination of eNOS monoclonal antibody, the present authors have found that the eNOS immunoreactivity in stromal cells of the chorionic samples of the control group was quite lower than the group of missed abortion. When analyzing decidual eNOS immunoreactivity in the group of missed abortion, the eNOS immunoreactivity in all decidual components has been observed to be relatively lower than the control group. The important role of NO in the process of implantation and pregnancy has been proved in animal studies [38, 39]. Haddad et al. have shown the role of NO in early pregnancy loss in their studies on mice decidua [40]. Fukurama et al. have indicated that the angiogenesis and hyperpermeability induced by VEGF would be inhibited in case that eNOS or its pharmacological blockade is genetically destroyed [20].

Inducible NOS (iNOS) can inhibit angiogenesis by means of the down-regulation of VEGF receptor. Brooks et al. have revealed that iNOS inhibits the neovascularization carried out by VEGF in a mouse model related to ischemic retinopathy [41]. In the examination of the decidual immunoreactivity of iNOS, the present authors have determined that there was a quite high immunoreactivity in the cytotrophoblast, syncytiotrophoblast, and stromal cells of the group of missed abortion. The immunoreactivity in the decidual vascular endothelium of the group of missed abortion was found to be relatively higher than the control group in this examination of the decidual immunoreactivity of iNOS. The study of Haddad et al. has indicated that NO in mice is produced by decidual macrophages and increases the synthesis of VEGF but iNOS inhibits this effect [40].

TSP-1 is a potential angiogenesis inhibitor with the ability of preventing new vessel formation in endothelial cells in response to the different types of angiogenic stimuli. In the examination of TSP-1 immunoreactivity of the group of missed abortion in the present study, trophoblast and stromal cells were found to be slightly stained whereas the chorionic stromal cells and vascular endothelium of the control group were found to have minor staining. In the evaluation of decidual components, decidual vascular endothelium, decidual cells, and endometrial glands in the control group were observed to have stained slightly while the thrombospondin immunoreactivity in the group of missed abortion was carried out in mild degrees. In the absence of TSP-1, VEGF, and the receptor VEGF-R1 have indicated to increase [17]. Jin et al. have stated that the abnormal expression of TSP-1 in decidua could cause unexplained recurrent pregnancy loss in some women [42].

In conclusion, this study has discovered the significant difference (p < 0.005) between the groups of controlled and missed abortion in the decidual and placental cell components, and has suggested that thrombospondin as iNOS has an impact on abortion through antiangiogenic effect in cases of missed abortions.

References


How does early cognitive behavioural therapy reduce postpartum depression?


Department of Life, Health & Environmental Sciences, University of L’Aquila, L’Aquila (Italy)

Summary

Postpartum depression (PPD) is a frequent mood disorder. Early identification of mothers at risk is crucial to successful prevention. Cognitive Behavioural Therapy (CBT) is an effective preventive therapy. Objectives of this study are to identify mothers at risk for PPD using the Edinburgh Postnatal Depression Scale (EPDS) and to evaluate the efficacy of CBT for the prevention of PPD in these mothers.

Women were recruited during their second postpartum day. Two groups were selected: mothers with high risk (EPDS score ≥ 10) and mothers with low risk (EPDS score <10) of PPD. The first group underwent CBT. Follow-up was carried out at 40 days, three, six, and 12 months after childbirth. APGAR score, neonatal hospitalization, delayed breastfeeding, and cesarean section were significant obstetric risk factors. Mothers at high risk of PPD presented a statistically valid improvement of EPDS score. Mothers with low risk of PPD did not have CBT and showed a higher EPDS score than mothers at high risk at 12 months. PPD prevention is possible through early identification of mothers at risk and early cognitive behavioural therapy.

Key words: Postpartum depression; Cognitive behavioural therapy; Prevention; Risk factors; Edinburgh Postnatal Depression Scale.
with low risk (score <10) of PPD. Both groups were contacted at 40 days, three, six, and 12 months after childbirth and they filled out the EPDS again. Mothers who screened positive with high risk of depression at the screening time (two days after delivery), were referred for a more thorough evaluation by psychiatrists of Postpartum Depression Prevention and Treatment Center of S. Salvatore Hospital. They underwent Cognitive Behavioural Therapy (CBT) in five to ten psychological weekly sessions. Women who scored 10 or more at 40 days, were screened as having PPD. Descriptive analysis were used with mode, frequency, and standard deviation, when it was necessary.

A Spearman non-parametric correlation was used to evaluate the association between continuous variables and EPDS score. Wilcoxon rank-sum test was used to test significant associations between other qualitative variables (mode of delivery, neonatal hospitalization, etc.), and EPDS score. Spearman and Chi-squared were used to test differences about demographic and obstetric variables between the two groups.

**Results**

The study resulted in 151 women of 513 who delivered between January and August 2012, signed informed consent, and completed questionnaire. A total of 252 refused to participate for various reasons (lack of time, lack of interest in the study protocol), and 102 patients did not complete follow up.

Mean maternal age was 33.64 years (DS 5.834), 17.3% were non-EU women, 75.90% were married, for the most part attained secondary educational level (62.30%), 39.40% were full time working, and 91.15% lived with new family (baby’s father and children). Obstetric and psychiatric data of the mothers are outlined in Tables 1 and 2.

Among the 151 new mothers who agreed to participate in the study, 74 (49%) returned the 40-days postpartum questionnaire, 72 participants (47%) returned the three-month postpartum questionnaire, 57 (38%) returned the six-month, and 49 (32%) completed the 12th month follow-up assessment.

At the first assessment, the authors identified 22 mothers (14.5%) with EPDS scores higher than cut-off (score ≥10) and 129 mothers (85.5%) with EPDS score lower than cut-off (score < 10), which represented “mothers at high risk” and “mothers at low risk” of developing PPD, respectively. Among the 74 women who completed EPDS 40 days after delivery, 11 (14.86%) were identified as having signs and symptoms of PPD, showing EPDS score ≥ 10.

Feeling of sadness during current pregnancy showed positive relationship with depressive mood during previous pregnancies (0.294; \(p = 0.010\)) and postpartum period (0.257; \(p = 0.023\)) and negative correlation with marital satisfaction (-0.242; \(p = 0.003\)). Moreover, good partner relationship was positively associated with desired pregnancy (-0.325; \(p = 0.000\), \(p \leq 0.05\)).

### Table 1. Obstetric characteristics of the sample.

<table>
<thead>
<tr>
<th>Obstetrical variables</th>
<th>Range or frequency (%)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic pathology</td>
<td>17 (11%)</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>11 (7%)</td>
<td></td>
</tr>
<tr>
<td>Abortions</td>
<td>38 (25%)</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>76 (50.3%)</td>
<td></td>
</tr>
<tr>
<td>Conception:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>139 (92%)</td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>12 (8%)</td>
<td></td>
</tr>
<tr>
<td>Multiple births</td>
<td>7 (5%)</td>
<td></td>
</tr>
<tr>
<td>Mather hospitalization during pregnancy</td>
<td>21 (14%)</td>
<td></td>
</tr>
<tr>
<td>Medical assumption during pregnancy</td>
<td>45 (30%)</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>28 w - 42 w</td>
<td>38.2 w</td>
</tr>
<tr>
<td>Preterm delivery (&lt;37 weeks)</td>
<td>20 (13%)</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>64(42%)</td>
<td></td>
</tr>
<tr>
<td>Urgent</td>
<td>29 (45%)</td>
<td></td>
</tr>
<tr>
<td>Hours of labor</td>
<td>1 h - 12 h</td>
<td>5.3</td>
</tr>
<tr>
<td>APGAR score &lt; 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1’</td>
<td>9 (6%)</td>
<td></td>
</tr>
<tr>
<td>5’</td>
<td>4 (3%)</td>
<td></td>
</tr>
<tr>
<td>Birth-weight</td>
<td>1700-4630</td>
<td>3230</td>
</tr>
<tr>
<td>Low birth weight (&lt; 2,500g)</td>
<td>6 (4%)</td>
<td></td>
</tr>
<tr>
<td>Neonatal hospitalization</td>
<td>14 (9%)</td>
<td></td>
</tr>
<tr>
<td>Postpartum complications</td>
<td>4 (3%)</td>
<td></td>
</tr>
<tr>
<td>Timing of breastfeeding:</td>
<td>1h 52 m</td>
<td></td>
</tr>
<tr>
<td>&lt; 6 h</td>
<td>95 (63%)</td>
<td></td>
</tr>
<tr>
<td>6-12 h</td>
<td>2 (13%)</td>
<td></td>
</tr>
<tr>
<td>12-24 h</td>
<td>6 (4%)</td>
<td></td>
</tr>
<tr>
<td>24-48 h</td>
<td>18 (12%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>12 (8%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Psychiatric characteristics of the sample.

<table>
<thead>
<tr>
<th>Psychiatric characteristics</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal history of mood disorders</td>
<td>20 (30%)</td>
</tr>
<tr>
<td>Previous prior medical consultation:</td>
<td></td>
</tr>
<tr>
<td>GMD</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Neurologist</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Psychiatrist</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Psychologist</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>Familiar history of psychiatric disorders</td>
<td>31 (20%)</td>
</tr>
<tr>
<td>Depression symptoms during previous pregnancy</td>
<td>18 (12%)</td>
</tr>
<tr>
<td>Depression symptoms during current pregnancy</td>
<td>22 (15%)</td>
</tr>
<tr>
<td>Previous treatment for depression</td>
<td>20 (13%)</td>
</tr>
<tr>
<td>Current psychiatric treatment</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Marital satisfaction</td>
<td></td>
</tr>
<tr>
<td>Excellent relationship</td>
<td>92 (61%)</td>
</tr>
<tr>
<td>Good</td>
<td>54 (36%)</td>
</tr>
<tr>
<td>Sufficient</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Poor</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Quality of sleep</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>30 (20%)</td>
</tr>
<tr>
<td>Good</td>
<td>63 (42%)</td>
</tr>
<tr>
<td>Sufficient</td>
<td>47 (31%)</td>
</tr>
<tr>
<td>Not sufficient</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Poor</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Unplanned/unwanted pregnancy</td>
<td>44 (29%)</td>
</tr>
<tr>
<td>Nausea during pregnancy</td>
<td>47 (31%)</td>
</tr>
</tbody>
</table>

The study resulted in 513 women of 513 who delivered between January and August 2012, signed informed consent, and completed questionnaire. A total of 252 refused to participate for various reasons (lack of time, lack of interest in the study protocol), and 102 patients did not complete follow up.
How does early cognitive behavioural therapy reduce postpartum depression?

The present results have shown that EPDS score at screening time appear to be related to history of depression, depressive symptoms in previous pregnancies and in current gestation, previous treatment for depression, familial history of psychiatric disorders, marital satisfaction, and quality of sleep. The authors noted a significant relationship between the mean EPDS at screening time with delayed breastfeeding (0.201, \( p = 0.01 \)) and between 40 days EPDS score and APGAR score (-0.342, \( p = 0.04 \)) \( (p \leq 0.05) \). In addition, mothers who underwent cesarean section showed more depressive symptoms (mean 48-hour EPDS 6.3, standard deviation 4.1) than mothers who delivered vaginally (mean 48-hour EPDS 5.4, standard deviation 4.8) \( (p = 0.05) \). Moreover, at screening time and at 40 days after delivery EPDS score of mothers of infant admitted to neonatal intensive care unit (mean 48-hour EPDS 8.1, standard deviation 4.6; mean 40 days EPDS 8.1, standard deviation 6.3) was significantly higher than mothers of healthy term infants (mean 48-hour EPDS 5.5, standard deviation 4.4; mean 40 days EPDS 5.1, standard deviation 4.4) \( (p = 0.02) \). Spearman and Chi squared test did not detected significant differences about demographic and obstetric variables between mothers with high risk and mothers with low risk of PPD. On the contrary, the authors identified a positive relationship between mothers with high risk and mothers with low risk of PPD. The present results showed a PPD prevalence in line with the literature (14.86\%) [5, 8]. Although many authors have identified specific risk factors for PPD, such as maternal age [9], race/ethnicity, education level [10], employment status, marital status, and unplanned/unwanted pregnancy [5], data from this study do not support these risk factors. According to most of the literature, the authors noted a significant relationship between PPD and obstetric variables: APGAR score (0.04) [11], neonatal hospitalization in Neonatal Intensive Care Unit (\( p = 0.02 \) ) [12], delayed breastfeeding (\( p = 0.014 \) ) [13,14] and cesarean section (\( p = 0.05 \) ) [15-18]. CBT and other psychological treatments are recommended by National Institute for Health and Clinical Excellence (NICE) guidelines for the treatment of both depressive and anxiety disorders [19]. Besides, numerous authors have demonstrated the effectiveness of CBT in the treatment of PPD. However, there have been few studies evaluating effectiveness of an early cognitive behavioural intervention for his prevention. The present study showed that mothers which underwent CBT had a significant decrease of EPDS score between the first (48 hours after delivery) and the second assessment (40 days after delivery) \( (p = 0.008) \) and between the second and the third assessment \( (p = 0.000) \). In fact, depressive symptom were significantly reduced from pre-intervention to post-intervention. On the contrary, mothers with low risk of PPD, which did not have CBT, showed a slight decline of EPDS scores during the follow-up period, and higher EPDS score than mother at high risk at 12 months.

Discussion

In this study the authors examined demographic, obstetric, and psychiatric risk factors of PPD in the population living in L’Aquila in order to identify mothers at risk for PPD and to evaluate if early CBT could prevent PPD in these mothers. The present results showed a PPD prevalence in line with the literature (14.86\%) [5, 8]. Although many authors have identified specific risk factors for PPD, such as maternal age [9], race/ethnicity, education level [10], employment status, marital status, and unplanned/unwanted pregnancy [5], data from this study do not support these risk factors. According to most of the literature, the authors noted a significant relationship between PPD and obstetric variables: APGAR score (0.04) [11], neonatal hospitalization in Neonatal Intensive Care Unit (\( p = 0.02 \) ) [12], delayed breastfeeding (\( p = 0.014 \) ) [13,14] and cesarean section (\( p = 0.05 \) ) [15-18]. CBT and other psychological treatments are recommended by National Institute for Health and Clinical Excellence (NICE) guidelines for the treatment of both depressive and anxiety disorders [19]. Besides, numerous authors have demonstrated the effectiveness of CBT in the treatment of PPD. However, there have been few studies evaluating effectiveness of an early cognitive behavioural intervention for his prevention. The present study showed that mothers which underwent CBT had a significant decrease of EPDS score between the screening time and the 40 days after delivery assessment and between the first- and
third-month assessments. This finding suggests that early identification and treatment of mothers at high PPD risk can reduce the development of more serious mood disorders. However, the decline of the mean EPDS distribution curve of women at high risk at 40 days’ postpartum might have been due to the fact that they could be affected by “baby blues”. This mood disturbance affects 50% to 80% of new mothers during first two weeks postpartum and usually resolves spontaneously [20]. The recent literature suggests that women with baby blues symptoms are at high risk of developing postpartum major depression [21]. This is the reason why the present authors chose to enroll all mothers in the early postpartum period proposing EPDS as a screening test 48 hours after delivery.

Conclusion

The present results showed that PPD prevention is possible through an early identification of mothers at risk submitting all new mothers to EPDS 48 hours after delivery and providing promptly cognitive behavioural therapy to these mothers. Obstetricians, midwives, and neonatologists should be able to good counseling and screen new-mothers in order to identify the presence of risk factors for PPD and ensure a psychiatric management if necessary. In fact, a multidisciplinary approach may reduce the development of serious mood disturbance and of life-threatening consequences to the mother and her baby.

Limits of the present study are, at first, the small number of the sample and the absence of a control group of high risk mothers that did not have CBT. Despite these, the obtained results are encouraging and show that a brief CBT intervention can prevent the onset of more severe mood disorders in high risk mothers, however, larger studies are needed.

References


Address reprint requests to:
F. PATACCHIOLA, M.D.
Via Tedeschini 7, 02100 Rieti (Italy)
e-mail: felice.patacchiola@libero.it
A different approach to placenta previa accreta: intrauterine gauze compress combined B-Lynch uterine compression suture

M. Kaplanoğlu¹, D.K. Kaplanoğlu², O. Koyuncu³

¹ Adiyaman University School of Medicine, Department of Obstetric and Gynecology, Adiyaman
² Adiyaman Training and Research Hospital, Department of Obstetric and Gynecology, Adiyaman
³ Mustafa Kemal University School of Medicine, Department of Anesthesiology, Antakya (Turkey)

**Summary**

**Objective:** To retrospectively evaluate the effectiveness of intrauterine gauze compress combined B-Lynch uterine compression suture in placenta previa accreta cases. **Materials and Methods:** Five patients who experienced postpartum hemorrhage (PPH) due to placenta previa accreta between January 2009 and March 2013 in the present clinics, who were irresponsive to medical therapy, and that had applied intrauterine gauze compress combined B-Lynch uterine compression suture were analyzed retrospectively. **Results:** Intrauterine gauze compress combined B-Lynch uterine compression sutures were applied in patients in whom medical therapy failed. Intrauterine gauze compresses were removed under sedation. No patients required hysterectomy or any complications. **Discussion:** B-lynch suture in combination with intrauterine gauze compress can be applied easily in placenta previa accreta cases. This is considered to be a highly successful method.

**Key words:** B-lynch suture; Intrauterine gauze compress; Placenta previa; Placenta accreta.

**Introduction**

Postpartum hemorrhage (PPH) is still a significant cause of maternal mortality and morbidity worldwide despite advanced medical and surgical developments. Various uterine compression sutures using similar mechanisms with B-lynch suture which were first described in 1997 have been defined for PPH control. The preferred technique varies, depending on the patient’s clinical condition and physician’s experience [1].

Approximately 18% of all deliveries are complicated with PPH. Furthermore, it is the cause of 25-30% of all maternal deaths and 64.7% of birth-related maternal morbidity are associated with PPH-related pathologies [2, 3]. The most important etiologic factor is uterine atony with the ratio of 75-90% [4]. On the other hand, complication rates are higher in case of placental adhesion anomalies, particularly if they are associated with placenta previa. General antepartum condition of the patient is as important as total blood loss for the prognosis of the patient [5-7]. Another important issue is the knowledge of uterus-preserving approaches as all of the patients are fertile-aged women.

Surgical options are fertility and life-saving in patients with PPH irresponsive to conventional medical therapies. B-lynch suture which makes a bimanual uterine compression-like effect has been used successfully both alone or in combination with other methods. Uterine balloon and gauze compress application in combination with B-lynch suture has been a successful alternative to hysterectomy particularly in placenta accreta-related PPH [8-10].

B-lynch suture application in combination with intrauterine gauze compress and its effectiveness have been evaluated retrospectively in placenta previa accreta-related PPH.

**Materials and Methods**

Five patients who had applied B-lynch suture in combination with intrauterine gauze compress due to placenta previa accreta-related PPH between January 2009 and March 2013 were retrospectively analyzed. A total of 31,697 deliveries occurred in the present clinic within that time and 74 patients who met primary PPH criteria were determined when patient records were analyzed retrospectively. Five patients who had applied B-lynch suture in combination with intrauterine gauze compress were determined. Patients who did not respond well to bilateral uterine artery ligation performed
in accordance with bimanual compression and O’Leary technique [11] was first applied a conventional B-lynch suture using no. 2 vicryl sutures; however final knotting was not done in the uterine segment below the lower segment transverse incision line. Concurrently used compresses were joined to each other and soaked with saline. Tightness and number of the placed gauze compress laces were controlled carefully. After the first gauze compress was passed from cervix, primarily in the cervical region, and thereafter uterine cavity was filled with gauze compresses (Figure 1-3). Afterwards, B-lynch suture was tied and lower segment transverse incision line was sutured as a single line so as not to pass from the compress (Figure 4). Uterotonics were maintained also after the operation. Compresses were gathered on the nurse desk and on the sterile nylon on the floor and they were weighed and counted at the end of the operation. The patient was closely monitored. Hemogram controls were taken two and six hours after the operation. All gauze compresses after counting were removed after sedation in lithotomy positions. Patients who were routinely monitored after the operation were discharged from the hospital. They were administered IV ceftriaxone one gr bid until discharge in postoperative period. Patients were scheduled for outpatient clinic follow ups one week and one month after the operation.

Results

Ratio of PPH was found to be 0.23% in the present clinic within the study period. All patients had the history of previous caesarean section and had undergone operation under urgent conditions due to placenta previa accreta. Ratio of the presented cases among PPH was found to be 6.75%. The common risk factor was considered to be the history of previous caesarean section. Preoperative hepatic and renal function tests were normal and mean he-
moglobin was 9.9 mg/dl (range, 8 - 11.2). Mean operative time was 55 minutes (range, 45-65) and mean blood loss was 1,550 ml (range, 1,050 - 2,100). Combination technique was successfully applied in all patients and hysterectomy was not required. Results of the patients are summarized in Table 1. Patients were not detected to have additional postoperative problems. Mean duration of hospital stay was 3.2 days. Mean duration of postoperative follow up was 20.4 months (range, 6 - 32). Patients did not have any other surgical or medical problems.

Discussion

PPH is a significant cause of maternal mortality and morbidity worldwide. Early and effective interventions are lifesaving. The most common cause is uterus atony. Today, increase in ratio of caesarean sections has led to a significant increase in postpartum hemorrhages developing due to placenta previa and accompanying adhesion anomalies [12]. PPH patients’ occurring in reproductive age has made development of organ-saving surgery mandatory besides being life-saving. For this purpose, B-lynch and various modifications have been successfully used for treatment of hemorrhages related with uterine atony. Aim of this method is to avoid hysterectomy in patients irresponsive to medical treatment. Another advantage of B-lynch suture is that it may be used successfully in combination with surgical methods, like major pelvic artery ligation and intrauterine balloons like Sangstaken-Blackmore tube [13-17]. Main purpose is to obliterate the cavity with sutures applied to uterine walls [18]. This is useful particularly in atony-related PPH. Pyometra, uterine synechia, uterine necrosis, and partial ischemic necrosis are the major complications of the procedure [19, 20].

Bleeding mainly arises from lower segment in placenta previa accreta cases. Therefore B-lynch uterine compression sutures which are in upper segments may be insufficient in these cases. Therefore the authors applied intrauterine gauze compression in combination with B-lynch suture in five cases who were detected to have placenta previa accreta-related PPH. Target of the procedure was direct compression on lower uterine segment which is the main source of hemorrhage and to obliterate uterine cavity. Additional procedures like iliac artery ligation which require surgical experience were not needed and not applied with this method. Uterine tampons were removed under sedation in operative room depending on general condition and mobilization of the patient. No bleeding or adhesion to uterus were encountered during removal.

Another key point of this method is the rapid recognition of the situation plus the quick preparation of gauze compression and sutures. In addition, initially inserted gauze compress should be absolutely pushed until it spans from the cervix to the vagina. This setting will facilitate the postoperative removal of the compress.

In conclusion, this method may be applied particularly in PPH arising from lower uterine segment. An additional surgical intervention like hysterectomy was not required as seen in the present patients. Its main advantages include being easily applicable and not requiring an advanced surgical experience. However infection risk due to intrauterine gauze compress should not be overlooked. Blood amount hidden by the gauze compress through absorption was tried to be avoided by soaking the compress with saline. Small

Table 1. — Summary of patient characteristics and clinical outcomes of use of method for the treatment of PPH.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Parity</th>
<th>Mode of delivery</th>
<th>Initial medical Treatment</th>
<th>Outcome</th>
<th>EBL (ml)</th>
<th>Blood products</th>
<th>Tamponized time (hours)</th>
<th>Number of tampons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>G2P1 (one caesarean)</td>
<td>Emergency LSCS</td>
<td>IV oxytocin infusion 40 units; IV Methylergonovine Rectal misoprostol</td>
<td>Uterus conserved. Uneventful postoperative recovery. Discharge on third POD</td>
<td>1,875</td>
<td>4 units PCT 2 units FFP</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>G3P2 (two caesarean)</td>
<td>Emergency LSCS</td>
<td>IV oxytocin infusion 40 units; IV methylergonovine Rectal misoprostol Intramyometrial methylergonovine</td>
<td>Uterus conserved. Uneventful postoperative recovery. Discharge on fourth POD</td>
<td>1,450</td>
<td>4 units PCT 2 units FFP</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>G5P3 (two caesarean)</td>
<td>Emergency LSCS</td>
<td>IV oxytocin infusion 40 units; IV methylergonovine Intramyometrial methylergonovine</td>
<td>Uterus conserved. Uneventful postoperative recovery. Discharge on third POD</td>
<td>2,100</td>
<td>4 units PCT 2 units FFP</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>G4P2 (two caesarean)</td>
<td>Emergency LSCS</td>
<td>IV oxytocin infusion 40 units; IV methylergonovine Intramyometrial methylergonovine</td>
<td>Uterus conserved. Uneventful postoperative recovery. Discharge on third POD</td>
<td>1,050</td>
<td>2 units PCT 2 units FFP</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>G3P1 (one caesarean)</td>
<td>Emergency LSCS</td>
<td>IV oxytocin infusion 40 units; IV methylergonovine Intramyometrial methylergonovine</td>
<td>Uterus conserved. Uneventful postoperative recovery. Discharge on third POD</td>
<td>1,275</td>
<td>2 units PCT 2 units FFP</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

EBL: estimated blood loss; LSCS: low segment caesarean section; POD: postoperative day; PCT: packed cell transfusion; FFP: fresh frozen plasma.
number of cases and short duration of follow up limit the suggestibility of this procedure. Therefore studies with large number of patients are required.

References


Address reprint requests to:
M. KAPLANOGLU, M.D.
Adiyaman University, School of Medicine,
Department of Obstetrics and Gynecology
Yesilyurt Mah, Sakarya Cad, Celikhan Yolu
Adiyaman (Turkey)
e-mail: mustafakaplanoglu@gmail.com
The outcome and course of pregnancies complicated with fetal neural tube defects

M. Steric1, J. Dukanac Stamenkovic1,2, L. Srbinovic1, T. Janjic1, S. Vrzic Petronijevic1,2, M. Petronijevic1,2, A. Cetkovic1

1 Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade
2 Faculty of Medicine, University of Belgrade, Belgrade
3 Clinic for Gynecology and Obstetrics, Narodni front, Belgrade (Serbia)

Summary

Purpose: The objectives of this study were as follows: to present the course and outcome of pregnancies complicated with neural tube defects, determine the association between prenatal ultrasound diagnoses, and definitive diagnoses after autopsy. Material and Methods: The survey was designed as a retrospective study and included 24 pregnant women who were attending a regular ultrasound examination at the Department of Gynecology and Obstetrics, Clinical Center of Serbia, or patients who were referred from other institutions in Serbia. Results: Neural tube defects are divided into five subgroups: spina bifida, meningocele, myelomeningocele, acrania, and anencephaly. The most frequent in the present study was spina bifida with 67%. All pregnancies complicated with neural tube defects were terminated. Conclusion: Their clinical severity and uncertain cause make them priorities for further research, whether to better target primary preventive measures, to improve in-utero surgery for prenatal repair, or to identify the causative genes to provide an objective basis for individual genetic counselling.

Key words: Neural tube defects; Fetus; Outcome; Pregnancy.

Introduction

The incidence of neural tube defects is two in 1,000 newborns that ranks this anomaly among the most common congenital malformations. The incidence of female children is four times higher than for male. Most of these malformations occur sporadically and are considered to be of multifactorial origin. Anencephaly and spina bifida are the most common neural tube defects [1]. It has long been pointed to the folate deficiency in pregnant women with neural tube malformations, which is why supplementation with folic acid is recommended for all women who plan to become pregnant during the three months before conception until 12 weeks of gestation. Also as risk factors for the development of these abnormalities are known folic acid antagonists such as methotrexate, valproic acid, vitamin A, diabetes, obesity, hyperthermia [2, 3]. However, 90% of children born with neural tube defects do not have any of the known risk factors. In recent years there has been a downward trend in the prevalence of neural tube defects which can be explained by successfully supplementation with folic acid, as well as improved prenatal sonographic diagnosis, and the use of screening protocols that imply the determination of alpha-fetoprotein in human serum. Since closed lesions do not increase the level of alpha-fetoprotein, screening in this case is not efficient. Serum alpha-fetoprotein varies with gestation, therefore it is expressed by the median. Cutoff value that is considered as a positive result in monofetal pregnancy is 2.5 MOM. Given this, the detection rate for anencephaly is over 95%, for open neural tube defects between 65% and 80%, and the false positive rate is between 1% and 3% [5]. Several studies, designed by the type of meta-analysis, suggested the fact that low mother serum vitamin B12 may be an important risk factor in the development of neural tube defects, therefore the authors recommend supplementation with synthetic vitamin B12 to the existing recommendations on the intake of folic acid [6].

The objectives of this study were as follows: to present the course and outcome of pregnancies complicated with fetal neural tube defects, to determine the association between prenatal ultrasound diagnoses, and definitive diagnoses after autopsy and additional analysis.

Matherial and Methods

Time and place of study implementation
The research was conducted at the Department of Gynecology and Obstetrics, Clinical Center of Serbia in the period from January 2002 until December 2012.
Respondents - monitoring units

The study included 24 pregnant women who were attending a regular ultrasound examinations at the Department of Gynecology and Obstetrics, Clinical Center of Serbia, or patients who were referred from other institutions in Serbia. The criteria for inclusion in this study were visualized neural tube defect by ultrasound and insight into the histopathological diagnosis if the pregnancy was terminated. Criteria for exclusion from the study were non-disclosure in the histopathologic diagnosis. Two patients were excluded from the study, because the authors did not have access to histopathologic diagnosis.

Clinical methodology

If during a routine ultrasound examination there was suspected neural tube defect, it was advisable to review patient by a multidisciplinary Consilium for fetal anomalies which consist of the perinatologist, child neurologist, neurosurgeon, and geneticist who followed the further course of pregnancy. In addition to the ultrasound examination, some examinees were advised additional diagnostic methods and analysis to determine the precise diagnosis and etiology of diseases, such as screening for infections toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus (TORCH), karyotyping, and magnetic resonance imaging (MRI).

Statistical methodology

The survey was designed as retrospective study. Information during pregnancy and their outcomes were collected from Consilium for fetal anomalies reports and fetal autopsy reports Observation characteristics that we followed were: maternal age, parity history, history of similar or other congenital anomalies in a family or in previous pregnancies, weeks of gestation at which women first was shown Consilium for fetal anomalies, the total number of consultantive examination, karyotype, MRI findings, mothers’ comorbidities, prenatal sonographic diagnosis of abnormalities, the diagnosis at autopsy or postnatal diagnosis after birth, institution where birth took place, way of delivery (vaginal delivery or cesarean section), child’s sex, and psychomotor development.

In order to determine the correlation between prenatal and final diagnosis, the authors divided them into three groups. In group 1 were pregnancies where the prenatal diagnosis and the final diagnosis after birth or autopsy fully matched, regardless of whether they were isolated or associated anomalies. Group 2 included pregnancies in which diagnosis at birth or after an autopsy confirmed prenatal diagnosis, but in which were discovered cerebral and extracerebral anomalies that were not seen by ultrasound. Group 3 classified anomalies that prenatally could not be diagnosed, so the diagnosis was made after the birth or autopsy or wrong diagnosed malformations.

Statistical analysis included descriptive statistics (integers, percentages of proportion, mean, and standard deviation). The results are presented as figures and in tabular form.

Results

Neural tube defects are divided into five subgroups: spina bifida, meningocele, myelomeningocele, acranius, and anencephaly, which is represented in Figure 1.

The total number of fetuses with spina bifida was 16 (67% of the total number of all diagnosed neural tube defects). The average age of mothers whose pregnancies were complicated with existence of spina bifida was 25.7 years (range 18-40 years). Among them, there were eight (50%) multiparous and it is important to note that none of the respondents had previous pregnancy complicated with anomalies of the central nervous system. The patients were examined by the Consilium team average in the 28th week of gestation. The earliest was sent for examination in the 18th week of gestation, and at the latest in the 37th week. Most of the pregnant women surveyed, 12 (75%), had only one medical consultations, and only one was not at all anticipated in the examinations. Karyotyping was performed in only two patients and the results of one respondent are known to the authors, in which were normal. MRI was performed in three patients, and two findings coincided with ultrasound, while the results of the third one is unknown. Three pregnancies were twin, one after artificial fertilization procedure, whereby findings of the central nervous system of the second twin was normal in both pregnancies.
One respondent mother had hypertension. Spina bifida was isolated in only two (13%) cases, while the remaining 14 (87%) fetuses were diagnosed with associated anomalies (Table 1) wherein internal hydrocephalus was diagnosed in all cases. Only one spina bifida was cervicothoracal.

All 16 pregnancies complicated by the presence of spina bifida were terminated (Table 2) and after termination autopsy was conducted. In the majority of fetuses, 14 (88%), autopsy confirmed the presence of spina bifida, while in two fetuses (12%), spina bifida was not seen; hence the authors can conclude that prenatal ultrasound diagnosis and postnatal final diagnosis were fully matched in ten cases (63%) (Figure 2). Three cases (18%) after autopsy presented associated anomalies such as talipes equinovarus in two fetuses and agenesis of corpus callosum in one fetus, but these anomalies would not have had a significant impact on decision making on further continuation of the pregnancy, as spina bifida anomaly dominates the findings, and in one fetus (6%) prenatal central nervous system anomalies was identified as Dandy Walker malformation, and after the final autopsy diagnosis, it was spina bifida associated with internal hydrocephalus.

In the present study, there were detected four (17%) fetuses with meningocele. The average age of women whose pregnancies were complicated with this malformation was 29.5 years, with the youngest respondent being 21-years-old and the oldest 38 years. Three respondents were nulliparous, and one in the second pregnancy had a history of previous pregnancy complicated with central nervous system anomaly. One respondent was first presented to Consilium in the 29th week of gestation, two in the 32nd, and one in 33rd week. Two respondents were presented to Consilium once, and the other two had two consultative examination, where only one out of four of them did not carry out all planned examinations. Fetal karyotype analysis was performed in none of the cases. MRI was performed in three respondents, while the result was known for two: in one fetal ultrasound fully coincided with the findings of MRI, while the second MRI showed the existence of corpus callosum agenesis that was not visualized by ultrasound. In only one fetus (25%), meningocele was isolated, and in the remaining three (75%) it was associated with other cerebral and extracerebral anomalies (Table 1). Three fetuses had ventriculomegaly and one fetus also had lissencephaly and cerebellum herniation, the second one had agenesis of corpus callosum body, and the third had agenesis of kidney and ureter. All four pregnancies complicated with meningocele were terminated with all the autopsies performed. Prenatal and postnatal diagnoses were fully matched in two respondents, while the result was known for two: in one fetal ultrasound fully coincided with the findings of MRI, while the second MRI showed the existence of corpus callosum agenesis that was not visualized by ultrasound. In only one fetus (25%), meningocele was isolated, and in the remaining three (75%) it was associated with other cerebral and extracerebral anomalies (Table 1). Three fetuses had ventriculomegaly and one fetus also had lissencephaly and cerebellum herniation, the second one had agenesis of corpus callosum body, and the third had agenesis of kidney and ureter. All four pregnancies complicated with meningocele were terminated with all the autopsies performed. Prenatal and postnatal diagnoses were fully matched in two respondents; the third after an autopsy, was diagnosed with agenesis of kidney and ureter, and in the fourth cerebellum herniation (Arnold Chiari type II) and lissencephaly (Figure 2).

This survey included two (13%) fetuses with myelo-meningocele. One respondent was 26-years-old and the first and only time she was presented to the Consilium was in the 34th week of gestation. The third pregnancy had no previous complicated by the existence of anomalies of the central nerv-

Table 1. — Associated anomalies with neural tube defects.

<table>
<thead>
<tr>
<th>Associated anomalies</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventriculomegaly</td>
<td>19</td>
</tr>
<tr>
<td>Corpus callosum agenesis</td>
<td>2</td>
</tr>
<tr>
<td>Cerebellum herniation</td>
<td>3</td>
</tr>
<tr>
<td>Lissencephaly</td>
<td>1</td>
</tr>
<tr>
<td>Talipes equinovarus</td>
<td>2</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>1</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>2</td>
</tr>
<tr>
<td>Ventricular-septal defect</td>
<td>1</td>
</tr>
<tr>
<td>Kidney agenesis</td>
<td>1</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
</tr>
</tbody>
</table>
Table 2. — Outcome of pregnancies complicated with neural tube defects.

<table>
<thead>
<tr>
<th>Anomalies</th>
<th>Number (percentage)</th>
<th>Pregnancy termination</th>
<th>Anomaly confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural tube defects</td>
<td>24 (16.8)</td>
<td>24 (100) 0</td>
<td>22 (92) 2 (8)</td>
</tr>
<tr>
<td>• Spina bifida</td>
<td>16</td>
<td>16 0</td>
<td>14 (88) 2 (12)</td>
</tr>
<tr>
<td>• Meningocele</td>
<td>4</td>
<td>4 0</td>
<td>4 0</td>
</tr>
<tr>
<td>• Myelomeningocele</td>
<td>2</td>
<td>2 0</td>
<td>2 0</td>
</tr>
<tr>
<td>• Acranius</td>
<td>1</td>
<td>1 0</td>
<td>1 0</td>
</tr>
<tr>
<td>• Anencephaly</td>
<td>1</td>
<td>1 0</td>
<td>1 0</td>
</tr>
</tbody>
</table>

Discussion

The greatest number of pregnancies complicated with the existence of open spina bifida were terminated. In 2008, the European Surveillance of Congenital Anomalies (EUROCAT) conducted an analysis of 12 European countries and came to the conclusion that the termination rate of pregnancies complicated with all neural tube defects was 88% [6]. In the study of Biggio et al., 38% of patients whose pregnancies were complicated with open spina bifida of fetus were terminated. In 75% of the patients whose pregnancies were terminated had lumbosacral spina bifida, and 25% thoracic spina bifida. In most cases, 65% were diagnosed with meningomyelocele and meningocele in 5% of the patients; the remaining 30% were myeloschisis. Talipes was diagnosed in 25% of cases where the pregnancy was terminated. The average width of the lateral ventricles where ventriculomegaly was associated with spina bifida was 11 ± 3 mm. The incidence of neural tube defects was four times higher in male than in female fetuses [7]. Ghi et al., showed that all the subjects in their study with open spina bifida had a banana and lemon sign, while they were absent in closed anomalies, so they concluded that on the basis of altered cranial anatomy, it is possible to differentiate open and closed neural tube defects [8]. Cameron et al. in their study showed that the 'lemon' and 'banana' sign were present in 97% of fetuses with spina bifida and in 75% of cases with ventriculomegaly [9]. Research shows that some fetuses with open spina bifida have a better prognosis, and it would be useful to select them prenatally based on sonographic findings [1, 7]. The most important single predictor of postnatal psychomotor development is the lesion level. High lesions have a worse prognosis and may have a lethal outcome. Neonatal mortality in high lesions is about 13%. In the first five years after surgical management of spina bifida, mortality was 35%. The best prognosis is seen in defects at the sacral and lumbosacral levels. Spinal lesions at higher levels are associated with more severe ventriculomegaly. The largest number of lethal outcome occurs as a result of surgical complications or as a result of herniation of the cerebellum. In addition to the level of lesion and presence of ventriculomegaly, as a potential predictor it is also mentioned with talipes, which is associated with open spina bifida in 50% of cases, but so far did not prove a significant correlation with postnatal prognosis [8]. It is important to note that if ventriculomegaly develops after 24 weeks of gestation, the degree of expansion of the lateral ventricles is smaller. According to the literature, ventriculomegaly is associated with open spina bifida in 80% to 95% of cases [10]. It was also observed that the ventriculomegaly degree has significant impact on intellectual and motor development, with this development marred in severe forms of dilated ventricles, but it is not possible to set a limit to the width of the ventricle, which can safely be an indicator of proper psychomotor development. In addition, complications that arise as a result of ventriculo-peritoneal shunt, such as obstruction or infection, can significantly affect the intellectual development than the ventriculomegaly degree [11, 12]. It is known that a large number of patients with open spina bifida have some degree of dysfunction of the sphincters. Only 17% of children do not have problems with urination. Paraplegia is present in 25%, severe paraparesis in 25%, and about 25% of children with no significant extremity dysfunction [13]. If a previous pregnancy was complicated with spina bifida, the risk of its recurrence is ten times higher [6, 14, 15].
The present survey included two (13%) fetuses with myelomeningocele and four (17%) with meningocele. There were only three cases (14%) with isolated spina bifida, meningocele, and myelomeningocele. The remaining 19 (86%) were associated with cerebral and extracerebral anomalies. In all 19 fetuses ventriculomegaly was also present and in 17 (90%) it was hydrocephalus, and the remaining 10% ventriculomegaly was of moderate type. Beside ventriculomegaly, the following malformations were associated: cerebellar herniation in three (16%) fetuses, corpus callosum agenesis, hepatomegaly, and talipes in two (11%), and one fetus (5%) was associated with hypotelorism, lissencephaly, facial dysmorphism, ventricular septal defect, and agenesis of the kidney and ureter. Irish authors have shown that 18% of cases were diagnosed with aneuploidies and that in almost every fetus presented associated structural anomalies. In the present study, all the fetuses had a neat karyogram [16].

Conclusion

The present study showed that all pregnancies complicated with neural tube defects were terminated, but it is important to mention that majority of them were associated with other structural abnormalities.

Neural tube defects provide a multifaceted challenge to epidemiologists, clinicians, and developmental biologists alike. Although their imminent eradication was predicted when prenatal diagnosis was introduced, and again after the discovery of the preventive effects of folic acid; in fact neural tube defects remain one of the commonest categories of birth defects worldwide. Their clinical severity and uncertain cause make them priorities for further research, whether to better target primary preventive measures, to improve in utero surgery for prenatal repair, or to identify the causative genes to provide an objective basis for individual genetic counselling.

References


Address reprint requests to:
M. STERIC, M.D.
Bulevar kralja Aleksandra 149/10
Belgrade (Serbia)
e-mail: steric.milen@gmail.com
Changes and clinical significance of peripheral blood helper T lymphocyte and natural killer (NK) cells in unexplained recurrent spontaneous abortion (URSA) patients after abortion and successful pregnancy

L.Y. Zhu¹, X. Chen¹, Z.Z. Xu¹, L. Xu¹, T. Mao², H. Zhang¹

¹ Department of Obstetrics and Gynecology, the 2nd Affiliated Hospital of Soochow University, Suzhou
² Department of Clinical Laboratory, the 2nd Affiliated Hospital of Soochow University, Suzhou (China)

Summary

Objective: This study aims to investigate the number changes and the clinical significance of the peripheral blood T lymphocyte subsets and NK (natural killer) cells in unexplained recurrent spontaneous abortion (URSA) patients before and after abortion, as well as after successful pregnancy. Materials and Methods: Thirty-nine URSA patients (URSA-abortion group), among who 22 patients were followed up until the final successful parturition (URSA-pregnancy group), 31 normal-pregnancy (NP) cases and 25 normal non-pregnancy (NNP) control cases in which the peripheral blood T lymphocytes and subsets, B cells, and NK cells were assessed flow cytometry. Results: Compared with the URSA-pregnancy group and the NP group, the Th cells and NK cells of the URSA-abortion group increased (p < 0.05); compared with the NNP group, the total number of T cells decreased after the first, second, and third month of the URSA abortion (p < 0.05); Th cells decreased within one to six months of the URSA abortion (p < 0.05); proportion of NK cells was significantly higher in URSA patients (p < 0.05). Conclusion: The abnormal numbers of the peripheral blood T cell subsets and NK cells were related with the occurrence of URSA.

Key words: Recurrent spontaneous abortion; Pregnancy; Helper T lymphocyte; NK cells.

Introduction

Recurrent spontaneous abortion (RSA) refers to the loss of conception product or fetus twice or more than twice before the 28th week of gestation (body weight ≤ 1,000 g) [1], accounting for 1% to 5% of the total pregnancy incidences. Clinical studies have found that the risk of spontaneous abortion of these patients on their subsequent pregnancy was up to 70% - 80% [2]. The repeated loss of fetus would bring great physical and psychological harm towards the pregnant women and families. Currently, there is lack of effective clinical control measures. The etiology is complex, which might be associated with fetal chromosomal abnormalities, maternal immune dysfunction, endocrine abnormalities, uterine anatomic abnormalities, infections, and environmental factors. After near 20 years of researches, it is gradually found that 80% of the unexplained recurrent spontaneous abortion (URSA) is related with the immune factors. Maternal-fetal interface is where the maternal tissues and the fetal components come into direct contact, the most important part of the immune response. Pregnancy is a homograft phenomenon. For the mother, embryo carries semi-allogeneic antigens, evading rejection by the maternal immune system during normal pregnancy. Several mechanisms contribute to immunoregulation of maternal-fetal interface. Abortion is a transplant rejection caused by the abnormal immune tolerance in maternal-fetal interface [3]. Currently, the known mechanism of the maternal-fetal interface immune tolerance is a complex regulatory system co-participated by the regulation of human leukocyte antigen and immune cells such as T lymphocytes, natural killer (NK) cells, macrophages, and dendritic cells [4]. T lymphocytes are the most important cell populations in the immune system, and divided into different subsets according to the different CD markers on their surface. The T lymphocyte subsets in normal body synergize with each other, maintaining the body’s normal immune function. When an exception occurs in the numbers and functions of different lymphocyte subsets, the body might encounter immune disorders [5]. Vujaklija et al. found that the peripheral blood T cell subsets in URSA patients were abnormal [6]. NK cells are the large granular lymphocytes derived from the lymphoid hematopoietic stem cells in bone marrow, accounting for 10% -15% of the total number of human peripheral blood lymphocytes. NK cells could participate the non-specific immune re-
response, without the stimulation of antigen and the participation of antibodies, and could directly kill the virus-infected target cells and tumor cells. Peripheral blood NK cells could migrate into the uterine local immune microenvironment and differentiate into the decidual NK cells through certain hormones or chemokines, and thus leading to the occurrence of URSA [7]. Carlino et al. pointed out that there was a dynamic balance between the peripheral NK cells and the NK which migrated and differentiated into the decidua on the maternal-fetal interface, the abnormal content and function of NK cell were closely related with URSA [8].

In this study, the compared detection was performed among RSA patients, normal-pregnancy (NP) women and normal non-pregnancy (NNP) women, aiming to understand the differences of T cell subsets and NK cells, and long-term follow-up was also performed towards the RSA patients for the comparison of the similarities and differences before and after the abortion, or the pregnancy at the next pregnancy, in order to analyze the relationship of the changes with pregnancy, and to investigate the possible mechanism and the monitoring significance of the changes of T-cell subsets and NK cells in RSA pathogenesis, providing the clinical basis for the diagnosis and prevention of URSA.

Materials and Methods

General information

The conditions of URSA: 1) continuous spontaneous abortions twice or more than twice; 2) chromosome karyotype of the couple were normal; 3) the determination of reproductive hormone was normal, with no endocrine disease history; 4) without the infections of chlamydia trachomatis and mycoplasma urealytium; 5) without organic disease in genital tract; 6) autoimmune antibody was negative; 7) the examination of semen was normal [9].

Grouping

(1) URSA-abortion group: 39 URSA patients were included, who were diagnosed in this hospital in 2008-2011 and met the above criteria after a detailed history and system checks. The pregnancy was confirmed through B-ultrasonic gestational sac-probe, and the pregnancy period was 8–10 weeks. The B-dynamic monitoring showed that the embryo had no growth, no fetal heart or the fetal heart impulse disappeared. (2) URSA-pregnancy group: 22 patients were included, who were hospitalized from 2008 to 2011 and met the above conditions of URSA. The patients were diagnosed as early pregnancy, the B-ultrasound probe reached the gestational sac, and the B-dynamic monitoring exhibited the normal embryo growth and fetal heart. The patients were all successfully followed-up and gave birth. (3) NP group: 31 patients were collected at the same period who accepted the voluntary termination of pregnancy in the clinics of this hospital; the pregnancy time was 8-10 weeks. The patients had no history of spontaneous abortion and had a normal history of pregnancy and delivery, and had all confirmed existence of gestational sac embryo and fetal heart beating through B-ultrasound. (4) NNP group: 25 patients were collected at the same period, without a history of spontaneous abortion while with NP history, when the patients were diagnosed they were not pregnant. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the 2nd Affiliated Hospital of Soochow University. Written informed consent was obtained from all participants.

Specimen collection

URSA-abortion group: three ml peripheral blood was extracted before the uterus-cleaning, the 1st, 2nd, 3rd, 4th, 5th, and 6th month after the abortion; URSA-pregnancy group: three ml peripheral blood was extracted on the third month (eight to ten weeks) of pregnancy; NP group: three ml peripheral blood was extracted on the third month (eight to ten weeks) of pregnancy as the control group. The above blood samples were all detected by flow cytometry.

Detection indicators

T lymphocyte subsets: T cell ratio, helper T lymphocyte (Th) ratio, suppressor T lymphocyte (Ts) ratio, Th/Ts (CD4 + T/CD8 + T) ratio, B cells ratio, and NK cells ratio.

Detection method

After the conventional anticoagulation with heparin, three ml peripheral blood of each group underwent density centrifugation for the peripheral blood mononuclear cells with Ficoll-Hypaque method. The cell suspension was treated with CD3-PERCP/CD4-FITC/CD8-PE/ CD19-PERCP/CD56-PE/CD16-PE/CD3-FITC, then after the incubation and hyalinization, FC500 flow cytometer, with Cell Quest software, was used to sort and calculate the T cell subsets, including T cells ratio, Th cells ratio, Ts cells ratio, Th/Ts (CD4 + T/CD8 + T), B cells ratio of NK cells ratio.

Statistical analysis

SPSS17.0 software was used. The counting data were expressed as mean ± standard deviation, the comparisons of inter- and inner-group were performed with ANOVA, with p < 0.05 considered as statistically significant.

Results

General information

Except the maximum numbers of abortion, there were no significant differences among the other indicators of the four groups (Table 1).

Table 1. Comparison of general information among the three groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Age (years)</th>
<th>Pregnant time (n)</th>
<th>Max abortion times (n)</th>
<th>Pregnancy duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URSA-abortion group</td>
<td>39</td>
<td>28.3 ± 3.22</td>
<td>2.8 ± 0.60</td>
<td>2.8 ± 0.60</td>
<td>65.6 ± 4.73</td>
</tr>
<tr>
<td>URSA-pregnancy group</td>
<td>22</td>
<td>27.4 ± 0.54</td>
<td>3.1 ± 0.40</td>
<td>2.1 ± 0.40</td>
<td>63.3 ± 4.98</td>
</tr>
<tr>
<td>NP group</td>
<td>31</td>
<td>26.6 ± 1.14</td>
<td>2.7 ± 0.46</td>
<td>0.8 ± 0.96</td>
<td>63.5 ± 5.12</td>
</tr>
<tr>
<td>NNP group</td>
<td>25</td>
<td>26.8 ± 2.34</td>
<td>2.6 ± 0.54</td>
<td>0.7 ± 0.34</td>
<td>-</td>
</tr>
</tbody>
</table>

P 0.340 0.094 0.004* 0.188

Note: * inter-group comparison, p < 0.05; - represented no data.
Comparison of immune cells of URSA-abortion group, URSA-pregnancy group and NP group

The comparison among the URSA-abortion group (before uterus-cleaning), the URSA-pregnancy group, and NP group revealed that, the ratios of Th cells and NK cells in the former significantly increased as 37.1 ± 6.87% and 15.2 ± 5.29%, with statistically significant difference (\( p < 0.05 \)); compared with URSA-pregnancy group, the T cells of URSA-abortion group (before curettage) significantly decreased as 66.9 ± 6.96%, with statistically significant difference (\( p < 0.05 \), Table 2, Figure 1).

Dynamic monitoring of a variety of immune cells in URSA-abortion group (after uterus-cleaning)

Compared with the NNP group, the T cells ratios in URSA-abortion group, on the 1\(^{st}\), 2\(^{nd}\) and 3\(^{rd}\) month after abortion, significantly reduced, as 67.1 ± 6.51%, 69.1 ± 5.95%, and 68.6 ± 7.12%, respectively, with statistical significance (\( p < 0.05 \)); the Th (CD4+ T cells) ratios of URSA-abortion group with 1~6 months after abortion significantly decreased as 36.0 ± 7.64%, 36.6 ± 5.43%, 35.0 ± 6.83%, 36.1 ± 6.91%, 36.0 ± 7.34%, 37.7 ± 4.01%, respectively, and the difference was statistically significant (\( p < 0.05 \)); the Th/Ts (CD4+T/CD8+T cells) ratios on the 3\(^{rd}\), 4\(^{th}\), and 6\(^{th}\) month after abortion decreased significantly, as 1.31 ± 0.43, 1.32 ± 0.46, and 1.33 ± 0.36, respectively, with statistically significant difference (\( p < 0.05 \), Table 3, Figures 2, 3).

Discussion

Currently, researches show that URSA is mainly related to immune factors. The immune tolerance on the maternal-fetal interface maintains the normal embryo not to suffer from maternal rejection; once this immune balance is broken, URSA would occur [10, 11]. CD4+T cells are auxiliary/inductive T lymphocytes (Th), which could mediating the cell immunity, and the increasing rate of Th could enhance the maternal immune function, and the immune rejection towards embryo would also increase, resulting in the pregnancy loss [12]. CD8+T cells belong to the killing/suppressive T cells (Ts), which could not only inhibit the B lymphocyte-mediated humoral immunity, but also in-

### Table 2. Comparison of peripheral blood immune cells in URSA-abortion group, URSA-pregnancy group and the NP group.

<table>
<thead>
<tr>
<th>Items</th>
<th>URSA-abortion group (n=39)</th>
<th>URSA-pregnancy group (n=22)</th>
<th>NP group (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells (%)</td>
<td>66.9 ± 6.96*</td>
<td>70.5 ± 5.67</td>
<td>70.4 ± 5.76</td>
</tr>
<tr>
<td>Th cells (%)</td>
<td>37.1 ± 6.87**</td>
<td>35.0 ± 8.24</td>
<td>34.4 ± 5.30</td>
</tr>
<tr>
<td>Ts cells (%)</td>
<td>25.7 ± 6.44</td>
<td>25.7 ± 6.63</td>
<td>25.3 ± 5.26</td>
</tr>
<tr>
<td>Th/Ts</td>
<td>1.5 ± 0.63</td>
<td>1.7 ± 1.03</td>
<td>1.41 ± 0.36</td>
</tr>
<tr>
<td>B cells (%)</td>
<td>11.6 ± 4.23</td>
<td>10.9 ± 3.57</td>
<td>10.0 ± 5.64</td>
</tr>
<tr>
<td>NK cells (%)</td>
<td>15.2 ± 5.29*</td>
<td>11.4 ± 4.73</td>
<td>11.9 ± 3.44</td>
</tr>
</tbody>
</table>

Note: *Compared with NP group, \( p < 0.05 \); **compared with URSA-pregnancy group, \( p < 0.05 \).

### Table 3. Dynamic monitoring of various immune cells (n=39).

<table>
<thead>
<tr>
<th>Items</th>
<th>1(^{st}) month after abortion</th>
<th>2(^{nd}) month after abortion</th>
<th>3(^{rd}) month after abortion</th>
<th>4(^{th}) month after abortion</th>
<th>5(^{th}) month after abortion</th>
<th>6(^{th}) month after abortion</th>
<th>NNP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells (%)</td>
<td>67.1 ± 6.51*</td>
<td>69.1 ± 5.95*</td>
<td>68.6 ± 7.12*</td>
<td>70.6 ± 4.10</td>
<td>70.3 ± 6.15</td>
<td>70.7 ± 6.42</td>
<td>72.8 ± 6.17</td>
</tr>
<tr>
<td>Th cells (%)</td>
<td>36.0 ± 7.64*</td>
<td>36.6 ± 5.43*</td>
<td>35.0 ± 6.83*</td>
<td>36.1 ± 6.91*</td>
<td>36.0 ± 7.34*</td>
<td>37.7 ± 4.01*</td>
<td>41.2 ± 8.53</td>
</tr>
<tr>
<td>Ts cells (%)</td>
<td>26.1 ± 6.78</td>
<td>28.2 ± 7.38</td>
<td>28.4 ± 6.62</td>
<td>29.0 ± 5.80</td>
<td>29.0 ± 5.59</td>
<td>29.0 ± 6.18</td>
<td>26.3 ± 5.81</td>
</tr>
<tr>
<td>Th/Ts</td>
<td>1.51 ± 0.69</td>
<td>1.40 ± 0.46</td>
<td>1.31 ± 0.43*</td>
<td>1.32 ± 0.46*</td>
<td>1.43 ± 0.80</td>
<td>1.33 ± 0.36*</td>
<td>1.69 ± 0.68</td>
</tr>
<tr>
<td>B cells (%)</td>
<td>11.3 ± 3.86</td>
<td>11.5 ± 4.58</td>
<td>10.6 ± 3.74</td>
<td>11.2 ± 3.73</td>
<td>12.4 ± 2.43</td>
<td>11.7 ± 3.31</td>
<td>11.7 ± 2.45</td>
</tr>
<tr>
<td>NK cells (%)</td>
<td>13.9 ± 4.09*</td>
<td>14.5 ± 5.27*</td>
<td>14.4 ± 5.87*</td>
<td>13.0 ± 4.54</td>
<td>15.3 ± 5.83*</td>
<td>14.2 ± 6.15*</td>
<td>10.8 ± 5.42</td>
</tr>
</tbody>
</table>

Note: *Compared with the NNP group, \( p < 0.05 \).
hibit CD4+T cell-mediated tardive hypersensitive reactions and proliferation, thus ensuring the embryonic semi-alloantigens would not be excluded. The above two factors would restrict each other mutually [13]. Under normal circumstances, the ratio of Th/Ts (CD4+T/CD8+T cells) would always maintain a dynamic equilibrium, no matter which one is too low or too high, the regulatory network would lose the balance, causing immune disorders. Warren et al. [14] found that Th/Ts <1.0 or > 2.0 could be regarded as immune dysfunction, leading to the occurrence of URSA. The study revealed that compared with the URSA-pregnancy group and the NP group, the number of Th of the URSA-abortion group (before curettage) significantly increased, therefore it could be considered that the increasing of CD4+T cells might lead to the immune disorders, and thus participating in the occurrence of URSA. Carbone et al. [15] found that the CD4+T cells, T lymphocyte subsets in URSA patients, significantly elevated. Szpakowski et al. [16] also found that the ratio of CD4+T/CD8+T cells changed, the significant increasing of CD4+T cells would cause the pathological pregnancy. These studies supported the results of this study, showing that the occurrence of URSA was related with the CD4+T cells-mediated immune enhancement.

In this study, the dynamic monitoring found that Ts cells of URSA-pregnancy group did not change significantly after abortion, while after abortion, T lymphocytes within three months and CD4+T cells within six months would be significantly lower than those of the NNP group were, and showed a gradual trend to approach near the NNP levels. This abnormality would gradually return to normal levels with time. Therefore, clinical diagnosis could detect the peripheral immune cells, especially the results within three months of abortion, to consider whether the RSA was caused by immune factors.

In recent years, the impacts of NK cells have been increasingly emphasized on in URSA patients, especially that the decidual NK cells are considered to play an important role in the immune tolerance in the maternal-fetal interface. Different from the activation of T cells, during the antigen process proposed by T cell receptor-specific antigen presenting cells (APC), it is necessary to simultaneously recognize the MHC molecule, which would combine antigen and form the complex, to produce the T cell activation signal, while the decidual NK cells, different from the T cells, are non-MHC restricted, with the main roles of inhibiting the KIR to bind non-classical HLA-class antigens, inhibiting the cytotoxic activity of NK cells, secreting a variety of cytokines, and providing nutrition and protection towards the embryo [17]. Peripheral NK and decidual NK are in a state of dynamic equilibrium, but in the current study conditions, there is no safe and noninvasive method to detect the number of decidual NK cells in the pregnant status. Peripheral NK cells are relatively easy to sample, and could perform long-term tracking, which would facilitate the study. According to the surface markers on the peripheral NK cells’ membrane, it could be divided into two subgroups, CD 56+CD 16+ (approximately 95%) and CD 56+CD 16- (about 5%). The former contains cytolytic granules, possessing the immune destruction and repulsion towards the embryo and the latter contains no cytolytic granules, and could produce a variety of cytokines, performing the immune protective and nutritional effects towards the embryo. Kim and Sachs [18] found that the peripheral blood NK cells in URSA patients and normal women did not change before and after pregnancy, and specific analysis towards the NK cell subsets revealed that peripheral blood CD56+CD16+ NK in URSA significantly increased [19]. In this study, compared with URSA-pregnancy group and the NP group, the NK cells of URSA-abortion group (before uterus-cleaning) was up to 15.26 ± 5.29%,
with significant difference, which was presumably consid-
ered as the performance of the maternal enhanced killing ef-
tects towards the embryo. Through dynamic monitoring, it 
was found that, within six months after abortion, NK cells 
significantly increased than the NNP group, and exhibited an 
upward trend, after considering that it would a longer period 
for NK cells to return to normal levels than Th cells after abor-
tion. This study showed that the peripheral blood NK cells of 
the NP group and the URSA-pregnancy group were less than 
12%, while >12% in URSA-abortion group before abortion 
and six months after abortion. Beer et al. [20] and Karami 
et al. [21] found that the patients whose NK cells increased < 
12%, usually could maintain the pregnancy to term, Paparis-
tidis et al. [22] also found that the increasing contents of the 
peripheral NK cells in RSA patients five days after pregnancy 
terminatation could indicate that the last abortion was likely 
 to be an immune abortion, and the patients with peripheral blood 
NK cells >12% would have nearly 90% probability of the oc-
currence of immune abortion, Yoo et al. [23] found that high 
concentrations of NK cells indicated that the subsequent preg-
nancy might still occur spontaneous abortion. It was consid-
ered that the detection of the peripheral blood NK cells level 
changes could diagnose whether the last abortion was immu-
ity abortion or not, and could forecast the outcome of next 
RSA pregnancy.

In addition, in the present study, patients in the URSA-
pregnancy group had a successful pregnancy and gave birth; 
compared with NP group, it was found that there was no sig-
nificant difference in the immune cells, indicating that when 
the peripheral blood Th cells and NK cells of URSA patients 
returned to the normal levels, it might be highly likely to get 
pregnancy and give birth. Therefore, the clinical treatment 
should focus on how to reduce the peripheral blood Th cell 
and NK cell levels of URSA patients in order to achieve the 
therapeutic effect of URSA.

References

standing the physiological mechanism of maternal immune tolerance to 
al.: “Ex vivo functional responses to HLA-A-G differ between blood and 
type of peripheral T lymphocytes, NK cells and expression of CD69 
activation marker in patients with recurrent spontaneous abortions, dur-
Medancic S.S.: “Immunoregulation by Cytolytic Pathways, Macins 
2011, 2, 31.
Sharma S.: “Evolution of non-cytotoxic uterine natural killer cells”. 
et al.: “Recruitment of circulating NK cells through decidual tissues a 
possible mechanism controlling NK cell accumulation in the uterus 
[9] Dokoshaki P., Moghaddam R., Rezvany M., Ghassemi J., Novin M.G., 
Zamani A. et al.: “Repertoire and clonality of T-cell receptor beta vari-
able genes expressed in endometrium and blood T cells of patients with 
[11] Practice Committee of the American Society for Reproductive Medi-
cine.: “Evaluation and treatment of recurrent pregnancy loss: a com-
“Decidual and peripheral blood CD4+CD25+ regulatory T cells in early 
pregnancy subjects and spontaneous abortion cases”. Mol. Hum. 
Reprod., 2004, 10, 347.
dence that CD8 T-cell homeostasis and function intact during 
murine pregnancy”. Immunology, 2010, 131, 426.
al.: “Quantitative abnormalities of peripheral blood distinct T, B, and 
natural killer cell subsets and clinical findings in obstetric antiphos-
[16] Szpakowski A., Malinowski A., Cieslak J., Nowak M., Wilczyński J.R., 
Banasiak M. et al.: “Influence of paternal lymphocyte immunization on the 
selected subpopulations of peripheral blood lymphocytes in women 
with recurrent spontaneous abortions of unknown etiology”. Ginekol. 
Pol., 2003, 74, 288.
C. et al.: “Soluble HLA-G dampens CD94/NKG2A expression and 
differentiation and modulates chemotaxis and cytokine and chemokine secretion in CD56bright and CD56dim NK cells”. Blood, 2011, 118, 5840.
[19] Emmer P.M., Nelen W.L., Steegers E.A., Veerhoek M., Veerhoek M., 
Joosten L.: “Peripheral natural killer cytotoxicity and CD56posCD16pos 
cells increase during early pregnancy in women with a history of re-
[20] Beer A.E., Kwak J.Y., Ruiz J.E.: “Immunophenotypic profiles of pe-
ripheral blood lymphocytes in women with recurrent pregnancy losses 
and in infertile women with multiple failed in vitro fertilization cycles”. 
hancement of peripheral blood CD56(dim) cell and NK cell cytotoxic-
tivity in women with recurrent spontaneous abortion or in vitro 
[22] Paparistidis N., Papadopoulou C., Chiotti A., Papaoannou D., Tsekoura 
C., Keramitsoglou T. et al.: “How valuable is measurement of periph-
eral blood natural killer cells at the time of abortion”? Am. J. Reprod. 
al.: “Peripheral blood NK cell cytotoxicities are negatively correlated 
with CD8(+) T cells in fertile women but not in women with a history 

Address reprint requests to: 
H. ZHANG, M.D. 
Department of Obstetrics and Gynecology, 
The 2nd Affiliated Hospital of Soochow University, 
No 1055 San Xiang Road, 
Suzhou 215004 (China) 
e-mail: hongzhangdoc@126.com
Introduction

Ectopic pregnancy is defined as the embryo implants outside the uterine cavity. It is a relatively common and potentially complicated condition of pregnancy. The incidence of ectopic pregnancy is two percent in all pregnancies [1]. Most ectopic pregnancies occur in the fallopian tube but implantation can also occur in the cervix, ovaries, and abdomen. Risk factors include: pelvic inflammatory disease (PID), infertility, use of an intrauterine device (IUD), previous exposure to diethylstilbestrol (DES), tubal surgery, smoking, previous ectopic pregnancy, and tubal ligation [2].

Ipsilaterally recurrent ectopic pregnancy after partial and total salpingectomy is a rare condition. Especially tubal surgery and previous ectopic pregnancy are risk factors for type of ectopic pregnancy.

In the present study, the authors report a rare case series of patients with a recurrent ipsilateral ectopic pregnancy after partial salpingectomy.

Materials and Methods

This retrospective study was performed between January 2008 and May 2013. In this period, the author detected 168 ectopic pregnancy in the present clinic. Five patients of all ectopic pregnancies were recurrent ipsilaterally ectopic. The demographic features, risk factors, and treatment modalities were recorded. The demographic features included age, smoking, parity, PID history, previous ectopic pregnancy, and treatment modalities. These results were reported as frequencies and percentages as descriptive statistics.

Results

In this study, recurrent ipsilaterally ectopic pregnancy incidence in the present clinic was 2.97%. Common risk factor for this patients were reported as previous tubal ectopic pregnancy and tubal surgery (partial salpingectomy). The demographic features, risk factors and treatment modalities are summarized in Table 1. The average time of previous ectopic pregnancy was 57.6 months. Two patients were smokers. All patients were evaluated with laparotomy and were treated with laparotomic salpingectomy (Figure 1). Average follow up of patients was 14 months. There were no complications in this period.

Discussion

Ectopic pregnancy is an important cause of maternal mortality and morbidity in first trimester pregnancy. The main risk factors for ectopic pregnancy include smoking, previous

<table>
<thead>
<tr>
<th>Age</th>
<th>PID History</th>
<th>Smoking</th>
<th>Location of ectopic pregnancy</th>
<th>Previous surgical procedure</th>
<th>Previous surgical procedure time (months)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>No</td>
<td>Yes</td>
<td>Left tubal remnant</td>
<td>Laparoscopy</td>
<td>48</td>
<td>Laparotomy</td>
</tr>
<tr>
<td>37</td>
<td>No</td>
<td>No</td>
<td>Left tubal remnant</td>
<td>Laparoscopy</td>
<td>72</td>
<td>Laparotomy</td>
</tr>
<tr>
<td>30</td>
<td>No</td>
<td>No</td>
<td>Left tubal remnant</td>
<td>Laparotomy</td>
<td>12</td>
<td>Laparotomy</td>
</tr>
<tr>
<td>28</td>
<td>No</td>
<td>Yes</td>
<td>Right tubal remnant</td>
<td>Laparotomy</td>
<td>60</td>
<td>Laparotomy</td>
</tr>
<tr>
<td>41</td>
<td>No</td>
<td>No</td>
<td>Right tubal remnant</td>
<td>Laparotomy</td>
<td>96</td>
<td>Laparotomy</td>
</tr>
</tbody>
</table>
tubal pregnancy, use of intrauterine device, and previous spontaneous abortion. Also fertility saving surgery in ectopic pregnancy is associated with recurrent ectopic pregnancy (such as recurrent ipsilateral ectopic pregnancy).

The most common site for ectopic pregnancy is in the fallopian tube, especially ampullary region of fallopian tubes. It accounts for 95% of all ectopic pregnancies. The classic triad of amenorrhea, abdominal pain and vaginal bleeding is presented in only 50% of patients with ectopic pregnancy [3]. Serum β-hCG level and especially transvaginal ultrasonography can be useful in early diagnosis of ectopic pregnancy. Ultrasonographic findings suggestive of ectopic pregnancy include an empty uterus with a serum β-hCG level greater than 1,500 mIU/ml cystic or solid tubal or adnexal masses, cul-de sac fluid.

Treatment of ectopic pregnancy is medical or surgical. Medical management may be attempted if the patient remains stable and is reliable. If the patient’s condition deteriorates, surgical management is indicated. The potential advances of medical treatment are the preservation of tubal patency and function. Hyperosmolar glucose, urea, cytotoxic agents (e.g., methotrexate), prostaglandins, and mifepristone can be used in medical treatment of ectopic pregnancy. On the other hand, the surgical intervention for tubal pregnancy can be radical (salpingectomy) or conservative (usually salpingostomy). The type of surgery is according to hemodynamic status of patient, experience of surgeon, and preservation of future fertility [4].

Recurrent ipsilateral ectopic pregnancy following partial salpingectomy is recognised as a potential complication. If conservative methods are not suitable for a patient with tubal pregnancy, total salpingectomy is the preferred option over partial salpingectomy. Although a salpingectomy does not necessarily eradicate all ipsilateral ectopics, it certainly minimises a tubal recurrence on the same side.

There are multiple theories postulated about the basis of recurrent ipsilateral tubal ectopic pregnancies. Firstly, spermatozoon passes through the patent tube into the pouch of Douglas, then travel to fertilize the ovum on the side of the diseased tube. Then the fertilized ovum implants on the stump of previous ectopic site. Second theory, transperitoneal migration is when the fertilized ovum on the side of the normal tube migrates and implants on the tubal stump. A third theory suggests that despite surgical excision, the lumina remain intact or recanalise in the interstitial portion and remnant of the fallopian tube. This permits communication between the endometrial and peritoneal cavities and hence passage of the fertilised ovum or sperm from the uterine cavity to the remnant of fallopian tube [5, 6].

Conclusion
Several etiologic factors are suspected in this disease. However most important factor is tubal surgery for ectopic pregnancy. Every women with a previous ectopic pregnancy would be at high risk for recurrent ectopic pregnancy. Abdominal pain is the most common symptom in ectopic pregnancy. Especially, if her complaint includes pain in the side of previous ectopic pregnancy, amenorrhoeic period, and vaginal spotting; these signs are important for recurrent ipsilateral ectopic pregnancy. Therefore if she has ectopic pregnancy in previous obstetric history, early and detailed ultrasound examination is mandatory. Therefore, clinical suspicion is important for diagnosis. Recurrent ectopic pregnancy is associated with increased risk of rupture and severe bleeding at an early gestational age of pregnancy. After the diagnosis, treatment protocol must be planned, but surgical treatment remains the safest and most effective option for recurrent ipsilateral ectopic pregnancy.

References

Address reprint requests to:
M. KAPLANOĞLU, M.D.
Adıyaman University School of Medicine, Department of Obstetric and Gynecology, Yesilyurt Mah, Sakarya Cad, Celikhan Yolu, Adıyaman (Turkey)
e-mail: mustafakaplanoglu@hotmail.com
Cervical ripening agent dinoprostone for delivery induction in late pregnancy mothers: experiences of 685 cases

C. Liang, D. Xu, J. He
Obstetrics and Gynecology Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang (China)

Summary
The failure of labor induction often requires following cesarean section and suffering of patients. Cervical ripening is therefore critical in clinical care of pregnant mothers. The present study demonstrated the use of dinoprostone in cervical ripening for delivery induction in 685 cases of pregnancy mothers. The authors conclude that dinoprostone is a very useful and safe drug for delivery induction. The combined use of oxytocin and careful monitoring of all body symptoms are important for the clinical safety.

Key words: Dinoprostone; Delivery; Cesarean section; Vaginal delivery; Cervical ripening.

Introduction
The failure of labor induction often requires following cesarean section and suffering of patients [1-3]. Cervical ripening is therefore critical in clinical care of pregnant mothers and the application of cervical ripening agents is with high usefulness [4-8]. The present study examined the use of cervical ripening agents in delivery induction for late pregnancy mothers, in order to provide the efficiency, safety, and clinical management of these agents.

Materials and methods
Clinical data
This study included 685 late pregnancy mothers admitted to the present hospital from April 30, 2009 to May 1, 2010, aged 28.5 ± 3.2 years: 639 cases were first mother, while 46 cases were with experience. Subjects with head disproportion, abnormal birth canal, fetal distress, uterine scar, more than three times full-term pregnancy, and cervical Bishop score less than 6, were excluded. All subjects had no history of allergy to prostaglandin preparations, no asthma, no glaucoma, and no history of cardiovascular disease. The ripening agent was dinoprostone (prostaglandin E2) 10 mg. All patients were informed with this drug administration and written consents were obtained. The study was approved by ethic committee of human medical studies in Zhejiang University School of Medicine (IRB No: ZJU0309-C-GB091).

Among all mothers, the gestational age was 37-42 weeks (mean gestational age 40.2 ± 1.1 weeks), including 150 cases 37-40 weeks, 221 cases of 40-41 weeks, 279 cases of 41-42 weeks, and 35 cases more than 42 weeks. The main reasons for induced delivery include: 458 cases close or exceeding the predicted delivery phase, 125 cases of suspected oligohydramnios, 35 cases of premature rupture of membranes for 24 hours without delivery, 31 cases of suspected fetal distress, 18 cases of gestational hypertension, 13 cases of gestational diabetes, three cases of pregnancy-nephritis, and two cases of thyroid disease during pregnancy.

Cervical ripening procedures
The cervical score was with improved Bishop Score: less than 6 suggests no ripening. Then 10 mg dinoprostone was placed in the posterior fornix, with the subject left for rest of 30 minutes. The dinoprostone was removed in case of (1) delivery or membrane eruption in 24 hours; (2) any overstimulation signs or stress of the babies; (3) no signs of regular contractions after 24 hours.

The following indices were monitored: (1) the Bishop score improvement: 3 was significant effective, and 2 was effective, with both counted for effective rates; (2) in case of any adverse effects such as nausea or vomiting, fever, hypotension, and tachycardia; (3) the time length of dinoprostone placement; (4) the time length since dinoprostone placement to delivery; (5) the approach used for delivery; (6) the mother conditions: postpartum hemorrhage, infection, and birth canal injury; (7) fetal conditions: fetal heart rate changes, amniotic fluid, and neonatal Apgar score.

If there were no signs of delivery 30 minutes after dinoprostone removal, the mothers were treated with intravenous oxytocin at 0.5% (2.5 mU/minute, eight drops per minute) for every half an hour until contractions appeared (maximum 30 drops/minute). The subjects without delivery after 72 hours of cervical ripening were evaluated as failure of induction.

Statistics
The data were analyzed with SPSS13.0 software and the count data were compared with t test across groups. The measurement data were with \( X^2 \) test, and \( p < 0.05 \) was considered as significant. For paired split comparisons, \( p < 0.025 \) was considered as statistically significant.

Results

The effects of cervical ripening by dinoprostone for mothers with different pregnancy weeks
The authors found that among 685 mothers with cervical ripening by dinoprostone treatment, 557 cases showed improved Bishop scores more than 3, 81 cases with more
Table 1. — The effects of cervical ripening by dinoprostone for mothers with different pregnancy weeks.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Cases</th>
<th>Improvement (cases, %)</th>
<th>Vaginal delivery (cases, %)</th>
<th>Cesarean section (cases, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37-40</td>
<td>150</td>
<td>131 (87.3%)</td>
<td>84 (56.0%)</td>
<td>66 (44.0%)</td>
</tr>
<tr>
<td>40-42</td>
<td>500</td>
<td>477 (95.4%)</td>
<td>324 (64.8%)</td>
<td>176 (35.2%)</td>
</tr>
<tr>
<td>&gt; 42</td>
<td>35</td>
<td>30 (85.7%)</td>
<td>20 (57.1%)</td>
<td>15 (42.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>685</td>
<td>638 (93.1%)</td>
<td>428 (62.5%)</td>
<td>257 (37.5%)</td>
</tr>
</tbody>
</table>

than 2, with a total effective rate 93.1%. Among these subjects, they found that the improvement rate for 40-42 weeks mother was significantly better than < 40 weeks group ($X^2 = 12.423, p = 0.001$), while the improvement rate for 40-42 weeks mothers was also better than > 42 weeks group ($X^2 = 6.187, p = 0.013$), suggesting for limited time window of this drug administration.

The modes of delivery included: 428 cases of vaginal delivery (62.5%, including 401 cases of normal delivery, 21 cases with prolonged delivery, and six cases with fetal distress and helper forceps), 257 cases of cesarean section delivery (37.5%). Among these subjects, the authors found that the vaginal delivery rate for 40-42 weeks mothers was significantly better than < 40 weeks mothers ($X^2 = 3.936, p = 0.05$), but not > 42 weeks group ($X^2 = 0.835, p = 0.361$) (Table 1).

The effects of cervical ripening by dinoprostone for mothers with different reasons

The reasons for induction of delivery included: 458 cases of approaching or exceeding the expected delivery phase, 125 cases of suspected oligohydramnios, 35 cases of premature rupture of membranes 24 hours without delivery, 31 cases of suspected fetal distress, and 36 cases of pregnancy complications or comorbidities (Table 2).

The effects of cervical ripening by dinoprostone on pre-delivery and final delivery

The mean time length from dinoprostone placement to delivery induction was 641.6 ± 365.9 minutes; average delivery time was 538.7 ± 256.5 minutes for vaginal delivery mothers (473.2 ± 244.0, 55.8 ± 44.7, and 8.0 ± 6.5 minutes, respectively, for first, second, and third phase of delivery).

The authors further analyzed patients without or with oxytocin addition (one or two days) during delivery into three groups. Interestingly, they found that dinoprostone alone group ($X^2 = 32.328, p = 0.001$) and dinoprostone + one day oxytocin group ($X^2 = 6.908, p = 0.009$) showed higher success rate of vaginal delivery in compared to dinoprostone + two day oxytocin group (Table 3).

The analysis for cases with failure of vaginal delivery

The authors further explored the potential cases of failure of vaginal delivery in the 257 cases of cesarean section in order to isolate risk factors for failure of vaginal delivery (Table 4). They found that the failure of vaginal delivery can be associated with age of the mother ($p = 0.023$), body weight index (kg/m²) ($p = 0.001$), neonatal weight ($p = 0.001$), cervical ripening improvement after dinoprostone application ($p = 0.001$), and the weeks of pregnancy ($p = 0.051$), but not the cervical ripening score before dinoprostone application ($p = 0.153$).

The adverse effects of dinoprostone induced cervical ripening

All body symptoms of pregnancy mothers were carefully monitored. The authors found no signs of nausea, vomiting, hypotension, and tachycardia; without tonic contraction of the uterus and uterine rupture; the average postpartum hemorrhage was 210.3 ± 86.1 ml, with 14 cases of postpartum hemorrhage (12 cases of vaginal delivery and two cases of cesarean section). Five cases showed puerperal infection and 100 cases showed newborn amniotic...
fluid II degrees or more turbid. Six cases showed children bruising asphyxia, with one case of full-month RDS. No other complications were observed.

Discussion

The increased percentage of cesarean section in recent years does not suggest improved neonatal conditions, especially in developing countries [1, 2]. The improperly performed cesarean section might lead to neonatal harm [9, 10]. The successful induction of delivery in prolonged pregnancy therefore is critical to reduce the percentage of cesarean section. The present study demonstrated that the administration of dinoprostone leads to cervical ripening improvement in 93.1% mothers, with 62.5% of vaginal delivery rate. These results proved the clinical usefulness of dinoprostone in cervical ripening induction and preparation for delivery.

It is critical to decide induction of delivery. The first cause was prolonged pregnancy as suggested, followed by suspicious oligohydramnios, and premature rupture of membranes for 24 hours without delivery. In some studies the induction was performed after 12-24 hours following membrane rupture [3, 11-14]; while for post-24 hours cases, the successful rate of induction was 82.9%. In cases of pregnancy complications, the authors believe that the pregnancy should be terminated when expected date is approaching while controlling the symptoms.

It is interesting that mothers at different weeks of pregnancy exhibited different responses to cervical ripening improvement and vaginal delivery rates. The association analysis showed that the age, body-weight index, and neonatal weight are important factors affecting the improvement rate, and therefore the successful rate of delivery induction. The authors further showed that with the addition of oxytocin for one day, the delivery induction was significantly improved [8, 12, 15]. Yet whether oxytocin should be provided further for one to two days when the induction failed is to be discussed.

Last but not least, the use of dinoprostone in cervical ripening induction showed few adverse effects and neonatal symptoms [9, 16-18]. The authors have provided consistent monitoring for contraction and body symptoms every two hours for any potential side effect detection. None of the present cases showed serious complications before and after delivery.

References


Address reprint request to:
J. HE, M.D.
Obstetrics and Gynecology Hospital,
Zhejiang University School of Medicine,
Hangzhou, Zhejiang 310006 (China)
e-mail: hej@zju.edu.cn
Essure microinsert hysteroscopic tubal sterilization: eight-years follow-up results

M. Sakinci1, T. Aksu2, O. Kuru3, M. Ozekinci1, C. Sanhal1

1 Department of Obstetrics and Gynecology, Akdeniz University Medical Faculty, Antalya
2 HRS Ankara Woman Hospital, Ankara
3 Department of Obstetrics and Gynecology, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul (Turkey)

Summary

Objectives: To evaluate the effectiveness and reliability of microinsert hysteroscopic sterilization method at short- and long-term. Materials and Methods: In the period between January 2004 and December 2005, 34 patients who submitted to the present gynecology outpatient clinic seeking for permanent contraception and accepted tubal sterilization with microinsert method were included in this prospective, interventional study. Results: Bilateral microinsert placement was successful in 28 (87.5%) of 32 patients that underwent the procedure. In all of the 30 patients (100%) in whom the placement procedure was attempted, bilateral tubal occlusion was documented by hysterosalpingogram (HSG) including the two patients in whom unilateral placement was carried out. First three procedures were performed under general anesthesia. Local or general anesthesia was not administered in any other cases (97.5%). The mean visual analogue scale score for pain felt during the procedure was 3.1. The mean procedure time was 11.5 ± 4.88 (5-22) minutes, the average time from beginning the procedure to discharge of the patients was 41.7 ± 18.5 (15-94) minutes. One intrauterine pregnancy was detected in one of the patients nine months after cessation of the alternative contraceptive period. This patient was excluded from the follow-up. At short-term all patients rated their microinsert-wearing tolerance as good or excellent. At eighth year, three patients were lost to follow-up. Mean follow-up time was 83.4 ± 15.0 (36-103) months. During 2,420 woman-months of follow-up, no other pregnancies were detected. Almost all of the patients were happy with the procedure and recommended it to a friend. Conclusion: Essure microinsert is a safe, effective, minimally invasive sterilization method which can be performed in outpatient settings without any anesthesia requirement. It appears to be a good alternative to laparoscopic tubal sterilization. The procedure time and the time to discharge are brief. Patient tolerance during the procedure and at long-term is very good.

Key words: Hysteroscopy; Tubal sterilization; Microinsert; Long-term results.

Introduction

In most of the countries, the vast majority of sterilization procedures are performed under general anesthesia by laparoscopy or mini-laparotomy [1]. As expected, these surgical procedures create potential complications of anesthesia. Even they are uncommon, there are some complications as vascular, intestinal, bladder, and uterine damage that necessitate a shift from laparoscopy to laparotomy [2]. The risk is higher in the event of the former abdominal surgery and pelvic adhesions. Additionally, postoperative pain and morbidity are other unintended states [3, 4].

Transcervical tubal sterilization or in other words, hysteroscopic tubal sterilization, eliminates the need for surgical incision or general anesthesia. Transcervical approach favors less pain and shorter time for recovery. Nevertheless, it was not straightforward to define a safe and effective transcervical method. For this purpose, electrocauterization (hot, cold), chemical materials (tissue glues, sclerotic agents), mechanical obstructive devices and polymer corks have already been suggested since mid 1970’s. Unfortunately, none of these methods were not used in daily practice because of the absence of their adequate safety and effectivity [5, 6]. Today, Essure Permanent Birth Control System with microinsert and Adiana with polymer matrix system are the two solely hysteroscopic FDA approved (2002 and 2009, respectively) sterilization methods in routine gynecological practice [7].

In this prospective-interventional study, the authors aimed to evaluate the reliability and effectiveness of Essure microinsert hysteroscopic tubal sterilization procedure with short- and long-term follow-up of the patients.

Materials and Methods

Thirty-four patients who submitted at the present gynecology outpatient clinic desiring permanent contraception and accepted tubal sterilization with microinsert method were enrolled in this prospective, interventional study in the period between January 2004 and December 2005. The study had been approved by the Institutional Review Board of Hacettepe University Medical Faculty and all women who accepted to take part gave written informed consent before enrollment to the study. The inclusion criteria for
the study were: willingness for permanent birth control method, ages between 28-46 years, with regular menstrual periods, a desire to complete the family (at least two or more children), and to accept using an alternative contraceptive method (barrier or oral) after placement of the device for three months. Exclusion criteria were: presence of a known anatomical anomaly precluding tubal cannulation, ambivalence towards permanent contraception, medical history of chronic pelvic pain, severe dyspareunia, severe dysmenorrhea, unexplained abnormal uterine bleeding, presence of any tubal, ovarian, cervical or endometrial pathology, and history of any allergic reaction against contrast agent. Detailed anamnesis was taken from all the participants and physical and pelvic examinations were performed. Cervical smears were obtained if not present during the last one year. A blood pregnancy test was obtained before the procedures. As far as possible, the procedures were scheduled during the proliferative phase of the menstrual cycle between days 7-14 to facilitate the visualization of tubal ostia and to rule out a luteal pregnancy.

Hysteroscopy procedures were performed by the same operator (M. Sakinci) using five-mm, continuous-flow, 30° hysteroscopy device, patients set in dorsal lithotomy position. All but three patients who were operated under general anesthesia were only given a premedication consisting of five mg diazepam and 100 mg flurbiprofen orally, one to two hours before the procedure. No prophylactic antibiotics were administered before the procedure. Hysteroscopies were carried out by vaginoscopic approach without using a tenaculum or speculum not to cause more pain or disturbance in the patients. As far as possible, mechanical dilatation of the cervix was avoided. Uterine cavity distention was provided with automatically controlled electronic irrigation/absorption device enabling 80-150 mm-Hg intrauterine pressure. All stages of the procedure were also visualized by the patient via the monitor of the hysteroscopy system. Tubal cannulation was performed through five Fr (inner diameter 1.7 mm) operative channel of the hysteroscopy. Optimal positioning of the microinsert in the fallopian tube was considered when the proximal end of the insert seen at the tubal ostium measured about five to ten mm. All hysteroscopy procedures were carried out in outpatient settings. The main purpose of the procedures was placement of the microinserts bilaterally in optimal position. A direct pelvic X-ray was obtained one day after the procedure for the first evaluation of the positions of the inserts. Procedure duration and the time to discharge from the beginning of the procedure was noted in all cases. The severity of the pain felt during the procedure was assessed through a questionnaire, assessing the level of pain as no pain, minimal pain, moderate pain, and severe pain and using a ten-cm visual analog scale (VAS). The participants were also contacted by telephone at the procedure day, one day after, and one week after the procedure to ask if there was any complaint of pain, bleeding, cramping, if they were happy or satisfied with the procedure, and the return to daily activities. They were reminded to continue an alternative contraceptive method and not to rely on microinserts until the hysterosalpingography (HSG) which would be performed three months later.

Three months after the microinsert placement procedure, all the patients underwent HSG for evaluation of tubal occlusion and position of microinserts. After HSG procedure patients were again asked if there were happy with the procedure, recommended it to a friend, which alternative contraceptive method they used, if they solely relied on microinsert for contraception, if they encountered an adverse effect or symptom, to what extent they were satisfied with the method, and if they recommend the method to anybody else.

<table>
<thead>
<tr>
<th>Properties</th>
<th>n  = 32†</th>
<th>Age (year)</th>
<th>38.3 ± 4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>67.3 ± 10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI² (kg/m²)</td>
<td>26.6 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravida</td>
<td>4.4 ± 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>2.8 ± 1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The patients microinsert placement procedure was not attempted are not included in the table. All values are given as mean ± standard deviation.
† Body mass index
‡ Age

<table>
<thead>
<tr>
<th>Reasons</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously blocked tube</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Uterine synechia</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Laterally placed tubes</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Tubal spasm</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Nonvisualization of tubal ostia</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>1 (2.9%)</td>
</tr>
</tbody>
</table>

Table 1. — Demographic characteristics of the patients.

Table 2. — Causes for failure to bilaterally place the device.

Results

The mean age of the study population, mean gravida, mean parity, and mean body mass index (BMI) were respectively, 38.3 ± 4.48 (28-46), 4.4 ± 1.79, 2.8 ± 1.00 (2-6), and 26.6 ± 3.72 (20.4 - 33.6) kg/m². Demographic characteristics of the participants are summarized in Table 1.

Placement of Essure microinsert system was attempted in 32 patients out of 34. No attempts were tried in two patients. The tubal ostia were not visualized in two separate hysteroscopy sessions in one of these patients. This patient underwent tubal ligation by laparoscopy. To reach the tubal ostia was impossible in the other patient due to obesity.

For the first three patients (7.5 %) who underwent the procedure, intravenous sedation anesthesia was provided by masked ventilation without endotracheal intubation. The reasons for using general anesthesia were: as this procedure was applied for the first time in the present center for the first two patients and due to the patient’s request in the third patient. Excluding premedication with flurbiprofen and diazepam, no local anesthetic agents or intravenous sedation were administered to the other patients (92.5%).

Tubal cannulation was not possible in two out of 32 patients who underwent a bilateral intervention of microinsert placement (attempted bilateral tubal cannulation). Tubal ostia were placed in an extreme lateral position in one of the patients, as it was impossible to line them onto...
Essure microinsert hysteroscopic tubal sterilization: eight-years follow-up results

the direction of the microinsert catheter, whereas in the other patient the catheter failed to proceed inside the tubes bilaterally due to a previous tubal occlusion or cornual spasm. Tubal ligation was performed in both of these patients by laparoscopy, as a result of their own request.

Microinserts were placed in a total number of 30 patients. Unilateral placement was performed in two patients. Accordingly, bilateral microinsert placement was achieved in 28 out of 32 (87.5%) patients. Positions of the devices were assessed by viewing direct pelvic X-rays of patients during the postoperative day one. All but four patients out of 28 who underwent a bilateral placement procedure (24 women), had positions of the microinserts that were bilaterally optimal and symmetric and bilateral cornual block was documented in their HSGs (Figure 1a, 1b). In one patient out of two who underwent unilateral placement, a microinsert was successfully placed in the right tube during the first session, however the authors failed to cannulate the left tube. After the primary procedure, cannulation process was also attempted during two other sessions, but unfortunately it was not possible. HSG was obtained three months later, and demonstrated no passage of the contrast agent bilaterally in both tubes with and without microinsert and was assessed as bilateral cornual blockage (Figure 1c). Possible pregnancy risk was explained, however the patient insisted that she would not use any contraceptive methods while trusting bilateral blockage. In the other patient, during hysteroscopy, right tubal ostium was monitored while the left tubal ostium was not. The cavity demonstrated a likewise unicornuate uterus appearance. In HSG, synechia totally obliterating the left side of the cavity in a form similar to the unicornuate uterus and unilateral block due to microinsert in the other tube was detected (Figure 1d). After the patient was informed, laparoscopic ligation was performed to the tube at the side where the synechia was present. No intervention was performed on the right tube which was already occluded due to the microinsert. The reasons related with the failure to place microinserts bilaterally are displayed on Table 2. Bilateral placement was performed in a single session in 24 patients out of 28. The remaining four patients underwent a successful microinsert placement procedure in the second session.

As mentioned above, positions of microinserts were evaluated as asymmetric in four patients. No passage of contrast agent bilaterally in HSGs were observed in three of them at third month (Figure 2a, 2b, 2c1). The first two patients who showed asymmetric placement and no passage on their HSGs demonstrated no significant problems during

Figure 1. — 1a-1b: Normal symmetrically-located bilateral microinserts, bilaterally no contrast passage; 1c: Normally placed unilateral microinsert, unilateral cornual block; 1d: Normally located unilateral microinsert, severe synechia obliterating totally left side of the cavity and causing a unicornuate uterus appearance; minimal passage from left uterine cornu.

Figure 2. — 2a-2b: HSG views showing asymmetrically-placed microinserts not allowing any contrast passage; 2c1-2: HSG views obtained one year apart in which no passage, despite asymmetrical microinsert location can be seen, belonging to the patient who conceived at follow-up.
M. Sakinci, T. Aksu, O. Karu, M. Ozekinci, C. Sanhal

their eight years follow-up period (Figure 2a, 2b). The third patient who displayed a HSG film that demonstrated no passage bilaterally is shown in Figure 2c1, became pregnant at month nine of abandoning the alternative contraception method. A six-week pregnancy with positive fetal cardiac activity was detected by transvaginal sonography. The pregnancy was terminated due to the request of the patient and she underwent hysteroscopy again six weeks after her curettage. The tail length of the right and left microinserts were two and six coils, respectively, and both two tails were monitored aligned at the tubal ostium. Despite this, to eliminate myometrial placement, bilateral cannulation of the tubes was re-attempted, unfortunately this attempt had failed. One day after office hysteroscopy a second HSG was performed. It was very interesting that no bilateral passage was monitored in the new HSG (Figure 2c2). Although passage could not be monitored by HSG, the authors thought that the tubal occlusion at the left asymmetric microinsert had not been completed or that the microinsert was placed intra-myometrially located at the cornual region, and therefore laparoscopic tubal sterilization was applied upon request by the patient. No perforating microinsert, extending towards the abdominal cavity from the myometrium was monitored during laparoscopy. Thereby patient ratio that trusted microinsert-insert as the contraceptive method reduced from 100% to 96.7%. This patient was withdrawn from the follow-up.

After bilateral microinsert was placed, the number of coils located inside the uterine cavity was counted and recorded for all the patients. In all but two patients, minimum three and maximum nine coils were left inside the uterine cavity. An excess proximal placement occurred in two patients due to difficulty during bilateral cannulation. Tail length counted in the right ostia was 11 and 12 coils while this number was 12 in the left ostia for both patients. The pelvic X-ray obtained at day 1 demonstrated too proximally but symmetrically located microinserts which were thought as satisfactory positions. Also, bilateral tubal occlusion was detected on HSGs of both patients obtained three months after (Figures 3a1-2 and 3b1-2). No pregnancies or any adverse effects were encountered in these two patients during their eight years follow-up.

In the fourth patient who had asymmetric microinsert placement, the right tube was detected as patent in three month-HSG (Figure 4a1). The authors assumed that the microinsert at the right side was located intra-myometrially.
Thus uterine perforation was encountered in one of the patients (3.3%). This patient was absolutely asymptomatic during and after the procedure. Two days after HSG was obtained, a microinsert was placed again. The right ostium was carefully monitored and the microinsert was successfully placed. The location of the new device was not monitored both in the uterine cavity and no bilateral passage was monitored on HSG at month 3 (Figure 4a1-2) and the patient was advised to abandon the alternative contraception method. No adverse intra-abdominal effect was detected in this patient related with perforation. No significant problems were encountered during the eight years follow-up period.

In the present series, additional to the patient mentioned above, three microinserts were placed in a second patient. However, the reason of the third device in this patient was excess distal placement in the right tube during the initial attempt. The trailing coils of the right microinsert had not been monitored in the uterine cavity. The authors assumed that the microinsert had migrated towards the ampulla section of the tube and therefore decided to perform cannulation again to this tube at the same session. A second microinsert was placed in the right tube in a satisfactory position. The trailing length of the device in the cavity was nine coils. No bilateral passage was monitored in the HSG obtained at month 3. No problems were encountered during the eight years terms of follow-up (Figure 4b1-2).

Obesity and previous abdominal surgical history which both can be accepted as a relative contraindication for laparoscopy did not lead to any negative impact during the placement of bilateral microinserts. Bilateral placement was performed in seven out of eight (26.7%) patients with a BMI more than 30; however unilateral placement was performed in one of the patients due to unilateral tubal occlusion. Fifteen (50%) patients demonstrated a previous history of intra-abdominal surgery. Eighteen (56%) patients had one or more medical problems that may compose a contraindication for anesthesia and laparoscopy, such as diabetes mellitus, hypertension, goiter, asthma, hearth valve disease, and Behcet’s disease.

The average duration regarding the procedures (hysteroscopy procedure, placement of microinserts, and finalization of hysteroscopy procedure) was calculated as 11.5 ± 4.88 (5-22) minutes. The mean duration starting from the beginning of the procedure until the time of discharge was calculated as 41.7 ± 18.5 (15-94) minutes.

Patients were requested to score their pain levels during the procedure and right after the procedure, using a ten-cm VAS. The mean pain score during procedure was 3.1 ± 2.4 (0-8) and 1.6 ± 1.5 (0-5) right after the procedure. Accordingly, 16.6% of the patients (five patients) stated that they felt almost no pain during the procedure while 56.7% of the patients (17 patients) felt a mild pain, 23.4% of the patients (7 patients) felt a moderate pain, and finally one patient (3.3%) felt severe pain. No postoperative analgesics were necessary in 83.4% of the patients. No negative symptoms were observed in patients during discharge and no analgesics were prescribed to any of the patients.

Patients were asked if they experienced complaints such as bleeding, pain, cramps, fever, nausea or dizziness, etc. during the first week of their follow-up period and 27% of the patients (8 patients) stated no bleeding events while 73% (22 patients) informed the authors they experienced bleeding in the form of minimal spotting. This spotting symptom easily recovered within approximately two days. Symptoms such as fever, nausea or dizziness was not observed in any of the patients. Patients were asked to classify the pain they felt as no pain, minimal pain, moderate pain, severe pain, and extreme pain considering the pain felt during their menstruation was classified as moderate pain as a reference point. Accordingly, 36.7% of the patients stated that, they felt no pain during their first week of follow-up while 56.6% felt a minimal pain, and 6.7% felt moderate pain.

When the patients were asked about the degree of satisfaction they felt from the procedure and the level of their happiness during the post-operative period, 97% of the patients found the procedure more simple than they thought and they were very happy of the outcome. Only one patient stated that she felt more pain during the procedure than estimated. After three-months follow-up period, HSG was obtained. 90% of the patients (27 patients) were very happy and 10% were happy with the procedure. All of the patients stated that they have recommended or would recommend the procedure to their friends or relatives. Excluding only one patient, all patients stated that they were able to continue their normal daily activities at the same day with the procedure while one patient had to rest at home for two days.

The mean follow-up period in the present study was 83.4 ± 15.0 (36-103) months. Twenty-six patients completed a eight-year follow-up time. Three patients were lost to follow-up during the various times of the eight years follow-up (two patients were lost at month 60 telephone call and one patient at month 90). Since 2004, excluding the intrauterine pregnancy in only one patient, no pregnancy was detected in 2,420 women-year follow-up period. In one patient, vaginal hysterectomy was performed 73 months after the procedure due to uterine prolapsus. One patient entered menopause during follow-up.

During the long term follow-up after the procedure, four patients (13.3%) stated an increase in the amount of their menstrual bleeding while five patients (16.7%) stated a decrease. No patients suffered from persistent pelvic pain, including the patients who had more than two microinserts placed. Only two (6.7%) patients stated that they felt a slight groin pain from time to time and they were not sure if the pain was related with the microinsert. All the patients interviewed informed the authors that they were quite happy with the result of the procedure and they have recommended it to their friends.
Discussion

To the authors’ knowledge, this is the first study evaluating the efficacy of sterilization and long term data by using Essure microinsert in Turkey. During the follow-up of 2,420 woman-months, no pregnancies except one, which was mentioned above, were detected in this study. In another trial conducted by Arjona et al., 1,630 women were examined for 42 months and three unintended pregnancies were reported [8]. Consistent with the literature, the present rate of tubal occlusion was almost 100% through the correct placement of devices with proper technique [9, 10]. On the other hand, one new gestation had occurred in one patient despite the documentation of tubal block twice via HSG performed one year apart in the same study. Compared to the first HSG, the microinsert at right tube seemed to locate more distally at the second year HSG. Interestingly, the laparoscopy procedure of this participant revealed no myometrial perforation by Essure and unintended gestation may be due to the inadequate fibrosis of tube uterina.

Adiana polymer matrix, another hysteroscopic sterilization method, has similar mechanisms for contraception like Essure, but, the main difference between the two is due to the success rates. In a study about Adiana evaluating the follow-up of 570 patients for five years, 12 unintended gestations were reported [7]. However, Essure, even after the inclusion of all unintended pregnancies in the literature, seems to be the most effective contraceptive method [11].

In this study, all the procedures were carried out in office conditions using only two per oral drugs (ibuprofen and diazepam) due to the minimally invasive nature and short duration of the procedure except in the first three patients. The absence of requirements like general anesthesia, incisions, and narcotic analgesics resulted in markedly decreased postoperative morbidity rate and very short recovery time. Previously reported time for hysteroscopic application of Essure was around 13 minutes in most of the studies [12-14]. Concordant with this data, the mean procedure duration in the present study was 11.5 minutes. The authors also found that the time between the beginning of the procedure and office discharge was only 42 minutes.

Another major advantage of this method was the absence of a marked pain during the procedure. In the present study, vast majority of the participants denied any pain or felt minimal pain at the time of procedure and 3.1 ± 2.4 were the mean pain score detected by using 10-cm VAS. Only five (16.6%) patients that demanded analgesics, underwent intramuscular diclofenac sodium injection. Neither of the patients needed narcotic analgesics nor suffered from postoperative nausea and vomiting. Also, majority of the patients returned to their daily activities at the same day.

Essure system seems as a perfect alternative, particularly for the patients who have unfavorable characteristics either for general anesthesia or laparoscopy. In the present study, the system was successfully performed in a woman with a cardiac arrest history during general anesthesia and another with serious mitral stenosis. Additionally, the number of patients suffering from co-existent diseases that created further risks was high. The procedure was also accomplished with success in patients with relative contraindications for laparoscopy (e.g. previous history of abdominal surgery, obesity).

Two of the disadvantages of Essure system was the necessity of a supplemental contraceptive method in the course of postoperative tubal occlusion for three months and the need for radiologic studies (HSG, X ray, and ultrasonography) in order to diagnose the blockage of tuba uterina. When this method was initially performed, HSG was solely used for the affirmation of intratubal correct placement and tubal blockage, at the end of the third postoperative month. Recent approach recommends an early subsequent pelvic X-ray, and in the event of bilateral symmetrical placements, withdrawal of alternative contraceptive method without a control HSG at the third postoperative month. To this approach, HSG is only indicated if the procedure is difficult, painful, and/or the placement of devices is unsatisfactory or asymmetrical on pelvic X-ray [12].

Complications are rarely seen during the implementation of Essure system, if seen these are expected to be minor and clinically insignificant. These can be classified under two headings; complications associated with hysteroscopy (fluid overload, cervical lacerations, and uterine rupture) and complications associated with the system itself (expulsion of device, rupture, and improper placement). The short duration of procedure and the use of small-sized hysteroscopies favor a low complication rate. In a retrospective study including 4,306 Essure microinsert procedure by Povedano et al., the complication rate was reported to be 2.7%. Additionally, the most common complication was vasovagal syncope (1.9%). Device expulsion was seen in 19 (0.4%) cases and 14 of these expulsions were within the first three months [15].

Fortunately, there were no expulsions in the present study. Although proximal placements were seen on control HSG in two cases, complete tubal obstruction was detected in both. A third device was introduced in two cases. First case was the patient with uterine perforation as a result of myometrial placement of one microinsert and the other case was due to the distal placement of one microinsert.

One of the limitations of hysteroscopic sterilization by microinsert method is the inability of bilateral successful placement of the devices due to unpredictable intrauterine or tubal pathologies or anatomic impediments. In this study, bilateral proper placements of devices were achieved in 87.5% of participants. The reasons for failure of bilateral placement of microinserts were formerly obstructed or stenotic tuba uterina, tubal spasm, intrauterine synchiea completely occluding tubal ostium, and laterally placed tubal ostia in which the microinsert catheter and tubal os-
tium could not be brought in the same direction. In a prospective study by Mino et al., 99% of 857 patients had successful placements; on the other hand 15% of procedures were reported as “difficult” by the operators. Anatomical tubal abnormality or tubal spasm were the major reasons of these difficulties [16].

The absence of persistent pelvic pain and low rate of any menstrual cycle changes associated with the procedure and the Essure system itself in follow-up were among the long-term advantages of the microinsert method.

In conclusion, the authors wish to emphasize that hysteroscopic tubal sterilization with Essure system is a minimal invasive and effective method which can be performed without any anesthesia and incisions in office conditions. Additionally, patients having contraindications for general anesthesia and laparoscopy are ideal candidates for Essure system.

Acknowledgement

The authors would like to thank to Ege Medikal firm for the donation of Essure microinsert devices used in the present investigation.

References


Address reprint requests to:
M. SAKINCI, M.D.
Akdeniz University Medical Faculty,
Obstetrics and Gynecology Department,
07059 Antalya (Turkey)
e-mail: mehmetsakinc@hotmail.com
Introduction

Chronic hypertension during pregnancy poses one of the greatest risks to pregnant women and their fetus. According to the Preface Bulletin of The American College of Obstetricians and Gynecologists, chronic hypertension in pregnancy is defined as hypertension present before pregnancy or before 20th week of gestation and not resolved by 12 weeks postpartum. Pregnant women with chronic hypertension are at increased risk for complications and the main complication is superimposed preeclampsia, which occurs in one-third of chronic hypertension patients and may further develop into more severe situation such as eclampsia if left without preventive treatment [1]. Chronic hypertension is also associated with several other adverse pregnancy outcomes, such as premature birth, fetal growth restriction, fetal demise, placental abruption, and cesarean delivery. Therefore anti-hypertensive treatments, although some of them carry risk, remain as a viable choice, which deserve cautious trials and assessment.

Calcium channel blocker is the second-line medicine used in pregnant women with chronic hypertension. Its effectiveness is a somewhat controversial but long-acting calcium blockers have been safely used [2]. In the trail of this article, two kinds of long-acting calcium blockers were used: nifedipine controlled-release tablets and amlodipine besylate tablets. The purpose of this trial is to further assess the efficacy of calcium channel blockers in reducing the morbidity of superimposed preeclampsia, mitigating the development into more severe situation such as eclampsia, placental abruption, and other adverse pregnancy outcomes, while improving neonatal outcomes.

Materials and Methods

Thirty-three pregnant women with chronic hypertensive were enrolled at the 1st People’s Hospital of Kunshan from March 2011 to June 2013. According to the criteria established by the Preface Bulletin of American College of Obstetricians and Gynecologists, (ACOG), gestational hypertension was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg on two or more consecutive occasions at six hours apart [3]. Preeclampsia superimposed on chronic hypertension was considered when new-onset or acutely worsening of proteinuria, sudden increase in blood pressure, thrombocytopenia, or elevated liver enzymes occurred after 20th week of gestation. Small for gestational age (SGA) baby was diagnosed with a birth weight lower than the 10th percentile according to a national standard curve for singleton births. Thirty-three pregnant women associated with chronic hypertension received evaluation during their first perinatal clinical visits. All of them received a previous history investi-
Table 1. — Baseline data collected during first clinical counselling (n=33).

<table>
<thead>
<tr>
<th>Items</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.3 ± 5.3</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
</tr>
<tr>
<td>Elementary, or high school</td>
<td>14 (42.42%)</td>
</tr>
<tr>
<td>College or above</td>
<td>19 (57.58%)</td>
</tr>
<tr>
<td>Pre-weight (kg)</td>
<td>68.6 ± 13.8</td>
</tr>
<tr>
<td>Pre-BMI</td>
<td>26.6 ± 5.0</td>
</tr>
<tr>
<td>Gestational week at 1st clinical counselling</td>
<td>16.0 ± 4.0</td>
</tr>
<tr>
<td>Systolic blood pressure at 1st clinical counselling</td>
<td>146.3 ± 12.1</td>
</tr>
<tr>
<td>Diastolic blood pressure at 1st clinical counselling</td>
<td>93.8 ± 7.9</td>
</tr>
<tr>
<td>Primipara</td>
<td>17 (51.5%)</td>
</tr>
<tr>
<td>Multipara</td>
<td>16 (48.5%)</td>
</tr>
</tbody>
</table>

Table 2. — After-medication data collected during delivery (n=33).

<table>
<thead>
<tr>
<th>Items</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure-delivery</td>
<td>148.7 ± 16.4</td>
</tr>
<tr>
<td>Diastolic blood pressure-delivery</td>
<td>97.9 ± 12.6</td>
</tr>
<tr>
<td>Gestational weeks at delivery</td>
<td></td>
</tr>
<tr>
<td>≥ 37</td>
<td>23 (69.7%)</td>
</tr>
<tr>
<td>&lt; 37</td>
<td>10 (30.3%)</td>
</tr>
<tr>
<td>Urine protein</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>20 (60.6%)</td>
</tr>
<tr>
<td>+</td>
<td>13 (39.4%)</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Delivery mode:</td>
<td></td>
</tr>
<tr>
<td>Natural delivery</td>
<td>5 (15.2%)</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>28 (84.8%)</td>
</tr>
<tr>
<td>Small for gestational age (SGA)</td>
<td></td>
</tr>
<tr>
<td>Less than 10th percentage</td>
<td>5 (15.1%)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3,008.8 ± 629.6</td>
</tr>
<tr>
<td>≥ 2,500 g n (%)</td>
<td>26 (78.8%)</td>
</tr>
<tr>
<td>Apgar (at 1 to 5 minutes)</td>
<td></td>
</tr>
<tr>
<td>9 to 10 points</td>
<td>33 (100%)</td>
</tr>
</tbody>
</table>

Discussion

A primary reason for treating chronic hypertension in pregnancy is to reduce maternal morbidity associated with hypertension [4]. Superimposed preeclampsia is the major adverse pregnancy outcome associated with chronic hypertension. A meta-analysis including 28 randomized trials showed that the antihypertensive treatment significantly reduced the risk of severe hypertension, but did not reduce the risk of super-imposed preeclampsia, placental abruption, or growth restriction nor did it improve neonatal outcomes [5]. The present trial indicated that calcium channel blockers as an antihypertensive medication, truly helped reduce the risk of severe hypertension as the blood pressure did not increase in obvious way after medication. Although 39.4% of the present patients developed super-imposed preeclampsia after medication, none of them developed more severe complications such as placental abruption and eclampsia, while Ellan and Heffrey cited that women with chronic hypertension have twice the frequency of placental abruption as normotensive women, 1.56% vs 0.58% [6], hence calcium channel blockers or early intervention may soften the progression into more severe situations. The only drawback to the present trail is that the authors had only 33 patients as a pool, therefore a greater number of patients in the future are required to make a statistical more solid judgment. Even though the antihypertensive treatment in the present trail did not reduce growth restriction as 15% patients gave birth to SGA infants, which just fall in the ballpark of 10-20% incidence Ellen and Heffrey cited from one study in Canada, US, and New Zealand for women with chronic hypertension [6], the Apgar score at one to five minutes for all neonatal infants 100% above 9 is considered a clear indication that neonatal outcomes are improved.
One study by ACOG reported that calcium channel blockers marginally increased the progression to superimposed preeclampsia [7]. In the present trial, 39.4% patients developed superimposed preeclampsia, which was higher than the 30% morbidity reported by ACOG. This result seems to support ACOG’s conclusion but the present authors still hesitate to give the conclusion because the patients’ baseline situations may be different from the ACOG patient group studied. If other abnormal test items were used as criteria on top of blood pressure for classifying mild or severe, several patients in the present trial group were already in a severe situation as baseline, which may induce the final results to be higher. Specifically, ten of these 33 patients were found to have medical diseases, as one was diagnosed with adrenal tumor, another two were diagnosed with retinopathy, and the remaining once complicated with GDM during their first clinical check. If these ten patients could be re-classified as highest risk category according to ACOG’s guidelines, the morbidity of superimposed preeclampsia would be 40% for this category (four out of ten patients) while ACOG reported 75% morbidity among this category [7]. It appears that the superimposed preeclampsia was reduced in this category by calcium channel blockers. In addition, only 30% (three out of ten patients) among this re-classified category had preterm labour before 34th week of gestation while ACOG reported 67%.

Even among the proponents of antihypertensive treatment for chronic hypertension during pregnancy, there is no consensus guideline of the threshold for initiating the use of antihypertensive medications or blood-pressure target in pregnancy in absence of conclusive data from randomized trials. There are only various professional guidelines providing disparate recommendation regarding indications for starting therapy [8], but predominant professional-opinions recommend commencing therapy for only severe hypertension while withholding the therapy for mild hypertension in pregnant women. For example, antihypertensive treatment guidelines from ACOG published in 2001 recommended that pregnant women with hypertension in the blood pressure range of 180 mmHg or greater systolic / 110 mmHg or greater diastolic should be treated with antihypertensive medications [9]; in ACOG’s updated version in February 2012, the threshold for medication was adjusted to 150-160 mmHg systolic / 100-110 mmHg diastolic [10]. However, the present trial adopted a different guideline by commencing therapy from even much milder hypertension levels of 90-95 mmHg diastolic as the threshold for initiating antihypertensive treatment. The reason the present authors began medication at such a mild level is that they believe that major adverse pregnancy outcome are related to the duration and to the severity of the hypertensions. To reduce the duration will soften the development into more severe hypertension and reduce the risk of developing other adverse pregnant outcome. The present trial results support this assumption in that the blood pressure was well-controlled without significant further increase and no placental abruption and eclampsia cases observed.

As nefidpine is a powerful arteriolar vasodilator, it appears to have much potential to lower blood pressure but with risk of being overshot to hypotensive level [11], therefore it requires further assessment of its effect on pregnancy. Amldipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure [12]. There was difference regarding the side effects of these two drugs based on some patients’ chief complaints in the present trial: headache complained in patients who took adalat while patients who took norvasc did not have these kinds of complaints. This seemed to support ACOG’s report on nifedipine’s side effect. However the present authors did not have statistically conclusive data to prove the difference between these two drugs. So further assessment on the side effects and efficacy difference between these two drugs is needed before a conclusion can be reached.

References


Address reprint requests to:
L. LIU, M.D.
Department of Obstetrics and Gynecology
The First People’s Hospital of Kunshan affiliated with Jiangsu University, Suzhou, 215300 (China)
e-mail: SL1012002322@126.com
Assessment of perioperative, early, and late postoperative complications of the inside-out transobturator tape procedure in the treatment of stress urinary incontinence

M. Bozkurt1, A.E. Yumru2, S. Salman2

1 Kafkas University School of Medicine, Department of Obstetrics and Gynecology, Kars
2 Taksim Education and Research Hospital, Department of Obstetrics and Gynecology, Istanbul (Turkey)

Summary

Objective: To evaluate the complications of urinary incontinence surgery with transobturator tape (TVT-O) system and to describe its diagnosis and management. Materials and Methods: A total of 156 patients who were diagnosed as having stress incontinence and mixed incontinence with stress predominance underwent a TOT operation under spinal anesthesia by one surgeon or two surgeons (MB, AEY) from the team. TVT-obturator inside out material was used in the operation. Urodynamic tests and pad tests were done on all the patients. This is a prospective and retrospective study of the complications of TVT-O. The operation was performed under regional anesthesia, as described by Deval et al. Patients were excluded from the study if they had been operated under general or local anesthesia, had undergone any vaginal operations except for anterior repair (cystocele), wanted to have a baby, had severe systemic diseases or had been diagnosed as having urge incontinence in urodynamic tests. These situations may affect the rate of complications, the authors also excluded slings that had materials other than monofilament polypropylene, and patients who were suspected of having neurologic bladder conditions. The bladder and urethra were evaluated using cystoscopy. The durations of the TOT procedure, cystoscopy, and if performed, the cystocele operation, were recorded. Perioperative, early, and late postoperative complications were analyzed by follow-up visits (after two months to four years). Results: Of the 156 patients included in the study, 100 (64.1%) had pure stress urinary incontinence and 56 (35.9%) had mixed incontinence, 20 (12.8%) had previous incontinence surgery. The mean duration of follow up was 30.3 ± 7.4 (range 17-42) months. The mean age of the patients was found to be 48.43 ± 6.24 years (range 42-68). The mean parity of the patients was 5.24 ± 2.86 (range 2-13), respectively. Mean maximum detrusor pressure was 10.30 ± 4.08 and the mean ALP value was 80.80 ± 25.57. Mean operative time was found to be 13.8 ± 5.16 min in patients who underwent only TOT and TOT-anterior repair. Vaginal injury including to the lateral fornix (4.4%), hemorrhaging of more than 200 ml (3.2%), vascular damage (1.9%), hematoma on the leg (1.9%), hemorhaging of more than 500 ml (0.064%), and bladder perforation (1.2%) were detected as perioperative complications. Urethral injury and perioperative nerve and intestinal injury did not occur. The most common complication in early postoperative period was inguinal pain extending the legs (30.7%), followed by headaches (23.7%), fever (12.8%), urinary tract infection (5.7%), and urinary retention (3.2%), respectively. Late postoperative complications included vaginal erosion (4.4%), de novo urge incontinence (8.9%), de novo dyspareunia (7.1%), perineal pain (4.4%), and worsening urgency (8.9%). Conclusion: Although the TVT-O technique is a minimal invasive surgery method applied to treat the urinary incontinence surgically, it does not imply that it is a complication-free surgical procedure. Despite the low incidence of intraoperative complications, there is a mild risk of early and late postoperative complications. Fortunately these complications can be taken under control by either conservative and simple medical treatments or surgical procedures.

Key words: Urinary incontinence; Complications of TVT-O; Bladder injury; De novo urge incontinence; Mesh erosion.

Introduction

The inside-out transobturator tape (TVT-O) (a monofilament macroporous, knitted, polypropylene mesh with pore size >75 mm) procedure, which is a simple and effective treatment modality for the treatment of female stress urinary incontinence, was first described by Deval et al. in 2003 [1]. Reduction of potential peri- and postoperative complications that may develop following the retropubic tension-free suburethral sling systems (TVT) widely used worldwide may be possible through newly developing surgical techniques (e.g. TVT-O) in treatment of female stress urinary incontinence. Complication rates of mid-urethral sling operations are low, however it is known that complications are reported less than those occurring [2, 3]. The transobturator tape procedure may be performed inside-out and also outside-in. Two types of transobturator approaches have been performed over the past ten years: the inside-out (TVT-O) and outside-in transobturator tape (TOT). Anatomic dissections have indicated that performing the TOT procedure inside-out significantly reduces bladder, external iliac, and epigastric vein injuries through reducing the probability of reaching the inferior part of pelvis. However, studies have shown that TOT procedures may lead to...
a risk to the obturator and vaginal vessels although they preserve the retropubic area [4, 5]. The TOT procedure is an effective surgical treatment for female stress urinary incontinence. However, data concerning its safety are rare, follow-up is frequent, and complications are probably under-reported. Several variables have an impact on the epidemiology of mid-urethral sling (MUS) related complications. Lack of worldwide national registers of all MUS procedures signifies that often investigators do not have the denominator for calculating the true incidence of complications; a discrepancy exists between complication rates in scientific reports and independent databases such as the Manufacturer and User Facility Device Experience (MAUDE), which monitors voluntary reporting of MUS-related complications. Deng et al. recently investigated the incidence of major MUS-related complications in the American population and found that they were underreported. A significant discrepancy emerged between scientific reports in English and Food and Drug Administration (FDA)/MAUDE reports, which collected four times as many major complications [6].

The aim of this paper was to describe perioperative, early and late complications associated with TVT-O procedures.

Materials and Methods

Patient selection

A total of 156 patients who were admitted with stress incontinence to the Gynecology and Obstetrics Clinics of Taksim Research and Training Hospital and Şırnak Idil State Hospital between May 2005 and January 2010 were included in this prospective study.

Preoperative assessment

A detailed anamnesis including the duration and severity of stress incontinence was obtained from the patients. All patients had symptoms for more than four years. Symptoms were found to be grade 2 (grade 1-3) according to the Ingelman-Sundberg scale. Vaginal examination and transvaginal ultrasonography were performed on all patients. All menopausal patients were administered local estrogen treatment and 112 of the patients participating to the research were sexually active. For urogynecologic evaluation, a stress test was applied in standing and lying positions after the urinary bladder had been expanded with 300 ml isotonic solution. Twenty-four and 48 hour pad follow-ups were performed. Cystometry and urodynamic tests were used on all patients for the discrimination of stress and urge urinary incontinence. Urodynamic evaluation was done using a specific device. A sterile 8 French dual channel cystometry catheter was placed into the urethra and a rectal catheter with a five-ml balloon was placed into the rectum when the patient was in the lithotomy position. Cystometric assessment was done after the residual urine measurement had been taken. The urinary bladder was filled with saline solution at room temperature at a rate of 50 ml/min and the patient was asked to cough after each 100 ml filling. Urinary incontinence occurred in the while was detected and diagnosis of stress incontinence was made. Urodynamic diagnosis of detrusor instability (urge incontinence) was made upon detection of an elevation of 15 cm H2O or above in basal detrusor pressure in cystometry. All patients were administered two grams of parenteral cefazolin preoperatively for antibacterial prophylaxis. While the patients who underwent only TVT-O did not receive vaginal lavage, this procedure was applied preoperatively to the patients who underwent additional surgery (cystocele). TVT-O procedure was performed as described by Deval et al.

Exclusion criteria

Patients were excluded from the study if they had received general or local anesthesia, had undergone vaginal surgery except for anterior repair, desired to have a baby or if they had severe systemic diseases or mixed urinary incontinence with urge incontinence predominance in urodynamic tests. These situations may affect the rate of complications, the authors also excluded slings that had materials other than monofilament polypropylene, and patients who were suspected of having a neurologic bladder conditions.

Concomitant surgery

Thirty patients diagnosed with anterior wall defect (cystocele) according to the POP-Q staging, underwent anterior wall repair without mesh. Regional (spinal) anesthesia was applied to all patients.

Surgical technique

The anterior wall of the vagina was incised two cm sagittally and two cm below the urethra in the dorsal lithotomy position. The ischiopubic bone was approached digitally by separating the pararectal areas with sharp and blunt dissections. The skin was incised on the line crossing the clitoris and one cm lateral to the ischiopubic ramus. A synthetic prolene band was placed (inside-out) using sloping trocars in order to pass from the suburethral area to near the medial part of the obturator foramen.

Cystoscopy was performed to evaluate the bladder and urethra after the procedure. Patients who had a cystocele operation underwent the additional intervention after the prolene band had been placed. Afterwards, a stress test was applied, the band level was adjusted and the operation was terminated. The duration of the TVT-O, cystoscopy and if performed, the additional operation, were recorded. All patients were monitored with a Foley catheter for bladder drainage for 24 hours. The catheter was removed if residual volume was below 100 ml at the end of 24 hours and intermittent catheterization was performed if post-voiding residual volume was above 100 ml. Similar protocols were applied for pre-, peri- and postoperative assessments in both clinics. In terms of diagnosing the patients with postoperative urinary tract obstruction, conditions such as Qmax below 12 ml/s and pdeQmax above 60 cm H2O have to be fulfilled. All surgical procedures were performed by one or two members (MB, AEY) of the surgical team.

Assessment of complications

An assessment of perioperative and postoperative complications was made for each patient. Perioperative complications were recorded after surgery by the surgeon. Immediate and late postoperative complications were also recorded during the patient’s hospital stay and by the follow-up visit (after two and six months, one and two years up to 42 months). A great majority of the patients had regular follow-ups and medical examinations during which their complaints and vaginal examination findings were reported. In the later years, the patients who were missing their follow-ups were contacted by phone and questioned for late operative complications.

Statistical analysis

Statistical analyses were done using SPSS 15.0. Mean values were estimated using a t-test and ratios were estimated using a Chi-square test. Constant data were given as mean ± standard deviation (SD).
Table 1. — Descriptive characteristics of the cases.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years) range</td>
<td>48.43 ± 6.24 (36-63)</td>
</tr>
<tr>
<td>Mean parity</td>
<td>5.24 ± 2.86 (2-13)</td>
</tr>
<tr>
<td>Mean body mass index</td>
<td>23.7 ± 5.2</td>
</tr>
</tbody>
</table>

Table 2. — Diagnosis and distribution.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress incontinence</td>
<td>100 (64.1%)</td>
</tr>
<tr>
<td>Mixed incontinence</td>
<td>56 (35.9%)</td>
</tr>
<tr>
<td>Previous incontinence surgery</td>
<td>20 (12.8%)</td>
</tr>
<tr>
<td>Concomitant surgery (only anterior repair)</td>
<td>30 (19.2%)</td>
</tr>
<tr>
<td>Sexually active patients</td>
<td>112 (71.7%)</td>
</tr>
<tr>
<td>Number of patients in menopause</td>
<td>64 (41%)</td>
</tr>
</tbody>
</table>

n = patient number.

Results

The mean duration of follow up was 30.3± 7.4 (range 17-42) months. The mean age of the patients was found to be 48.43 ± 6.24 (range 42-68) years. Mean parity of the patients was 5.24 ± 2.86 (range 2-13) and mean body mass index was found to be 23.7 ± 4.8 (Table 1). Diagnosis and distribution of the patients are shown in Table 2 and also diagnosis and distribution of the patients are shown as a graph in Figure 1.

Mean operative time was found to be 13.8 ± 5.16 minutes in patients who underwent only TVT-O and TVT-O anterior

Table 3. — Perioperative complications.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal injury concerning lateral fornix</td>
<td>7 (4.4%)</td>
</tr>
<tr>
<td>Haemorrhage more than 200 ml</td>
<td>5 (3.2%)</td>
</tr>
<tr>
<td>Vascular damage</td>
<td>3 (1.9%)</td>
</tr>
<tr>
<td>Haematoma on the leg</td>
<td>3 (1.9%)</td>
</tr>
<tr>
<td>Haemorrhage more than 500 ml</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Bladder perforation</td>
<td>2 (1.2%)</td>
</tr>
<tr>
<td>Urethral damage</td>
<td>-</td>
</tr>
<tr>
<td>Intestinal injury</td>
<td>-</td>
</tr>
<tr>
<td>Nerve injury</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. — Early postoperative complications.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal pain extending to legs</td>
<td>48 (30.7%)</td>
</tr>
<tr>
<td>Headache</td>
<td>37 (23.7%)</td>
</tr>
<tr>
<td>Fever</td>
<td>20 (12.8%)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>9 (5.7%)</td>
</tr>
<tr>
<td>Urinary retention</td>
<td>5 (3.2%)</td>
</tr>
</tbody>
</table>

Table 5. — Late postoperative complications.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspareunia</td>
<td>8 (7.1%)</td>
</tr>
<tr>
<td>De novo urge incontinence</td>
<td>14 (8.9%)</td>
</tr>
<tr>
<td>Worsening urgency</td>
<td>14 (8.9%)</td>
</tr>
<tr>
<td>Vaginal erosion</td>
<td>7 (4.4%)</td>
</tr>
<tr>
<td>Perineal pain</td>
<td>7 (4.4%)</td>
</tr>
<tr>
<td>Obturator abscess</td>
<td>-</td>
</tr>
</tbody>
</table>
repair. Perioperative, early, and late postoperative complications are shown in Tables 3, 4, 5, and Figures 2, 3, and 4.

Although IUGA/ICS classifications did describe a specific terminology particularly for mesh erosions (mesh erosion into the urinary tract, mesh exposure through the original incision, and mesh extrusion through the vagina other than the incision) in this research, the authors decided to use the most commonly utilized definition, the “mesh erosion” to specify the mesh complications.

Discussion

Retropubic and transobturator midurethral synthetic slings are now considered gold standard procedures for primary surgical treatment of stress urinary incontinence in women. The less invasive nature of MUS has significantly reduced many forms of surgical morbidity. Unfortunately, minimal invasive incontinence surgery does not necessarily guarantee minimal risks. Although the transobturator midurethral sling was designed to minimize bladder and bowel perforation, complications such as thigh pain and neurological pain are known to occur. Bladder perforation is one of the more common intraoperative problems encountered with retropubic midurethral sling placement, occurring in 2.7%–6% of a large series [7-9].

In a multi-center study carried out by Costa et al., the overall perioperative complication rate of the TOT operation was found to be 2.2%. Vascular damage, nerve, and intestinal injury did not occur. Postoperative urinary retention was detected at a rate of 3.3% [10]. Urinary retention was found to be 2.4% in another study and vascular damage, nerve, and intestinal injury did not occur either in this study [11]. In order to definitely ensure that no bladder injury has occurred during the TVT-O procedure a cystoscopy would be necessary and can be recommended although is not the current practise with TVT-O users. The present authors performed a cystoscopic examination in all of their patients to assess the urethra and bladder.

Two perioperative bladder injuries occurred in the present study (0.12%). One of these cases was 64-years-old and had severe atrophic vaginitis and bladder injury occurred during creation of the lateral dissection towards the right obturator area. This case was associated with marked cystocele secondary to a lateral defect and was injury discovered during the cystoscopic examination done for bladder injury. The other patient had a history of previous incontinence surgery and diagnosis was made with urine originating from the vagina. As for the two patients in the present study, bladder perforation usually does not need any further therapy except catheter drainage for two to four days. Concomitant procedures and previous vaginal surgery appear to be risk factors for low urinary tract (LUT) injuries with TVT-O and therefore extra care should be taken during incision and dissection. It should be kept in mind that bladder injury cannot always be detected in cystoscopic examinations; some of these patients may be admitted to the clinic with complaints of urinary tract infection or chronic pelvic pain. LUT injury is seen at a rate of around 1% in the literature and distributed as 0.5% urethral injury and 0.5% bladder injury. Bladder injury was mostly seen in cases operated on using the outside in the technique [12]. None of the present patients developed urethral damage, nor did they experience intestinal or nerve injury. There are multiple reports of bowel injury during urinary incontinence surgery [13, 14]. Fortunately, this is a rare complication. Bowel injury may occur during entry into the retropubic space during an autologous pubovaginal sling or urethroplication, or during passage of needle passers or trocars during midurethral slings. These can be devastating complications leading to sepsis, abscess, and even death [13].

Sivanesan et al. did not encounter bladder or urethra injury in the 167 cases that underwent TOT. The mean operative time (14.7 min., range 12-28 min, median 14 min.) was similar to that found in the present study (13.8 min.). Blood loss was found to be less than 100 ml in 97.8% of the patients [15]. While hemorrhaging more than 200 ml was 3.2%, hemorrhaging more than 500 ml was found to be 0.64% in the present TVT-O procedures. Hemorrhaging is relatively rare in TVT-O applications. Hemorrhage, defined as blood loss greater than 200 ml or postoperative hematoma, occurs in approximately 2% of patients and can usually be managed by observation or local compression. When the causes of hemorrhaging were analyzed in the present study, vaginal plexus injury or absence of a dissection line between the vaginal wall and bladder, or both were seen. One of the intraoperative complications was vaginal bleeding and intramuscular hematoma due to vascular injury. In the present research, vascular injury bleeding cases were higher than the expected, a total number of three patients bled during surgery due to the vascular injury; two of these patients received erythrocyte suspension treatment and the parenteral iron supplementation was adequate to treat the third patient.

In a study by Krauth et al., 0.5% bladder injury, 0.3% vaginal wall injury, 0.8% hemorrhage of 200-300 ml, and 0.33% perineal hematoma were detected. Urethral wall injury was not seen. In the postoperative period, temporal retenption (1.5%), temporal pain (2.3%), urinary tract infection (2.5%), and temporal dysuria (1.3%) were detected [16]. In the present series, urinary retention occurred in seven cases (4.4%).

One of the early postoperative complications was urinary system infections. A urinary tract infection occurred in nine (5.7%) and was managed with oral antibiotics. None of the present patients ever suffered from surgical site infections. The low rate of surgical site infection is due the preoperative antibiotic administration, meticulously applied hemostasis, and overall high fidelity to the antisepsis rules. Postoperative obstruction and urinary retention are another
challenging complication. Urinary retention may first be recognized when the patient fails to empty her bladder at the first voiding after MUS surgery.

While urinary retention rate is 8-17% in TVT, this rate is around 1-3% in TOT [11]. However, high rates as 13.3% which were considered to develop due to urinary retention have also been reported [16]. Of these cases, while four recovered with intermittent catheterization, urethral dilation was needed in two patients and mesh removal was needed in one patient [16]. The most important factor for urinary retention development is thought to be extensive stretching of the suburethral sling system [17]. When the patient cannot void after MUS surgery, many surgeons prefer indwelling bladder catheterization up to one week (three to seven days) and retesting the patient after catheter removal.

In the present study, urinary retention, which the authors evaluated in the postoperative early complications, occurred in five cases (3.2%). While three of these cases recovered with intermittent catheterization, urethral dilation was required in one and mesh removal was required in another. A clinical improvement was obtained in the latter case although the mesh was removed partially. Urinary retention is temporary in most cases as in three cases in the present study.

Subcutaneous and intramuscular hematoma in the inguinal region occurred in three patients (1.92%). Multiple manipulation of the guide and manipulations that cannot be made towards the targeted outlet site have led to this condition.

Some of the most common early postoperative complications can be listed as inguinal pain radiating to the leg, headache, and fever. In the present research, headaches related to the spinal anesthesia can be taken under control by the administration of caffeinated drinks and increased fluid intake. To alleviate the leg pain and fever non-steroidal anti-inflammatory drugs were administered to the patients.

Persistent groin or thigh pain is one of the most important complications of the transobturator route and its incidence after TOT ranges from 8% to 17% [18-20]. A higher incidence of pain in the transobturator approach could be due to the passage of trocars through the tissues (muscles, tendons, and sometimes nerves) in case of TVT-O. The reasons explained for pain in TOT tapes are adductor muscle injury/strain, osteitis pubis, obturator/groin abscess, inflammation, and edema or nerve entrapment of the anterior branch of the obturator nerve, and structural adhesions [21]. To explain the possible reason and pathophysiology of perineal and groin pain, they are associated to recent operation related pain which persists and exist initially or may develop years after the operation.

Postoperative inguinal pain was reported at a higher rate than other complications in TOT procedures. Inguinal pain was reported to disappear within few weeks and two months [11, 18]. In the present study, the most common early postoperative complication was inguinal pain that tended to the legs. Patients experience pain particularly with movements. In the present authors’ opinion, this condition may be explained with the proximity of the band outlet site to the origin of the gracilis and adductor muscles. This unwanted condition, seen in 48 of 156 patients (30.7%), was the most common condition requiring analgesic together with headaches which are a complication of spinal anesthesia.

Pain in the upper part of the thigh was seen in 5% - 26% in the literature [19, 22]. This pain is a subjective finding - pain scales often thought to be objective may vary between individuals - may explain the variability of complication rates.

A regional or local anesthesia is often preferred in retropubic operations and the ones who received spinal anesthesia were analyzed in the present study. Spinal anesthesia-related headache was seen at a rate of 23.7%. Headache was controlled by increasing fluid intake and caffeine containing analgesics. In the present authors’ opinion, use of local anesthesia will lead to a reduction in headache complications.

Vaginal erosion seen in broad range and its incidence is reported to range from 1.7% to 20% in the literature [23-25]. Some possible reasons for vaginal extrusion have been suggested to be wound infection, impaired wound healing, an improper vaginal dissection plane, and vaginal atrophy. Symptoms of vaginal erosion include vaginal discharge, a palpable rough surface in the vagina, sexual discomfort (usually partner related), and lower urinary tract symptoms including hematuria. The mean time to erosions varies, which emphasizes the need to pay attention when symptoms like vaginal discharge, pain or dyspareunia occur even after a long period.

A high index of suspicion is required. The management options are not standardized and range from observation to partial and complete tape excision and reapproximation of the vaginal mucosa over the exposed tape. Mesh erosion that the present authors evaluated in postoperative late complications occurred in seven out of 156 cases (4.4%). One of these patients wanted the mesh to be removed. In six other patients, vaginal repair had been realized using vaginal rhomboid flap technique.

Gambriosio et al. applied TOT to 233 cases and followed up for 27 months. Mesh erosion was found at a rate of 7.6% in this series. The rate of mesh erosion varied between 0% and 17% with varying brands of mesh use [25]. Comparisons with other mesh types could not be made as the same type of mesh was used in the present study.

Another important concern is emergence of de novo urgency after TVT-O procedure. De novo urgency, although claimed to be the complication with the strongest negative impact on quality of life, is sometimes self-limiting. When a patient complains of urgency after surgery, the surgeon must rule out and remove specific causes of urgency, such as urethral erosion, intravesical tape, urinary retention, or
recurrent urinary tract infections. If urgency and urinary urge incontinence persist, oral antimuscarinic agents are first-line therapies. Should they fail, alternatives (intravesical vanilloids, intradetrusor injection of botulinum toxin, and sacral neuromodulation) may be proposed. The hypothesis that tape location closer to the bladder neck correlated with a higher risk of dysfunction.

De novo urge incontinence was investigated by Juma and Brito. In their study, while a persistent urge was detected in 21 out of 130 patients (16%), de novo urge incontinence developed in only one (2%) patient [26].

De novo urge incontinence was found to be 2% - 22.1% in the different series in the literature [26-28]. In another study done in 2011, de novo urge incontinence was reported to be seen 13.4% in the first six months, 19.3% at the 12th month, and 22.1% at the 36th month in patients who had undergone TOT and TVT. De novo urge incontinence was found to be significantly higher in the TVT group compared to the TOT group. De novo urgency was significantly more frequent in the TVT group than in the TOT group at 12 (22.2% vs. 11.2%, \( p = 0.025 \)), 24 (24.8% vs. 12.3%, \( p = 0.033 \)), and 36 (0% vs. 24.7%, \( p = 0.034 \)) months [28]. This study indicates that frequency of de novo urge incontinence increases in parallel with postoperative periods. Recognition of this condition requires subjects to be examined meticulously in postoperative checks. De novo urge incontinence was found to be 8.9% in the present study. Worsening injury was found to be 8.9%. Worsening urgency was reported to be 5-25% following mid-urethral sling surgery [29]. Given that 35.9% of the patients in the present study group had mixed incontinence, 8.9% is an acceptable rate. Mixed incontinence is a risk factor for de novo urge incontinence. In four other patients, the present authors had to prescribe anticholinergic treatment. The symptoms improved in all these four patients who had a good compliance to treatment.

Persistent urge rate was found to be 16.4% in patients who underwent a mid-urethral sling procedure for mixed incontinence [30]. In a review it was reported that mixed incontinence could be treated successfully with a mid-urethral sling however persisting urge symptoms could significantly affect the quality of life of the patients [31].

One of the postoperative complications that impair the quality of life after the mid urethral sling technic is sexual dysfunction. Dyspareunia is only one form of sexual dysfunction but it may occur following anti-incontinence surgery as vaginal anatomy is altered by these types of treatments. The vaginal axis can be shifted, changing the angulation of the vaginal canal, and narrowing of the vagina may occur as a result of aberrant scarring. Dissection along the anterior vaginal wall may result in nerve injury and neuroma formation. In the present study, most women were sexually active (71.8%) and among them 7.1% reported de novo dyspareunia after the operation. In a retrospective cohort study, Cholhan et al. found that none of the 25 patients undergoing retropubic tapes reported dyspareunia whereas four of 17 (24%) of the patients in the TOT group complained of de novo dyspareunia [32]. Cholhan et al. reported the finding of paraurethral bands—anterior vaginal wall banding in the paraurethral folds immediately adjacent to the midurethral placement of the sling in all patients in the TOT group complaining of dyspareunia and in none of the patients in the retropubic group. Contrary to the studies reporting that dyspareunia did not develop following the TOT procedure, studies are also available reporting relatively high rates such as 24% [33, 34].

Finally, in another study investigating complications similar to the present study, 363 patients were followed up for 36 months and 50 (13.8%) patients were seen to develop complications. Twenty-one patients (42%) developed irritative symptoms of the lower urinary tract, ten (20%) developed externalization of the mesh to the vagina, three (6%) developed necrotizing fasciitis, three (6%) developed obturator or vaginal abscesses, five (10%) developed chronic pelvic pain (thigh pain or dyspareunia), two (4%) experienced bruising and bleeding, three (6%) experienced urinary tract infection, and one (2%) mesh entering the bladder, which showed signs ten months after surgery [35]. Urinary tract infection rates were also similar in the present study (5.7%).

Tape-related infections include swelling, redness, and pus formation in the skin puncture area; abscess formation in the skin, retropubic space, deep tissues around the obturator membrane, and adductor compartment of the thigh; and necrotizing fasciitis. Infections in the deep tissues around the obturator membrane are particularly dangerous because of the presence of a foreign body, confined space, and relatively low oxygen tension can lead to rapid abscess formation resulting in necrotizing fasciitis [21]. Inguinal abscess was reported in TOT and it is more frequent with certain sling materials [19, 36]. Obturator abscess which is one of the late postoperative complications did not develop in the present series.

In the present research, identifications of some complications in a retrospective manner, subjective evaluation of perioperative complications as headache, pain radiating to the leg, and postoperative complications as dyspareunia and perineal pain are the limitations of the present research. Another limitation of this research is the admission of patients with previous incontinence surgery to the research; in this way the present authors attempted to form relatively heterogeneous patient groups and ensure the admission of pa-
tients with concomitant surgery. Because of the aforementioned reasons, the severity and degree of the patient complaints cannot be evaluated and the research results may present high complication rates due to the heterogeneity of the patient group. Although aforesaid limitations of the research, long patient follow-ups, determination of complications by clinical follow-ups, being a prospective study, realization of the research only in two centers, execution of the surgeries by two surgeon experienced in urology either side by side or one by one, admission of patients with similar demographic background, utilization of one type mesh, and same type of surgical kit are the main factors that increase the validity of this research. As a result, although TVT-O is a minimal invasive technique, contrary to the common belief, the complication rates of the surgery are not low. As seen in this research, urinary bladder and vascular injury were minimal/lower than expected; it has been also shown that major perioperative complications are rare with TVT-O surgery: in the present research urethral, intestinal, and neural injuries never took place. However this research revealed that early and late postoperative complications are not a rare case. It is very important to evaluate the patients meticulously and frequently to elicit the late complications. Most of the early and late postoperative complications can be cured or treated with simple applications and medical treatments or surgical interventions, therefore omission of these complications can be facilitated. According to the present authors, perioperative complications can be minimized with the experience and suspiciousness of the surgeon although it is impossible to state the same for early and late postoperative complications. The authors recommend that patients be thoroughly informed preoperatively about the possible complications of TVT-O surgery.

Conclusion

Although the TVT-O technique is a minimal invasive surgery method applied to treat urinary incontinence surgically, it does not signify that it is a complication-free surgical procedure. Despite the low incidence of intraoperative complications, there is a mild risk of early and late postoperative complications. Fortunately these complications can be taken under control by either conservative and simple medical treatments or surgical procedures.

References


Address reprint requests to:
M. BOZKURT, M.D.
Kalkas Üniversitesi Kampüsü,
Sağlık Araştırma ve Uygulama Hastanesi,
Kadın Hastalıkları ve Doğum AnaBilim Dalı
Kars (Turkey)
e-mail: jindrmb@yahoo.com
Post-partum management in a patient affected by thrombotic thrombocytopenic purpura: case report and review of literature

A.S. Laganà¹, V. Sofo², F.M. Salmeri², B. Chiofalo¹, L. Ciancimino¹, O. Triolo¹

¹Department of Pediatric, Gynecological, Microbiological and Biomedical Sciences. University of Messina, Messina
²Department of Environmental Sciences, Safety, Territory, Food and Health. University of Messina, Messina, (Italy)

Summary
Thrombotic thrombocytopenic purpura (TTP) is a rare and potentially lethal syndrome characterized by severe thrombocytopenia, microangiopathic haemolytic anaemia, and aspecific neurologic symptoms. This syndrome is the result of an abnormal intravascular platelet aggregation which induces transient ischemia in various organs, especially in the central nervous system. Platelet aggregation causes also fragmentation of erythrocytes, thus leading to the characteristic anaemia. The exact cause of TTP is unknown, but a large body of evidence suggest that this syndrome might be due to acquired (immunological) or congenital ADAMTS13 deficiency. The dysregulation of ADAMTS13 activity could promote massive release of high molecular weight multimers of von Willebrand factor (VWF) from endothelium and, as a consequence, could cause intravascular platelet aggregation. Pregnancy is commonly associated with numerous metabolic, immunological, and haemostatic changes which could increase thrombotic risk: during pregnancy, in fact, it is generally observed an increase of procoagulant activity and a decrease of fibrinolytic activity; moreover, at the end of pregnancy, it is not rare to find thrombocytopenia. All these reasons lead us to consider pregnancy itself as a triggering event for the onset of TTP. The authors describe a case of TTP occurred during puerperium, in a patient who underwent caesarean section.

Key words: Thrombotic thrombocytopenic purpura; Pregnancy; Plasma exchange; Plasma infusion; Post-partum complications.

Introduction
Thrombotic thrombocytopenic purpura (TTP), as described for the first time by Moschcowitz [1], is a rare and potentially lethal syndrome characterized by severe thrombocytopenia, microangiopathic haemolytic anaemia, and aspecific neurologic symptoms [2]. Patients diagnosed with classical TTP had a severely deficient activity of this von Willebrand factor (VWF) cleaving protease (VWF-cp) (<5% of normal) [3-6]. Two forms of classical TTP are distinguished. Acquired TTP is caused by circulating autoantibodies, mainly IgG, generally neutralizing ADAMTS13 activity [5-7], while hereditary TTP (Upshaw-Schulman syndrome) is caused by severe constitutional deficiency of ADAMTS13 [8-15]. The incidence of TTP is estimated at 4.5 per one million people a year, mean age at diagnosis is 35 years, and is more frequent among women (male/female ratio is 2 : 3) [16]. While at the beginning of the last century TTP’s mortality rate was 90%, with the introduction of the plasma-exchange (PE) technique, it fell between 8% and 30% depending on associated diseases [17]. After the first episode, relapses are frequent in the following years [18], especially when the patient undergo pregnancy [19], transplantation of hematopoietic progenitor cells [20], infections [21], metastasis [22], use of ticlopidine, and clopidogrel [23]. This syndrome is the result of an abnormal intravascular platelet aggregation which induces transient ischemia in various organs, especially in the central nervous system. Platelet aggregation causes also fragmentation of erythrocytes, thus leading to the characteristic anaemia. The first indication that VWF was involved in the pathogenesis of TTP originated from the observation by Moake et al. [24] of unusually large VWF multimers in the plasma of patients with a chronic relapsing form of TTP. VWF is a multimeric glycoprotein composed of identical disulfide-linked 250 kD subunits synthesized by endothelial cells and megakaryocytes and plays an important role in primary hemostasis by mediating initial platelet adhesion to the subendothelium of the damaged vessel wall at high shear rates. From the storage organelles (Weibel-Palade bodies) of endothelial cells, VWF is secreted in the form of extremely adhesive ultralarge VWF multimers into the circulation, where they are slowly...
but constantly attacked by plasma protease(s) and degraded into multimers ranging in size from 500 to ~20,000 kD [25]. Proteolytic cleavage occurs physiologically between the tyrosine residue at position 842 and the methionine residue at position 843 within the A2 domain of the mature VWF subunit [26]. The multimeric structure of VWF is strictly regulated by a VWF-cp, a new member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 motifs) family of metalloproteases, denoted as ADAMTS13 [27] and to locate the gene to chromosome 9q34 [28]. Levy et al. [8], performing a genome-wide linkage analysis in patients with hereditary TTP which displayed severe VWF-cp deficiency and their family members, detected the same gene, ADAMTS13. They identified several different mutations presumably responsible for the severely deficient protease activity and hereditary TTP in homozygous or double heterozygous carriers of mutated alleles. On the contrary, patient’s family members with a heterozygous mutation had about 50% of protease activity and were clinically asymptomatic [29]. Numerous hypotheses regarding the aetiology and pathogenesis of TTP have been put forward over the years [27, 30-32]. Endothelial injury, decreased prostacyclin production, reduced fibrinolytic capacity of the vessel wall, anti-endothelial cell, and -platelet autoantibodies, specifically antibodies toward glycoprotein IV (CD36) [33, 34], and capacity of plasma of TTP patients to induce apoptosis of microvascular endothelial cells [35] have been proposed as pathogenetic factors. Moreover, a 37-kDa protein [36] and a 59-kDa protein or a calcium-dependent cysteine protease (calpain) [37, 38] were identified in serum or plasma from patients with acute TTP and suggested to be responsible for in vivo platelet aggregation. TTP acquired form could be also due to a massive endothelial damage which causes releasing of VWF multimers in blood vessels. If these multimers overcome their physiological degradation rate, they could trigger severe microvascular thrombosis [24]. TTP diagnosis is based on the presence of schistocytes at peripheral blood smear [16] and blood tests, which show decrease of haemoglobin and haptoglobin, increase of haemolysis indexes, lactate dehydrogenase (LDH) and unconjugated bilirubinemia, negative Coombs test, severe thrombocytopenia with normal prothrombin time (PT) and activated partial thromboplastin time (aPTT). Another test which can be performed is the enzyme-linked immunosorbent assay (ELISA) immunoassay for IgG anti-ADAMTS13 [39], although its specificity is low. In fact, it has been observed that about 5% of healthy individuals and 13% of patients with systemic lupus erythematosus (SLE) result positive to this immunoassay, nonetheless showing normal activity of ADAMTS13 in plasma [40]. The risk of recurrence after the first episode depends on ADAMTS13 activity rate or anti-ADAMTS13 level at onset. When ADAMTS13 activity rate is <10% the risk of relapse is 40%, while when it is >10%, the risk decreases to 4% [41]. The differential diagnosis must include

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Measurement unit</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>mm/mmc</td>
<td>208,000</td>
<td>Women: 4,000,000 - 4,500,000</td>
</tr>
<tr>
<td>White blood cells</td>
<td>mm/mmc</td>
<td>92,000</td>
<td>4,500 - 9,000</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>gr/ mmc</td>
<td>6.2</td>
<td>Women: 12.0 - 16.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>19</td>
<td>38.0 - 46.0</td>
</tr>
<tr>
<td>Platelets</td>
<td>mm/cell</td>
<td>78,000</td>
<td>150,000 - 350,000</td>
</tr>
<tr>
<td>Prothrombin time (PT)</td>
<td>%</td>
<td>102.0</td>
<td>70.0 - 120.0</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (aPTT)</td>
<td>sec</td>
<td>30.4</td>
<td>21.0 - 35.0</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>mg/dl</td>
<td>217</td>
<td>180 - 350</td>
</tr>
<tr>
<td>International normalized ratio (INR)</td>
<td>-</td>
<td>1.03</td>
<td>-</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>mg/L</td>
<td>3.37</td>
<td>&lt; 0.50</td>
</tr>
<tr>
<td>Antithrombin III (AT III)</td>
<td>%</td>
<td>72</td>
<td>80 - 120</td>
</tr>
<tr>
<td>Fibrin degradation products (FDP)</td>
<td>ug/ml</td>
<td>10</td>
<td>0 - 10</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>mg/dl</td>
<td>86</td>
<td>65 - 110</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN)</td>
<td>mg/dl</td>
<td>114</td>
<td>10.0 - 50.0</td>
</tr>
<tr>
<td>Creatinine</td>
<td>mg/dl</td>
<td>1.4</td>
<td>0.5 - 1.2</td>
</tr>
<tr>
<td>Uric acid</td>
<td>mg/dl</td>
<td>5.0</td>
<td>2.5 - 7.2</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/dl</td>
<td>4.2</td>
<td>6.0 - 8.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>31</td>
<td>34 - 48</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>mg/dl</td>
<td>1.3</td>
<td>0.0 - 1.2</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>U/L</td>
<td>30</td>
<td>0 - 42</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>U/L</td>
<td>23</td>
<td>0 - 50</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>U/L</td>
<td>1170</td>
<td>150 - 460</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>U/L</td>
<td>5992</td>
<td>4,500 - 14,500</td>
</tr>
<tr>
<td>Sodium</td>
<td>mmol/L</td>
<td>142</td>
<td>130 - 148</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>3.7</td>
<td>3.5 - 5.2</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg/dl</td>
<td>7.98</td>
<td>8.2 - 10.4</td>
</tr>
</tbody>
</table>

Table 1. — Blood test results at hospital admission.
IVF) procedure, delivered with caesarean section at 34th week for a twin pregnancy obtained with eggs donation and subsequent in-vitro fertilization with the suspect diagnosis of TTP. She had a twin pregnancy but went in the puerperium on the third day postpartum. She was sent to the authors’ observation from another hospital and admitted to our obstetric emergency unit on the third day postpartum. The authors describe a case of TTP occurred during puerperium, in a patient who underwent caesarean section.

Case Report

The authors report a case of a 50-year-old female who was admitted to our obstetric emergency unit on the third day postpartum. She was sent to the authors’ observation from another hospital with the suspect diagnosis of TTP. She had a twin pregnancy obtained with eggs donation and subsequent in-vitro fertilization (IVF) procedure, delivered with caesarean section at 34th week for gestational hypertension unresponsive to medical therapy (nifedipine and methylprednisolone). Both newborns were admitted to neonatal intensive care unit, without reporting any subsequent complication. At admission, the patient was informed in a comprehensive and complete way about clinical condition and procedures that the authors were going to perform, and signed an informed consent to allow the data collection for research purposes. A subsequent approval by the Hospital’s Ethic Committee was obtained before initiating the report. During the admission procedure, the patient had treatable abdomen, valid peristalsis, and first passage of flatus, uterus in regular puerperal involution and physiological lochia. The vital parameters were normal, except for the high blood pressure (155/90 mmHg). She complained of mild headache. Family anamnesis showed that a sister was affected by SLE; obstetric history highlighted unexplained infertility, and for this reason she underwent two IVF procedures hesitated in both cases in early miscarriages at fifth week; personal medical history was negative for other disease. Blood tests showed anaemia, thrombocytopenia, high levels of blood urea nitrogen (BUN), LDH, creatinine, and total bilirubin; low levels of antithrombin III (AT III), total proteins, albumin, calcium; PT, aPTT, international normalized ratio (INR) were in normal range (Table 1). Moreover urine test evidenced massive proteinuria and high level of urobilinogen (2.0 mg/dl; normal range 0.0 ÷ 1.0 mg/dl), and 40-50 erythrocytes were present at urinary sediment analysis. Routine and available in the laboratory autoimmunity tests, including antinuclear antibodies (ANA), antiDNA antibodies (nDNA), extractable nuclear antigens (ENA), antimitochondrial antibodies (AMA), anti-smooth muscle antibody (ASMA), anti-neutrophil cytoplasmatic antibody (ANCA), IgG and IgM anti-cardiolipin antibodies, were negative. The peripheral blood smear analysis, performed by haematologist, showed the presence of schistocytes (10%). Neurological exam was negative, as well as brain CT scan. Considering all these findings, the authors confirmed the diagnosis of TTP. In full agreement with the haematologist, nephrologist and neurologist, they decided to administrate therapy with nifedipine 30 mg/die per os, methylprednisolone 500 mg/die per os, Dexamethasone sodium phosphate four mg/die i.m., methylprednisolone hemissuccinate 20 mg/die e.v., acetylsalicylic acid 1g/die e.v., enoxaparin sodium 4,000 UI/die s.c.; additionally, on the basis of daily blood tests, the patient underwent treatment with calcium folinate 25 mg/die i.m., AT III 1000 UI/die e.v., albumin 20% e.v. when required. Moreover, she was treated daily with PE procedure, and multiple plasma infusion (PI) or blood transfusion when required. Nevertheless, on the seventh day of post-partum, the authors noticed a severe bleeding from the caesarean section scar and found a massive subfascial hematoma: therefore, they performed a revision of laparotomic incision, in order to drain the hematoma. Besides this complication, the clinical condition and the results of blood tests progressively improved following the medical treatment and PE procedures, and patient was discharged home on the 13th post-partum day.

Discussion

TTP is a life-threatening systemic illness of abrupt onset and unknown cause(s) which can occur also during pregnancy or puerperium. When it occurs during pregnancy, it could cause fetal death for placental infarction due to thrombotic occlusion of the decidual arteries. More specifically, pregnancy itself can be considered a triggering event for the onset of TTP. Pregnancy is commonly associated with numerous metabolic, immunological, and haemostatic changes which could increase thrombotic risk. During pregnancy, in fact, an increase of procoagulant activity and a decrease of fibrinolytic activity occur [43, 44], and at the end of pregnancy it rare not rare to find thrombocytopenia (prevalence between 6.6 and 11.6%) [45]. According to the Oklahoma TTP-HUS registry, pregnancy-associated TTP accounts for 13% of all cases of TTP [43] and is related to high rates of obstetric complications [46,47]. Some authors [48, 49] suggest that delivery could resolve TTP, although this point of view is not universally shared in literature. In the present reported case, the delivery did not resolve the pathology but it was the triggering event. Nowadays the treatment of this syndrome with PE and PI, together with corticosteroid administration, reduces the mortality and morbidity rate and improves the medium- and long-term maternal-foetal outcomes [50]. This is what occurred in the present patient who improved her condition in one week. PI is effective both in immune-mediated form, because it removes the anti-ADAMTS13 antibodies, as well as in congenital ADAMTS13 deficiency, because it replaces the lacking protease [51]. Although the authors could not perform any dosage of anti-ADAMTS13 antibodies or ADAMTS13 activity, they hypothesized that this reported case is not due to congenital ADAMTS13 deficiency, because the patient had no previous episode of TTP and personal medical history was negative for other related diseases. Since TTP is rare and shares some common features with other more frequent obstetric diseases as pre-eclampsia, eclampsia and HELLP syndrome, the authors suggest that accurate differential diagnosis be mandatory in order to commence treatment as soon as possible and avoid adverse outcomes.

References


Address reprint requests to:
A.S. LAGANA, M.D.
Department of Pediatric, Gynecological, Microbiological and Biomedical Sciences
University of Messina
Via C. Valeria 1, 98125 Messina (Italy)
e-mail: antlagana@unime.it
Investigation of short- and long-term effects of ovarian hyperstimulation syndrome on ovarian reserve: an experimental study

S. Pala¹, R. Atilgan¹, Z.S. Ozkan¹, N. Akpolat², N. Ilhan³, E. Sapmaz¹

¹Firat University School of Medicine, Department of Obstetrics and Gynecology, Elazig
²Firat University School of Medicine, Department of Pathology, Elazig
³ Firat University School of Medicine, Department of Biochemistry, Elazig (Turkey)

Summary

Purpose: To investigate the short and long term effects of ovarian hyperstimulation syndrome (OHSS) on serum levels of vascular endothelial growth factor (VEGF) and endothelin-1 and ovarian follicular reserve (OFR).

Materials and Methods: An experimental case-control study was conducted on a university animal laboratory with 20 immature (22-day-old) virgin female Wistar Albino rats. Firstly, rats were divided into two groups. Group 1 (n = 10): control and Group 2 (n = 10): experimental OHSS induced rats. Secondly, Group 2 was randomly divided into two groups on the day of OHSS development (27th day) as follows: Group 3 (n = 5): 27-day-old OHSS induced rats and Group 4 (n = 5): 27-day-old OHSS induced rats supervised for seven days. Group 1 was divided into two groups to constitute age-matched controls as follows: Group 5 (n = 5): 27-day-old rats, Group 6 (n = 5): 35-day-old rats. The comparisons of Group 3 vs Group 5 and Group 4 vs Group 6 were performed. Main outcome measures were OFR, serum levels of VEGF, and endothelin-1.

Results: While the OFR and primordial follicle number (PFN) of Group 3 were significantly lower than those of Group 5 (p < 0.05); VEGF and endothelin-1 levels and atretic follicle number (AFN) were significantly higher in Group 3 compared to Group 5 (p < 0.05). In Group 4, PFN was significantly lower (p < 0.05) and AFN was significantly (p < 0.05) higher than Group 6. However, there were no statistically significant difference between Group 4 and Group 6 regarding the parameters of OFR, serum levels of VEGF, and endothelin-1.

Conclusion: This experimental OHSS model revealed increased serum VEGF and endothelin-1 levels and decreased OFR during short-term of OHSS. OHSS showed detrimental effect on PFN of rats during long-term.

Key words: Endothelin-1; Ovarian follicle reserve; Ovarian hyperstimulation syndrome; VEGF.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication of ovulation induction with gonadotropin and human chorionic gonadotrophin (hCG). This iatrogenic condition is potentially lethal and occurs in 0.3 to five percent of stimulated ovarian cycles [1]. Some forms of OHSS may arise from the following conditions: [1] pregnant women with polycystic ovary syndrome that respond excessively to endogen gonadotropin; [2] abnormally high serum hCG levels in molar pregnancies; [3] women with primary hypothyroidism; [4] gonadotroph adenoma inducing co-secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH), causing a rise in estradiol (E2) levels with ovarian enlargement without ascites [2, 3]. Clinical manifestations of OHSS are massive extravascular fluid accumulation and hemoconcentration similar to that in syndromes due to capillary leakage. The patients may be complicated by renal failure, hypovolemic shock, thromboembolic episodes, and adult respiratory distress syndrome. The pathophysiology of this syndrome has not been completely evaluated, the increased capillary permeability triggered by the release of vasoactive substances secreted by the ovaries under hCG stimulation plays a key role in this syndrome [4].

The angiogenic molecule, vascular endothelial growth factor (VEGF) is the most important mediator of hCG-dependent ovarian angiogenesis. It is known that VEGF is expressed in human ovaries [5] and that VEGF mRNA levels increase after hCG administration in granulosa cells [6, 7]. A circulatory dysfunction has been described in every woman treated with gonadotropins for in vitro fertilization. It is not known, whether the gonadotropins up-regulate VEGF receptor-2 (VEGFR-2) expression and whether increased vascular permeability is also found with mild stimulation [8]. High concentrations of VEGF have been demonstrated in ascitic fluid from patients with OHSS [6].

Endothelin-1, an endothelial derived peptide, is a potent vasoconstrictor that increases capillary permeability in several tissues [9]. High levels of endothelin-1 were found in follicular fluid in patients undergoing ovulation induction.
[10]. Endothelin-1 concentration was found to be 100–300 fold higher in the follicular fluid than in the plasma. Moreover, a positive correlation between endothelin-1 and FSH concentration in the follicular fluid were found, suggesting that endothelin-1 may play a role in ovarian function as well as in OHHS [11]. In this study the authors aimed to investigate the short and long term effects of OHSS on ovarian histology and serum levels of VEGF and endothelin-1 in a rat model.

Materials and Methods

Experimental design

This study was approved by Firat University Animal Use Committee and conducted at Firat University Animal Laboratory (FUTDAM). Twenty immature (22-day-old), weighing 41-49 grams, female Wistar Albino rats were used for all experiments. They were housed individually as quinary groups in plastic cages with chip bedding, and ad libitum access to rat chow (pellet) and water. They were maintained on a 12:12 light:dark cycle (lights on at 07:00 AM) at room temperature.

Twenty immature (22-day-old) female rats were randomly divided into two groups. Group 1 (n = 10): 22-day-old rats. Group 2 (n = 10): experimental OHSS induced rats. These rats were randomly divided into two groups on the day of OHSS development (27th day); Group 3 (n = 5): 27-day-old experimental OHSS induced rats euthanised and then ovarian tissue and serum samples were collected. Group 4 (n = 5): 27-day-old experimental OHSS induced rats supervised spontaneously for seven days. Group 1 divided into two groups to constitute age-matched controls; Group 5 (n = 5): 27-day-old normal rats group, Group 6 (n = 5): 35-day-old normal rats group. The comparisons of Group 3 vs Group 5 and Group 4 vs Group 6 were performed. The rats were divided into four age-matched groups. Ovarian histopathologic evaluation and serum level analysis of VEGF and endothelin-1 of all rats were performed.

OHSS induction and ELISA assays

To prepare the OHSS model, immature female Wistar rats were stimulated with 10 IU of FSH for four consecutive days followed by 30 IU of hCG on the 26th day of life. The manifestation of the OHSS was demonstrated with daily weight gain and hematocrit elevation as illustrated by Ohba et al. [12]. All rats were euthanised with decapitation. Approximately three cc blood were collected from all rats and centrifuged at 2,500 rpm for four minutes to obtain serum samples. The serum samples were stored at -20°C until the analysis of VEGF and endothelin-1. The extracted serum samples were assayed by an enzyme linked immunosorbent assay (ELISA) using commercially available kit for VEGF and endothelin-1, according to the manufacturer’s instructions.

Ovarian morphology

After laparatomy, ovaries were removed and cleaned of adhering tissue in culture medium, weighed, and used for subsequent assays. Ovarian tissue was fixed with ten percent formaldehyde and then paraffin-embedded tissue samples were cut into four μm sections for estimation of mean ovarian follicle count. The sections were stained with masson trichrome to determine ovarian follicle reserve under light microscope. The four μm step sections were mounted at 50 μm intervals onto microscope slides to prevent counting the same structure twice, according to the aforementioned method described [13]. Follicles were classified as primordial, primary, secondary, and tertiary follicles. An atretic follicle was defined as the follicle that presented more than ten pycnotic nuclei per follicle; in the smallest follicles, the criterion for atresia was a degenerate oocyte, precocious antrum formation, or both [14].

Main outcome measures were as follows: age of rat (days), weight of rat (gr), hematocrit of rat (%), weight of ovary (mgr), serum levels of VEGF (pg/ml) and endothelin-1 (ng/ml), total follicle count with determination of primordial, primary, secondary, and tertiary follicle numbers [15]. Atretic follicle, corpus luteum (CL), and corpus albicans were also determined. CL was investigated for regression of angiogenesis and ovarian stromal fibrosis and these findings were scaled as 0 = absence, 1 = moderate presence, and 2 = high presence. Ovarian follicle cysts were counted macroscopically and scaled as 0 = absence and 1 = presence [16].

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) version 12.0. Results were presented as mean and standard deviation and number and percentage where applicable. Age-matched comparison of Group 3 vs Group 5 and Group 4 vs Group 6 were performed. Differences between groups for ordinal variables were analyzed using Mann-Whitney U test and differences in the categorical variables of groups was assessed using Chi-squared test. P values < 0.05 were considered as statistically significant.

Results

All of the experiments were completed successfully in all of the groups. Main outcome measures of the groups were weight, hematocrit and ovarian weight of rats and are presented in Table-1; ovarian morphology (Figure 1) and follicle counts are presented in Table 2, and serum levels of VEGF and endothelin-1 are presented in Table 3. The comparison of Group 3 vs Group 5 showed significantly low ovarian follicle reserve and primordial follicle count in Group 3 (p < 0.05) and significantly high atretic follicle count and serum VEGF and endothelin-1 levels in Group 3 (p < 0.05). The weights and hematocrits of rats on days four and six were significantly high in Group 3 and total ovarian weight on day six was significantly high in Group 3, too. Primary and secondary follicle counts of Group 3 were lower than of Group 5, but the difference was not significant.

The comparison of Group 4 vs Group 6 showed significantly high total ovarian weight, hematocrit, and weights of rats on days four, six, and 13 in Group 4 (p < 0.05). While primordial follicle count was significantly low, AFN was significantly high in Group 4 (p < 0.05). In Group 4, serum VEGF and endothelin-1 levels were higher than Group 6 and ovarian follicle reserve was lower than Group 6, but the differences were not significant.

Discussion

The present study is the first one to evaluate the short- and long-term effects of OHSS on ovarian reserve in an experimental model. It was shown that OHSS increased the serum levels of VEGF and endothelin-1 and had detrimen-
Investigation of short and long term effects of ovarian hyperstimulation syndrome on ovarian reserve: an experimental study

Table 1. — The weight, hematocrit % and ovarian weight of all rats in the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G3 (n=5, OHSS)</th>
<th>G5 (n=5, control)</th>
<th>p value</th>
<th>G4 (n = 5, OHSS)</th>
<th>G6 (n = 5, control)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight on day 0 (gr)</td>
<td>44.4 ± 3</td>
<td>45 ± 2.3</td>
<td>NS</td>
<td>45.6 ± 2.8</td>
<td>47 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Weight on day 4 (gr)</td>
<td>63.4 ± 3</td>
<td>54 ± 1.4</td>
<td>&lt; 0.05*</td>
<td>58.6 ± 2.6</td>
<td>53.4 ± 1.1</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Weight on day 6 (gr)</td>
<td>69 ± 4.1</td>
<td>60 ± 2.1</td>
<td>&lt; 0.05*</td>
<td>71.8 ± 2.1</td>
<td>61.4 ± 2.3</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Weight on day 13 (gr)</td>
<td>decapitation</td>
<td>decapitation</td>
<td>-</td>
<td>105.6±3.6</td>
<td>83.6 ± 1.1</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Htc on day 0 (%)</td>
<td>37 ± 1</td>
<td>37 ± 1.3</td>
<td>NS</td>
<td>37.2 ±1.4</td>
<td>37.6 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Htc on day 4 (%)</td>
<td>40.6 ± 1</td>
<td>37 ± 0.6</td>
<td>&lt; 0.05*</td>
<td>40.6 ± 1.3</td>
<td>37.4 ± 0.9</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Htc on day 6 (%)</td>
<td>42 ± 1.2</td>
<td>37 ± 0.8</td>
<td>&lt; 0.05*</td>
<td>42.2 ± 0.8</td>
<td>37.5 ± 1.8</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Htc on day 13 (%)</td>
<td>decapitation</td>
<td>decapitation</td>
<td>-</td>
<td>42.2 ± 0.8</td>
<td>38.2 ± 1.3</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Ovarian weight (mgr)</td>
<td>69.4 ± 7</td>
<td>53 ± 7</td>
<td>&lt; 0.05*</td>
<td>123±18.2</td>
<td>60.6 ± 5.8</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

Note: The values are presented as mean ± SD and %; * = MWU test; NS = Non-significant; Htc = hematocrit

Table 2. — Ovarian histopathology and follicle counts of all rats in the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G3 (n=5, OHSS)</th>
<th>G5 (n=5, control)</th>
<th>p value</th>
<th>G4 (n = 5, OHSS)</th>
<th>G6 (n = 5, control)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial follicle count</td>
<td>11 ± 10.2</td>
<td>25±12</td>
<td>&lt;0.05*</td>
<td>8.4 ± 5.0</td>
<td>17.8±13.8</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Primary follicle count</td>
<td>16.4 ± 2.8</td>
<td>19 ± 4</td>
<td>NS</td>
<td>20 ± 7.5</td>
<td>20.6 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary follicle count</td>
<td>10.8 ± 3.1</td>
<td>12 ± 2.8</td>
<td>NS</td>
<td>14.4 ± 3</td>
<td>9.6 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Tertiary follicle count</td>
<td>4.4 ± 1.1</td>
<td>2.8 ± 3.1</td>
<td>NS</td>
<td>2.2 ± 0.8</td>
<td>1.4 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian follicle reserve</td>
<td>42.6 ± 10</td>
<td>60 ± 1.5</td>
<td>&lt; 0.05*</td>
<td>45±11.1</td>
<td>49.4 ± 7.6</td>
<td>NS</td>
</tr>
<tr>
<td>CL count</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>1.8 ± 1.8</td>
<td>0 ± 0</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Corpus albicans count</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>0±0</td>
<td>0 ± 0</td>
<td>NS</td>
</tr>
<tr>
<td>Total corpus count</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>1.8 ± 1.8</td>
<td>0 ± 0</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Follicle cyst count</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
</tr>
<tr>
<td>Angiogenesis in CL</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>0.4 ± 0.5</td>
<td>0 ± 0</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
<td>0.4 ± 0.5</td>
<td>0 ± 0</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Atretic follicle count</td>
<td>3.4 ± 1.9</td>
<td>0.6 ± 0.9</td>
<td>&lt; 0.05*</td>
<td>2.6 ± 2</td>
<td>0.4 ± 0.5</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean±SD; * = MWU test; NS = Non significant; CL = Corpus luteum.

Table 3. — Serum VEGF and endothelin-1 levels of all rats in the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G3 (n = 5, OHSS)</th>
<th>G5 (n = 5, control)</th>
<th>p value</th>
<th>G4 (n = 5, OHSS)</th>
<th>G6 (n = 5, control)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF(pg/ml)</td>
<td>2717± 324</td>
<td>1,596 ± 1113</td>
<td>&lt; 0.05*</td>
<td>2,250 ± 952</td>
<td>1,777 ± 1016</td>
<td>NS</td>
</tr>
<tr>
<td>Endothelin-1 (ng/ml)</td>
<td>0.8 ± 0.01</td>
<td>0.55 ± 0.1</td>
<td>&lt; 0.05*</td>
<td>0.9 ±0.1</td>
<td>0.8 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean±SD; * = MWU test; NS = Non significant
significantly increased serum VEGF and endothelin-1 levels especially during short-term of OHSS. This increment might be due to relative hypoxia of stromal tissue of hyperstimulated ovary [24, 31]. The comparison of serum VEGF and endothelin-1 levels between the non-stimulated rats showed increment with age.

The preovulatory follicle provides a unique physiological example of rapid growth accompanied by neovascularization: two processes that are generally characteristic of pathologies such as wound repair or malignancy. During the hours preceding ovulation, follicular growth is accompanied by elevated levels of messenger RNA for VEGF. Following ovulation, rapid infiltration of capillaries through the follicular wall is essential for the formation of the CL [24]. Growth and regression of CL are accompanied by growth and regression of the luteal vascular bed. VEGF is the main regulator of angiogenesis, inducing endothelial cell proliferation, migration, vascular permeability, and vessel lumen formation [32]. VEGF-dependent angiogenesis is crucial for follicular growth, and corpus luteum formation and function [33]. In the ovary VEGF can be hormonally regulated, but in other systems, the main regulator of VEGF expression is hypoxia [34-36]. The mediator of this process is hypoxia-inducible factor-1alpha (HIF1A) [34]. Avascularization and decrement of local oxygen concentrations of granulosa cells are related to ovulation [24, 36].

VEGF inhibition in the mid- or the late luteal phase induces functional luteolysis due to premature and selective death of endothelial cells [37]. Most of the studies reported that disruption of ovarian blood supply resulted with ovarian follicular reserve decrement [38-40]. Atilgan et al. reported that unilateral total salpingectomy induced atretic follicles, stromal fibrosis, and macroscopic follicular cystic formation on the same side ovary [40]. In another study, the researchers reported that bilateral tubal ligation performed with uni/bipolar cautery increased the numbers of CL, but decreased the regression level of angiogenesis in CL only during short-term of surgery [16]. Both of two experimental study showed the effect of hypoxia on ovarian follicular reserve and development. In the present study, angiogenesis in CL was significantly higher in rats with OHSS than normal age-matched rats during long-term. Hypoxic conditions increased the vascularisation of CL and decreased ovarian follicular reserve. These results indicate the relationship between hypoxia-induced angiogenesis and VEGF. The present study observed significantly decreased ovarian follicular reserve especially during short-term of experimental OHSS.

In the present study, endothelin-1 levels were high in rats with OHSS. It is reported that in addition to VEGF, hCG may trigger activation of the renin-angiotensin system and kinin-kallikrein system together with releasing of endothelin-1, that also increases vascular permeability [41].

In conclusion, according to the similarity of OHSS between human beings and rats, it can be said that VEGF and endothelin-1 might have a trigger function on the onset of OHSS. Multiple follicular development in OHSS may bring out relative hypoxia and induce the expression of angiogenic substances. These substances induce the manifestation of OHSS while leading to damage on ovarian follicular reserve.

Acknowledgement

This study was supported by Firat University Scientific Research Foundation.
References


Address reprint requests to:
Z. OZKAN, M.D.
Firat University School of Medicine, Department of Obstetrics and Gynecology, 23119 Elazig (Turkey)
e-mail: zehrasema@yahoo.com
Massive haemorrhage secondary to placenta percreta in the first trimester: a case report

H.A. Hamid¹, R. Zulida¹, M. Norhafizah²
¹ Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM), Serdang
² Department of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM), Serdang (Malaysia)

Summary
Placenta percreta detected in the first trimester is a very rare condition. It is a known obstetric condition leading to serious maternal morbidity and mortality. High index of clinical suspicion and anticipation of placenta percreta is highly essential in early pregnancy as it is difficult to diagnose. The authors report on a patient who presented with heavy per vaginal bleeding in week 9 of pregnancy. Pelvic examination showed a 12-week sized uterus. Ultrasonography revealed a non-viable fetus. The subsequent emergency curettage performed was complicated by massive haemorrhage which required an abdominal hysterectomy performed as a life-saving procedure.

Keywords: Placenta percreta; First trimester; Pregnancy; Miscarriage; Haemorrhage.

Introduction
In general, placenta accreta is a condition defined as abnormal placental adherence in which there is presence of deep penetration of the placenta through the uterine wall. There are three forms of abnormal placentation classified according to the depth of penetration. Placenta accreta is the mild form of abnormal placentation in which placental villi adhere to the underlying myometrium without an intervening layer of decidua. The other two forms are placenta increta and placenta percreta. Placenta increta shows villous infiltration into the myometrium. As in the present patient, the villi extend through the whole thickness of the myometrium to the serosa that leads to uterine perforation. This condition is known as placenta percreta [1]. It is commonly encountered in third trimester of pregnancy and rarely in early pregnancy.

The authors report an interesting case of placenta percreta in the first trimester that potentially endangered the woman’s life, where placenta percreta leads to massive postabortal haemorrhage and uterine perforation necessitating hysterectomy.

Case Report
A 36-year-old woman at nine weeks of gestation in her eighth pregnancy presented with active per vaginal bleeding and abdominal pain. The bleeding began at home about one ‘sarong’ that was soaked, associated with passing of blood clots. She had significant obstetric history of two previous lower segment caesarean sections (LSCS) and one dilatation and curettage for missed miscarriage. She was a healthy lady with no medical illness.

On presentation, she was pale and hemodynamically unstable with blood pressure of 88/52 mmHg, pulse rate of 110 beats per minute and with a body temperature of 37°C. The abdomen was soft and non-tender. The uterus was not palpable per abdomen. Pelvic examination revealed bulging membrane through a two-cm dilated os of partially effaced cervix with active bleeding. Uterus was of 12-weeks size anteverted, mobile, non-tender, and with no adnexal mass palpable. Her haemoglobin level was 9.8 g/dl with hematocrit of 27.3%. Urine pregnancy test was positive. Ultrasound scan of the abdomen revealed a small fetal pole within a collapse intrauterine gestational sac.

Curettage was performed under general anaesthesia. She was resuscitated with fluid and one pint of packed cells was transfused before the procedure. Profuse bleeding occurred soon after commencing the suction procedure. The bleeding continued profusely despite 80 units of oxytocin, followed by two doses of 250 mcg carboprost, vigorous bimanual compression, and balloon tamponade. A total abdominal hysterectomy was performed in view of persistent bleeding. There was a highly vascularised area at the lower part of the uterus which bulged anteriorly (Figure 1). All other intraperitoneal organs were intact. The total blood loss was estimated at 3,500 millitres and one cycle of disseminated intravascular coagulopathy (DIC) regime and seven pints of packed cells were transfused in the operation theatre. She was then monitored in intensive care unit (ICU) postoperatively. She was discharged well on day four post-operation.

Grossly, the uterus weighed 131 grams and measured 100 mm from the fundus to cervix and 60 mm bicornu. The uterine cavity was filled with blood clot. There was a perforation noted at the neck of uterus measuring 20 mm in length. Microscopically, the uterine sections showed presence of chorionic villi which adhered to the underlying myometrium without intervening layer of decidua. In areas there was infiltration of chorionic villi through the whole thickness of the myometrium. Extensive areas of haemorrhage were noted (Figure 2).
Placenta accreta is on the rising trend for the past decade. The increasing incidence of placenta accreta is multifactorial but the most important factor is the increase number of caesarean sections in current obstetrics practice. Review of the literature reported incidence of the disorder of abnormal placentation to vary between one in 540 and one in 93,000 with an average of one in 7,000 pregnancies. [2] Among the three types of abnormal placentation, placenta percreta is the most severe form and very rare even in late pregnancy. Massive haemorrhage due to placenta percreta is one of the most serious obstetric complications which are more commonly seen in the third trimester classically recognised during an attempt to remove the placenta following delivery of the baby in vaginal birth and caesarean section. Excessive bleeding is expected if the placenta is forcibly removed. However, it rarely occurs in the first trimester. In a survey of literature, it is usually following surgical management of miscarriages which resulted in profuse per vaginal bleeding which had occurred in this patient. [3-5]

Bleeding from first trimester miscarriage due to placenta percreta is one of the severe obstetric complications and a potentially life threatening condition. Other related abnormal placentation complications resulting in high mortality are organ injury, amniotic fluid embolism, thromboembolism, DIC, multiple organ damage, and sepsis. [1]

The exact etiology of placenta accreta is unknown. However, there are well known risk factors associated with abnormal placentation. An essential factor contributing to this disorder is prior caesarean section. The risk ranges from 3% for patients suffering
with single caesarean delivery and can be up to 40% with the third caesarean delivery, and majority of women with antepar tum haemorrhage coexist with placenta previa [2]. Furthermore, the predisposing factors include scarred uterus following uterine surgery, other than caesarean section as myomectomy, endometrial ablation, prior uterine curettage, uterine irradiation, endometritis, uterine leiomyomata, uterine anomalies, advanced maternal age, multiparity, and smoking [4].

The clinical presentation of placenta accreta depends on the severity of the abnormal placent al adherence and the site of implantation such as bleeding, acute abdomen, uterine rupture, uterine inversion, bladder invasion, and others. Greatest among them are recognised intrapartum with post-abortal haemorrhage or postpartum haemorrhage and asymptomatic during antepartum period.

Early diagnosis with early intervention is essential to prevent or at least to minimise the complications related to placenta accreta. It is difficult to diagnose placenta percreta that morbidly adhered to the uterus especially in early pregnancy and rarely can be recognised during the curettage procedure. There is still lacking evidence through randomized controlled trial and large cohort studies regarding the diagnosis and treatment. However, there are several imaging technique can assist in the diagnosis of placenta accreta such as ultrasound, computed tomography scan (CT scan) and magnetic resonance imaging (MRI). There is no completely sensitive and specific test for the diagnosis especially in the first trimester. [2, 6-7] However, the sonographic features that might suggest placenta accreta in the first trimester include the presence of sac at lower uterine segment with thin myometrium surrounding it and presence of large irregular lacunae, hypervascularity periplacenta, and dilated intraplacental vessels [2, 7]. The present authors could not perform other imaging modalities as the patient presented with hypovolemic shock.

Essential differential diagnoses in suspected cases of profound bleeding in early trimester include septic miscarriage, cervical ectopic pregnancy, hydatidiform mole, invasive mole and even choriocarcinoma. The gold standard of making the final diagnosis is by histopathological examination (HPE).

The management of placenta percreta is a real obstetric challenge. It should be a combined multidisciplinary team approach in a tertiary centre with appropriate anaesthetic, surgical, and haematological facilities [1, 2]. Successful management depends on immediate blood transfusion therapy and prompt surgical intervention, traditionally with hysterectomy. Conservative managements such as uterine packing, uterine compression sutures, uterine tamponade, and medical therapy using methotrexate has been described as an option especially appropriate for partial placenta accreta with minimal bleeding.

However, with the recent development of diagnostic and treatment modalities, alternative measures of management have shown satisfactory results in control of bleeding as well as preserving the uterus. The alternative management of placenta accreta include uterine or hypogastric ligations, uterine artery embolisation (UAE), and transcatheter arterial chemoembolisation (TACE). There were few cases of placenta accreta that has been successfully treated with these modalities [8, 9]. In this case the present woman presented with active bleeding and the management was challenging due to damage that might have already existed. High risk informed consent should be taken for hysterectomy as part of the treatment for placenta accreta [4].

Placenta percreta is a serious obstetric condition leading to maternal mortality and severe morbidity. High index of clinical suspicion and anticipation relying on the risk factors are very crucial in optimizing management strategies as an early recognition of the condition may improve the clinical outcome. It provides an opportunity to the obstetrician to deal with the problem in the best way and to manage the obstetric emergency promptly.

Acknowledgements

The authors would like to thank the woman presented in this paper for giving permission for her case to be reported.

References


Address reprint requests to:
H.A. HAMID, M.D.
Department of Obstetric and Gynaecology,
Faculty of Medicine and Health Science,
Universiti Putra Malaysia (UPM),
43400 Serdang, Selangor (Malaysia)
e-mail: habibah@upm.edu.my
dribb76@yahoo.com
Late postpartum hemorrhage due to placental and fetal membrane residuals: experience of two cases

A. Luo¹,²,³, P. Mao¹,²,³

¹Department of Obstetrics and Gynecology, The Third Xiangya Hospital, Central South University, Changsha
²School of Public Health, Central South University, Changsha
³Key Laboratory of Medical Information Research, Central South University, College of Henan Province, Changsha (China)

Summary

Purpose: To investigate the cause and preventative measures of late postpartum hemorrhage resulted from placental and fetal membrane residuals.

Materials and Methods: Retrospective analysis on 161 cases of late postpartum hemorrhage resulting from residuals of placenta and fetal membrane from 2002 to 2012. Results: Among the 161 cases, there were 148 cases of vaginal delivery and 13 cases of cesarean section delivery. One hundred twenty-one cases (4.77%) of placental and fetal membrane residuals were present in 2,535 cases of pregnant women with history of abortion; 40 cases (2.01%) of placental and fetal membrane residuals were found in 1,989 cases of pregnant women without history of abortion. Conclusion: Placental and fetal membrane residuals are the major cause of late postpartum hemorrhage. Repeated abortion will increase the incidence of late postpartum hemorrhage resulting from placental and fetal membrane residuals.

Key words: Late postpartum hemorrhage; Residual; Placental and fetal membrane; Management.

Introduction

Late postpartum hemorrhage refers to massive uterine bleeding occurred during puerperium after delivery in 24 hours as a serious complication in obstetrics which will endanger the life of the puerperant [1].

Materials and Methods

Retrospective analysis of 161 cases of late postpartum hemorrhage resulting from placental and fetal membrane residuals treated in the present hospital during the last previous ten years was conducted and reported. Based on the diagnostic criteria for late postpartum hemorrhage as blood loss ≥ 500 ml after fetal delivery in 24 hours, 161 cases of late postpartum hemorrhage resulted from placental and fetal membrane residuals have been treated in the present hospital from January 1st, 2002 to December 31st, 2011. All cases belonged to term delivery, of which 148 cases were vaginal delivery, and 13 cases were cesarean section delivery. Seven cases were preoperatively diagnosed as placenta previa.

Results

The clinical manifestations were mainly present after incomplete inspection of placental separation, incomplete artificial separation, or long-lasting lochia rubra after uterine curettage. The characteristics of B ultrasonic imaging were present as echogenic mass inside the uterine cavity with intensive light spots as well as relatively clear edge and outline, indicating placental and fetal membrane residuals which was verified by pathological examination and blood beta-human chorionic gonadotropin (ß-HCG).

For full-term vaginal delivery, uterine contraction, uterine hardness, and uterine fundus height were observed every half hour within postpartum two hours. At postpartum two hours, uterine fundus height equalled the width of two fingers below the umbilicus, which slowly rose at postpartum 12 hours. When uterine fundus was inspected on the next day, it could have resulted above the umbilicus. Thus, if uterine fundus was found to be above the umbilicus within postpartum two hours, problems with involution of uterus were present. Vaginal bleeding and discharge were observed and samples were collected for examination if necessary.

The medical treatment applied was mifepristone 7.5 mg on an empty stomach in the morning for six days consecutively, while 15 g of biochemical granules were additionally administered, three times a day for three to six days. Follow up occurred after 12-40 days to assess the effect. The standard of efficacy was classified as cured: complete cessation of lochia rubra, B ultrasonography indicated normal echo or no tissue residues and normal blood ß-HCG. Ineffective: lochia rubra had not ceased, B ultrasonography still showed patchy strong echo, and blood ß-HCG was still higher than normal level. Results: among 57 cases of patients who received medication, 42 cases were cured and other 15 cases underwent uterine curettage.
Table 1. — The relationship between abortion and placental residual.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of cases</th>
<th>Number of cases with placenta residual</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with history of abortion</td>
<td>2,535</td>
<td>121</td>
<td>4.77%</td>
</tr>
<tr>
<td>Patients without history of abortion</td>
<td>1,989</td>
<td>40</td>
<td>2.01%</td>
</tr>
<tr>
<td>Total</td>
<td>4,524</td>
<td>161</td>
<td>3.56%</td>
</tr>
</tbody>
</table>

The surgical treatment in patients with late postpartum hemorrhage with severe anemia accompanied by shock, symptomatic treatments such as antishock, fluid infusion, hemostasis, anti-infection, etc. was prescribed. For patients that underwent vaginal delivery with massive bleeding, most of them required immediate uterine curettage. One hundred four patients in this group were treated by uterine curettage, in which the scraped material was identified by pathological examination as placental and fetal membrane residuals. The patients were treated postoperatively with oxytocin, hemostatic agents, and antibiotics, etc. until symptoms were alleviated.

Sexual activity was prohibited for one month, and contraception was prescribed for six months. The patients were counselled to choose proper methods of contraception, re-examined after one month, and followed up if bleeding continued.

The incidence of placental residual in patients with history of abortion was higher than those without history of abortion as shown in Table 1 (comparison made between the two groups, p < 0.05), indicating the endometrium repairs incompletely after operation and the ratio of abnormal placental attachment and placental adhesion is high during re-pregnancy, while postpartum hemorrhage is easily present.

Discussion

The primary causes of late postpartum hemorrhage are tissue residues of placenta or fetal membrane, intrauterine infection, and subinvolution of placenta attachment site, etc, while tissue residues of placental and fetal membrane is one of the most common causes of postpartum hemorrhage [2]. This kind of hemorrhage is mostly present at approximately postpartum two weeks when a portion of placental and fetal membrane residuals degenerate, organize, then blood vessels at the basal part are exposed due to drop of the tissue residues leading to hemorrhage which are mostly related to improper handling at the third stage of labor [3]. Midwives are prohibited to drag the umbilical cord by force during the third stage of labor, and manual removal of placenta should be conducted if the placenta is not removed 30 minutes after delivery. Careful inspection should be performed when the placenta and fetal membrane are completed discharged or not after operation, and intrauterine inspection or bedside color B ultrasonography should be conducted to make a definite diagnosis if necessary. The possibility of placental implantation should be carefully noted if residues of placenta or fetal membrane are present after cesarean section [4].

For the causes placental residual, besides related to repeated delivery, abortion, especially if repeated, will lead to increase incidence of the disease, because the endometrium will be damaged by dilatation and curettage during abortion, possibly leading to endometrial scarring and atrophy [5]. When fertilized egg is implanted, in order to obtain sufficient nutrition, placental area increases and its attachment site expands downwards, therefore, incomplete removal of placenta is easily present during delivery, directly resulting in severe postpartum hemorrhage [6]. It is suggested by the present data that disease resulting from residual of placenta is significantly related to abortion directly, therefore, great attention should be paid to technical guidance for women at child-bearing age with contraceptive method, reducing unplanned pregnancy and incidence of abortion.

References


Address reprint requests to:
P. MAO, M.D.
The Third Xiangya Hospital, Central South University
Changsha, 410013 Hunan (China)
e-mail: maoping627@126.com
Intrauterine endometriotic cyst at the site of previous cesarean scar; scar endometriosis

H. İsci¹, G. Gonenc², A.B. Yigiter¹, N. Guducu¹, İ. Dünder¹
¹ Department of Obstetrics and Gynecology, Istanbul Bilim University, Istanbul
² Department of Obstetrics and Gynecology, Beykoz State Hospital, Istanbul (Turkey)

Summary
Uterine scar endometriosis is an extremely rare entity. As the surgical procedures of the uterus increases through time, scar endometriosis may be diagnosed more often in the future. A case of uterine scar endometriosis is presented with complaints of menstruation lasting one day with associated pelvic pain. When a cystic mass in the site of previous surgery is diagnosed, scar endometriosis must be considered.

Key words: Cesarean scar; Endometriosis; Uterine scar.

Introduction
Endometriosis is described as the presence of functioning endometrial tissue outside the uterine cavity [1]. Nonetheless the incidence of scar endometriosis is found to be 0.03% to 0.15% in different studies; scar endometriosis located inside the uterine wall is much rarer and is difficult to diagnose [2]. The symptoms are often nonspecific. Non-ovarian endometriomas typically present as a slow-growing, painful mass inside or around the site of previous surgery concurrent to menstrual cycle [3].

Case Report
A 23-year-old gravida 0, parity 0 women admitted to the present clinic with the complaints of pelvic pain during her menstruation periods for two years. Her menstruation was lasting one day. Her medical history revealed unremarkable except cesarean section, performed four years prior. Pelvic examination was normal. Transvaginal ultrasound revealed a 15-mm, regular, cystic mass in the uterine cavity placed in the previous cesarean incision (Figure 1). During operative hysteroscopy a cystic mass approximately 2 cm at the site was detected. The cyst was full of chocolate-like endometriotic fluid (Figure 2). The cyst was hysteroscopically drained and endometriotic scar base was cauterized (Figure 3). One week after surgery, transvaginal ultrasound revealed normal uterine appearance (Figure 4).

Discussion
Although most frequently found in the pelvis, reports citing extrapelvic endometrial locations range from the lungs to the extremities. Incisional or scar endometriosis has also been described, however, with a much rarer incidence [4]. The reported incidence of abdominal scar endometriosis following hysterectomy is 1.08 %, whereas after cesarean section the incidence is 0.03 – 0.4 % [5]. However, endometriosis of the uterine wall scar is an extremely rare entity, hence no statistics are available regarding its incidence and prevalence [6]. Many theories of the cause of scar endometriosis have been postulated; however, the most generally accepted theory is the iatrogenic transplantation of endometrial implants to the wound edge during an abdominal or pelvic surgery [7]. The presence of endometriotic deposits in previous cesarean section scars in hysterectomy specimens is likely to have been under-reported by pathologists. In a retrospective study, analyses of hysterectomy specimens for the endometriosis confined to cesarean scar revealed an incidence of 28% [8].

Sholapurkar et al. published a case with severe life threatening hemorrhage six weeks after cesarean due to uterine scar endometriosis [8]. In the literature, severe pelvic pain, dyspareunia, and menorrhagia has also been published as complaints. Uterine rupture at the third trimester of pregnancy in nulliparous was reported after endocervical endometriotic cyst excision in the literature. However cesarean is not indicated after cervical cystic lesion excision; previously ruptured cervical endometriosis may be considered as indication for cesarean section [9]. Cervical smears in cases of cervical endometriosis may be misinterpreted as high-grade intraepithelial lesions, atypical glandular cells, and adenocarcinoma in situ [10]. Management includes both surgical excision and hormonal suppression. Oral contraceptives, progestational, and androgenic agents are agents for medical therapy [11]. It is believed that hormonal suppression is only partially effective and surgical excision of the scar is the definitive treatment [12].

In conclusion, the cystic masses at the site of previous cesarean section should give an impression regarding incision endometriosis. Hysteroscopic excision is a minimally intra-
sive technique which is satisfactory in patients with incision endometriosis.

References


Address reprint requests to:
G. GÖNENÇ, M.D.
Mehmetçik Cad. Hüseyin Cahit Yalçın Sok.No:1 Avrupa Florence Nightingale Hastanesi, Kadin Hastalıkları ve Doğum Anabilim Dalı, Fulya, Mecidiyeköy, İstanbul (Turkey)
e-mail: gokcenur82@hotmail.com
Successful pregnancy and breastfeeding in a woman with mucopolysaccharidosis type I while receiving laronidase enzyme replacement therapy

M. Castorina1, D. Antuzzi2, S.M. Richards3, G.F. Cox3, Y. Xue1

1Dipartimento di Tutela della Donna e della Vita Nascente, Pediatria; Università Cattolica del Sacro Cuore, Rome (Italy); 2Laboratorio di Neonatologia, Dipartimento di Tutela della Donna e della Vita Nascente, Pediatria; Università Cattolica del Sacro Cuore, Rome (Italy); 3Genzyme, a Sanofi company, Cambridge, MA (USA)

Summary

The authors describe the first mother-infant pair to complete an on-going, prospective, open-label, Phase 4 trial (ALID 01803, NCT00418821) determining the safety of laronidase enzyme replacement therapy (ERT) in pregnant women with mucopolysaccharidosis type I (MPS I) and their breastfed infants. The mother, a 32-year-old with attenuated MPS I (Scheie syndrome), received laronidase for three years and continued treatment throughout her second pregnancy and while lactating. A healthy 2.5 kg male was delivered by elective cesarean section at 37 weeks. He was breastfed for three months. No laronidase was detected in breast milk. The infant never developed anti-laronidase IgM antibodies, never had inhibitory antibody activity in a cellular uptake assay, and always had normal urinary glycosaminoglycan (GAG) levels. No drug-related adverse events were reported. At 2.5 years of age, the boy is healthy with normal growth and development. In this first prospectively monitored mother-infant pair, laronidase during pregnancy and breastfeeding was uneventful.

Key words: Mucopolysaccharidosis type I; Laronidase; Pregnancy; Breastfeeding; Scheie syndrome.

Introduction

Mucopolysaccharidosis type I (MPS I) is a life-threatening, autosomal recessively inherited lysosomal storage disorder (LSD) that affects approximately one in 100,000 births. The disease is caused by a deficiency in lysosomal α-L-iduronidase activity, which results in the cellular accumulation of the glycosaminoglycans (GAGs) dermatan and heparan sulfate with consequent progressive multi-organ dysfunction and disability. Clinical features may include developmental delay followed by cognitive decline, coarse facial features, corneal clouding, an enlarged tongue, recurrent upper respiratory tract and ear infections, obstructive airway disease, cardiac disease, hepatosplenomegaly, skeletal deformities, short stature, joint contractures, and progressive disability. MPS I represents a disease continuum described by three main forms based on age of onset, rate of disease progression, and degree of central nervous system (CNS) involvement. The most severe form, Hurler syndrome, is characterized by early and rapidly progressive somatic and CNS involvement with death usually within the first decade in untreated patients. The most attenuated form, Scheie syndrome, presents in later childhood to adulthood with fewer and less severe somatic symptoms, such as corneal clouding, joint contractures, and valvular insufficiency. Scheie syndrome progresses slowly, is not associated with coarse facial features or CNS decline, and may have near-normal life expectancy. Hurler-Scheie syndrome, the intermediate form of MPS I, has mild to moderate CNS involvement, moderate to severe somatic involvement, variable coarsening of facial features, and a lifespan that extends into early adulthood [1].

The two main therapeutic approaches for MPS I are enzyme replacement therapy (ERT) and allogeneic hematopoietic stem cell transplantation (HSCT). Laronidase ERT (recombinant human α-L-iduronidase) was approved in 2003 to treat the non-CNS symptoms of MPS I. Laronidase is administered intravenously as a weekly infusion of 100 U/kg (0.58 mg/kg) for the life of the patient. The enzyme contains oligosaccharide chains terminating with mannose-6-phosphate residues that bind to specific cell surface receptors and enable its uptake and delivery into lysosomes. Laronidase has been shown to reduce urinary GAG levels and hepatomegaly and to improve pulmonary function, endurance, mobility, and quality of life [2, 3]. Due to its inability to cross the blood-brain-barrier, laronidase does not address CNS disease in MPS I [4]. For patients with progressive CNS involvement and cognitive decline, HSCT is considered the treatment of choice despite associated risks of morbidity and mortality. Optimal HSCT outcomes have been observed in Hurler patients who

Revised manuscript accepted for publication December 11, 2013

are under two years of age and have normal developmental quotients. When successful, early HSCT can preserve intellectual development and prolong survival [5].

Pregnancy in women with MPS I has been reported rarely. To the authors’ knowledge, there are no reports in the English language literature of pregnancies in untreated women with MPS I. This may be attributable to disease rarity, high disease burden, and shortened lifespan. Case reports describe pregnancy outcomes in two women with MPS I who underwent HSCT early in life. One, a woman with Hurler syndrome, elected to terminate her pregnancy due to concerns about her health [6]. The other, a woman with Hurler-Scheie syndrome treated with HSCT at 14 months, received HSCT at three years of age and had four children later in life [7]. Another case report describes a woman with Hurler-Scheie syndrome who became pregnant while receiving laronidase as part of a clinical trial and then discontinued treatment. At 29 weeks gestation, she went into spontaneous labor and delivered a premature but healthy baby. Her clinical condition worsened rapidly during treatment withdrawal [8].

Animal studies of laronidase using over six times the human dose have not demonstrated direct or indirect harmful effects on embryonic/fetal development, parturition, or postnatal development. In the absence of adequate and well-controlled clinical trials, laronidase is recommended for use during pregnancy ‘only if clearly needed’. Since it is unknown whether laronidase is excreted in human milk, ‘caution’ is recommended when administering laronidase to breastfeeding women [9].

As treatments for MPS I become increasingly available, more affected females are expected to survive to reproductive age and to consider having children. Without clinical data to address risk/benefit, the question remains whether to interrupt laronidase treatment during pregnancy and/or breastfeeding, which may incur a risk of disease worsening for the mother [8, 10, 11], or to continue treatment with unknown consequences for the developing fetus and breastfeeding infant.

Although cross-placental transfer of laronidase is expected to be minimal because of the enzyme’s high molecular weight (approximately 83 kD [9]), it is unknown whether laronidase might be transferred and present a risk to the fetus. It is also unknown whether laronidase is secreted into breast milk, and whether it could harm the infant. Most patients with MPS I who receive treatment develop Immunoglobulin G (IgG) antibodies to laronidase [4], but no correlations have been demonstrated between the presence of antibodies and clinical response, as measured by the six-minute walk test (6-MWT) and forced vital capacity (FVC), or with the occurrence of allergic reactions. IgG antibodies are able to cross the placental barrier [12], but it is unknown whether trans-placental IgGs, or any potential exposure of the fetus/infant to laronidase that results in the formation of antibodies against normal endogenous enzyme, might cause an acquired enzyme deficiency.

To learn more about the effects of laronidase during pregnancy and breastfeeding, a prospective clinical trial in pregnant women with MPS I and their infants has been initiated. The authors present the clinical and obstetric history of the first mother-infant pair to complete the study.

## Materials and Methods

This is an ongoing, prospective multicenter, multinational, 12-to 18-month, open-label Phase 4 trial (ALID 01803, NCT00418821) of pregnant women with a confirmed diagnosis of MPS I, who plan to receive laronidase during pregnancy and while breastfeeding (http://clinicaltrials.gov). This study was approved by the local ethics committee, conducted in accordance with the declaration of Helsinki, and performed according to Good Clinical Practice. The objectives are to determine if laronidase is present in the breast milk of postpartum women receiving laronidase, and to assess the effects of laronidase on the growth, development, and immunologic response of their breastfed infants. Mothers and their infants undergo periodic clinical, immunological and biochemical evaluations (Table 1) during the pregnancy and up to 12 months of life (if no antibodies are detected in the infant for three months), or

### Table 1. — Schedule of evaluations in mother and infant.

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Mother</th>
<th>Infant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical exam</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IgG antibody titer</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Inhibition of enzyme uptake</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Urinary GAG</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Breastfeeding status</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Laronidase in breast milk*</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>1 month postpartum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IgG antibody titer</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IgM antibody titer</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Inhibition of enzyme uptake</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Urinary GAG</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>12 weeks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental Screening Test [17]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>End of study</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Samples collected within 24 hours preceding a laronidase infusion and within 60 minutes of completing an infusion; **Obtained from umbilical cord blood at birth; ***Blood sample collection from infant is optional. IgG and IgM antibody titers were assessed by a direct colorimetric enzyme-linked immunosorbent assay (ELISA) using specific anti-human IgG or IgM as a detector reagent. Reactivity was confirmed by radioimmunoprecipitation. Titer values are the reciprocal of the last dilution that has an absorbance value above the ELISA cut point for positivity. Enzyme cellular uptake inhibition titer was assessed using flow cytometry by determining the interference of specific antibody with intracellular incorporation of fluorescently-labeled laronidase. Urinary GAG measurements were done as previously described [2]. Laronidase activity in breast milk was measured using a 4-methylumbelliferone iduronidase enzyme activity assay with a sensitivity of 25 ng/ml.
The patient first became pregnant at 30 years of age. Laronidase therapy was discontinued at four weeks gestation due to insufficient safety information about the use of laronidase during pregnancy. At 11 weeks gestation, fetal data corresponded to gestational age (active fetal heart rate, active fetal movement, crown rump length, and biparietal diameter). At week 16, prenatal ultrasound showed oligohydramnios and pulmonary hypoplasia (33-52% of normal). A non-viable fetus was expelled at 17 weeks gestation. ERT with laronidase was resumed after four weeks gestation but returned to pre-pregnancy levels after delivery. Lactation was normal and she reported no difficulties with breastfeeding for three months. At that time, the mother stopped breastfeeding because she was prescribed an antihypertensive drug that is contraindicated during breastfeeding. Her sacroiliitis symptoms remitted four weeks after delivery, and her 6-MWT distance improved to 270 meters. She experienced a herniated disc (L4/L5), and postnatal depression documented using the Edinburgh Postnatal Depression Scale (15) (data not shown). Depression may have been influenced by the patient’s spinal complications, but neither the disc herniation nor the sacroiliitis prevented the mother from caring for her child independently. Quality-of-life (QoL), monitored using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) [16], worsened during the pregnancy but returned to pre-pregnancy scores within 14 weeks of delivery.

Two days before delivery, the mother was found to be seropositive for anti-laronidase IgG antibodies (titer of 25,600) (Table 2). At birth, the infant’s blood in the umbilical cord tested positive for anti-laronidase IgG antibodies (titer of 6,400) and negative for anti-laronidase IgM antibodies. The infant was seronegative for anti-laronidase IgG and IgM antibodies at three months of age and at all subsequent time points during the study. A cellular enzyme uptake assay showed no inhibitory effects of antibodies in the infant throughout the study. Laronidase was not de-

### Table 2. — Laboratory values in mother and infant.

<table>
<thead>
<tr>
<th>Time point</th>
<th>IgG titer</th>
<th>Enzyme cellular uptake inhibition titer</th>
<th>IgM titer</th>
<th>Urinary GAG µg/mg creatinine (reference range for age)</th>
<th>Laronidase in breast milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (delivery)</td>
<td>25,600</td>
<td>40</td>
<td>N/A</td>
<td>28 (3-36)</td>
<td>Negative</td>
</tr>
<tr>
<td>3 months postpartum</td>
<td>51,200</td>
<td>20</td>
<td>N/A</td>
<td>27 (3-36)</td>
<td>Negative</td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (birth)</td>
<td>6,400 (in umbilical cord blood)</td>
<td>Negative</td>
<td>Negative</td>
<td>76 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>3 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>194 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>5.7 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>29 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>8.3 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>55 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>12 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>44 (30-300)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Results

The patient presented at 11 years of age with carpal tunnel syndrome requiring surgery. The rarity of carpal tunnel syndrome in children raised the suspicion for a mucopolysaccharidosis. Although the patient’s urinary GAG level was normal, which may occur in patients with MPS I Scheie, genetic testing confirmed compound heterozygosity for two known MPS I mutations: p.Ala327Pro (proline for alanine substitution at amino acid 327) and c.886_887ins12 (12-base pair in-frame insertion at position 886 of iduronidase cDNA). The genotype is associated with the MPS I Scheie phenotype [13]. The patient had a history of joint stiffness and reduced mobility, which improved after initiation of ERT with laronidase (100 U/kg/week) [5] when she was 30-years-old.

Seven months later, the patient became pregnant again. During the 37-week pregnancy, she received 28 laronidase infusions (100 U/kg/week). Treatment was interrupted during weeks 9 and 10 while the patient was deciding about participation in the clinical trial; during weeks 19 to 22 when she experienced premature labor and was treated with complete bed rest and tocolytics; and during weeks 35 to 37, when complete bed rest was advised by the treating obstetrician because of sacroiliitis, which precluded visits to the hospital for laronidase infusions. During the episode of sacroiliitis, the patient experienced reduced mobility as shown by a decline in her 6-MWT results from a peak of 275 meters to 50 meters. The patient had no radiocarpal radiation or evidence of root compression deficits on MRI.

At 37 weeks gestation, a healthy 2.5 kg (2nd to 5th percentile for gestational age on the USA Centers for Disease Control and Prevention (CDC) growth charts [14]) male infant was delivered via elective caesarian section because of the mother’s underlying MPS I disease. Apgar scores were 7 and 8 at one and five minutes, respectively. Physical examination of the infant at birth revealed bilateral single palmar creases, but no other dysmorphic features.

The mother resumed ERT with laronidase one week after delivery. Lactation was normal and she reported no difficulties with breastfeeding for three months. At that time, the mother stopped breastfeeding because she was prescribed an antihypertensive drug that is contraindicated during breastfeeding. Her sacroiliitis symptoms remitted four weeks after delivery, and her 6-MWT distance improved to 270 meters. She experienced a herniated disc (L4/L5), and postnatal depression documented using the Edinburgh Postnatal Depression Scale (15) (data not shown). Depression may have been influenced by the patient’s spinal complications, but neither the disc herniation nor the sacroiliitis prevented the mother from caring for her child independently. Quality-of-life (QoL), monitored using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) [16], worsened during the pregnancy but returned to pre-pregnancy scores within 14 weeks of delivery.

### Table 2. — Laboratory values in mother and infant.

<table>
<thead>
<tr>
<th>Time point</th>
<th>IgG titer</th>
<th>Enzyme cellular uptake inhibition titer</th>
<th>IgM titer</th>
<th>Urinary GAG µg/mg creatinine (reference range for age)</th>
<th>Laronidase in breast milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (delivery)</td>
<td>25,600</td>
<td>40</td>
<td>N/A</td>
<td>28 (3-36)</td>
<td>Negative</td>
</tr>
<tr>
<td>3 months postpartum</td>
<td>51,200</td>
<td>20</td>
<td>N/A</td>
<td>27 (3-36)</td>
<td>Negative</td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (birth)</td>
<td>6,400 (in umbilical cord blood)</td>
<td>Negative</td>
<td>Negative</td>
<td>76 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>3 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>194 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>5.7 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>29 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>8.3 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>55 (30-300)</td>
<td>N/A</td>
</tr>
<tr>
<td>12 months</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>44 (30-300)</td>
<td>N/A</td>
</tr>
</tbody>
</table>
tectable in breast milk, before or 60 minutes after the completion of the mother’s laronidase infusions at one month and three months postpartum (Table 2). Urinary GAGs were within normal range for age throughout the study for both mother and infant. No drug-related adverse events were reported.

By three months of age, the infant’s weight increased to the 25th-50th percentile (Figure 1). By six months, and until the end of the study, the infant was at or above the 50th percentile for both weight and length. Denver II Development assessments [17] showed appropriate development for age. The boy, now 2.5 years old, has continued to develop normally.

Figure 1. — Growth chart of the infant. The infant’s length and weight during the evaluation period were plotted on a standard USA CDC growth chart showing the length-for-age and weight-for-age percentiles for boys from birth to 24 months of age. The chart was published by the Center for Disease Control and Prevention (www.who.int/childgrowth/en).

Published by the Centers for Disease Control and Prevention. November 1, 2009

SAFER • HEALTHIER • PEOPLE®
Discussion

This is the first report of a successful pregnancy and lactation in a woman with MPS I Scheie while receiving ERT with laronidase. The pregnancy was uneventful and resulted in the birth of a healthy male infant. Physical examination at birth was normal with no significant congenital abnormalities. The infant was breastfed for three months, had normal growth and development throughout the 12-month study period, and continued to do well through 2.5 years of age. Laronidase was not detectable in breast milk.

Anti-laronidase IgG antibodies were detected in maternal blood and umbilical cord blood at birth, but not in the infant’s blood throughout the first year of life. The infant never developed IgM antibodies to laronidase. These results are consistent with the passive trans-placental transmission of maternal IgG antibodies into the fetus and the lack of an active immunological response in the infant. The infant’s normal growth, development, and urinary GAG results provide additional evidence that the mother’s treatment with laronidase had no untoward effects on the infant during pregnancy and while breastfeeding.

MPS I is a progressive disorder in which early and sustained ERT may help stabilize disease and improve clinical status [4]. Treatment withdrawal may result in clinical deterioration [8, 10, 11], although the response to treatment interruption is likely to be influenced by the disease severity, rate of progression, and duration of time without therapy. A previous report involving a woman with MPS I Hurler-Scheie noted that laronidase treatment interruption for 24 months during pregnancy and postpartum resulted in hepatomegaly (liver edge 8.5 cm below the costal margin compared with a non-palpable liver edge before treatment withdrawal), reduced endurance (decrease from 340 to 259 meters on the 6-MWT), and worsening of pulmonary function (decrease in predicted FVC from 45% to 38% of normal [8]). A 13-week treatment withdrawal during the present patient’s first pregnancy did not result in any apparent worsening of clinical status. The only observed deterioration during the present patient’s second pregnancy (in the 6-MWT) coincided with sacroiliitis and was probably due to this spinal complication rather than MPS I-related functional deterioration. This assumption is supported by a return in 6-MWT results to pre-pregnancy values soon after delivery.

There are insufficient data to conclude that laronidase contributed to the successful outcome in this patient’s second pregnancy. However, evidence from another lysosomal storage disorder, Gaucher disease, suggests that ERT with imiglucerase before and during pregnancy may be beneficial. A survey of pregnancy outcomes in women with Gaucher disease showed a statistically significant increase in the number of live births in treated mothers, fewer spontaneous abortions, and fewer complications at delivery and postpartum [18]. In Europe, women with Gaucher disease are advised to consider continuation of imiglucerase therapy before and during pregnancy and while breastfeeding, and if untreated, to initiate imiglucerase therapy before attempting to become pregnant [19]. Although data are limited for other LSDs, available evidence suggests no adverse events in infants delivered by mothers receiving ERT during pregnancy for Pompe disease [12] and Fabry disease [20-22].

In contrast to treatment for Gaucher [23] and Pompe diseases [12], where case reports showed the presence of enzyme in breast milk, laronidase was not detected in breast milk collected within 60 minutes of laronidase infusion when the pharmacokinetics of the laronidase predict peak plasma concentrations. However, peak enzyme concentration in breast milk from a woman with Pompe disease occurred two hours later than in plasma [12]. A potentially similar delay for peak laronidase concentration in breast milk may have contributed to a negative result in the present patient. Nevertheless, exogenous enzyme ingested by infants through breast milk is likely to have limited bioavailability because of its large molecular size, and enzymatic degradation, adsorption, and denaturation in the gastrointestinal tract [19].

Conclusion

The results of this case appear encouraging and may help reassure patients who may be inadvertently exposed to laronidase during pregnancy or who may consider continuing treatment throughout pregnancy and breastfeeding. Further data are required to confirm the findings presented in this report. Patients considering laronidase treatment during pregnancy should undergo an individualized risk/benefit assessment. The clinical study remains open for enrollment worldwide.

Acknowledgements

The authors would like to thank Prof. em. P.W.J. van Dongen, Stellenbosch University, South Africa, for helpful discussions on the manuscript; Crystal Sung, PhD, D (ABMLI) and Karen Welch, MS, Genzyme Clinical Specialty Laboratory, for clinical sample analysis; Pam Pickering for assistance with medical writing, and Shari Fallet, DO, Lisa Underhill, MS, and Iva Ivanovska Holder, PhD, Genzyme Global Medical Affairs, for critical review of the manuscript. MC and DA are investigators in the clinical trial. SR, GFC, and YX are employees of Genzyme, a Sanofi company.

References


Introduction

Holoprosencephaly (HPE), a complex brain malformation resulting from incomplete cleavage of the prosencephalon into distinct cerebral hemispheres, is rare in newborns. Two preterm male neonates were born at 34 weeks’ and five days’ gestation in the monochorionic diamniotic twin pregnancy complicated with pre-eclampsia and intrahepatic cholestasis of pregnancy, and one of them was prenatally diagnosed with alobar HPE by ultrasonography with frontal bossing, hydrocephaly, hypotelorism of eyes, flat nasal bridge, macroGLOSSIA, and cheilo/palatoschisis at birth. Karyotyping by G-banding of amniocentesis specimens in normal twin and fetal umbilical blood in both fetuses showed 46, XY. This report expands discordant alobar holoprosencephaly in monochorionic diamniotic twins.

Case Report

The authors present a case of a male infant with alobar holoprosencephaly in a monochorionic diamniotic twin pregnancy conceived naturally. To the authors’ knowledge, this is the first report on alobar HPE in one twin and the co-twin well developed in monochorionic diamniotic twin pregnancy with normal karyotype. Meanwhile the pregnancy was complicated with pre-eclampsia and intrahepatic cholestasis of pregnancy.

The patient was a 21-year-old woman, gravida 1 para 0. Initial ultrasound at eight weeks’ gestation showed monochorionic twin pregnancy and gestational age was determined by last menstrual period consistent with ultrasound. At 24 weeks’ and six day’s gestation the patient was transferred to the present hospital because of developmental abnormality of the brain in one twin. Ultrasonography in this hospital showed alobar HPE (thalamus fusion, single brain tissue), hydrocephalus, cheiloschisis, and nose abnormality in one twin (Figures 1A, 1C) and co-twin was normal (Figure 1B). Karyotyping by G-banding of amniocentesis specimen in normal twin showed 46, XY. Despite the poor prognosis of the affected fetus, the couples declined an invasive procedure for selective fetocide of the abnormal fetus and opted to continue with the pregnancy. The maternal history was unremarkable for any infections, drug abuse, prenatal trauma or any other chronic disease. No significant family or obstetric history was elicited. Follow-up ultrasound evaluations were performed every two weeks. The hydrocephalus aggravated gradually. Polyhydramnios was found for the abnormal fetus at 31 weeks’ gestation. At 32 weeks’ and four day’s gestation the woman was admitted to hospital for severe pre-eclampsia, intrahepatic cholestasis of pregnancy, and threatened premature labor. The woman’s clinical state was stable when nifedipine GITS, magnesium sulfate, ursodeoxycholic acid, and dexamethasone were given. However, the degree of hydrocephalus in the HPE twin gradually aggravated (from 2.6 cm to 10.8 cm). Cesarean section was performed because of spontaneous preterm labor at 34 weeks’ and five day’s gestation. Normal twin was male, weighing 2,190 g with apgar scores of 9 at one minute and 10 at five minutes and transferred to NICU. Malformed twin was male and 2,829 g, and died immediately after birth. The 924 g placenta was delivered. Macroscopic and histological examination confirmed a...
monochorionic diamniotic pregnancy, with velamentous insertion of umbilical cord in malformed twin and margin insertion in normal twin.

Physical examination of the malformed twin revealed frontal bossing, hydrocephaly (head circumference 41 cm), hypotelorism of eyes, flat nasal bridge, macroglossia, and cheilo/palatoschisis (Figure 1D). The anterior fontanel was 7×7 cm. Further post-mortem examination of the malformed twin was not performed because the couples did not consent to it. Karyotyping by G-banding of fetal umbilical blood specimen at birth in both twins verified 46, XY. The normal twin stayed in NICU for four days and discharged. The infant is being followed up and achieves developmental milestones as is appropriate for the age. Written informed consent was obtained from the patient for publication of this case report and accompanying images.

**Discussion**

HPE is estimated to occur in one in 10,000 to 20,000 live births [3]. However, the incidence of HPE in twins is still unclear. The etiology of HPE is heterogeneous and has been shown to be associated with chromosome aberrations, maternal diabetes, exogenous teratogens, cytomegalovirus, ethanol, and salicylate [4-8]. Trisomy 13 and 18 are the most frequently identified chromosomal abnormalities accounting for 40% of the HPE cases. The genes known involved in the pathogenesis of HPE include HPE gene 1 (HPE 1), HPE 2 (SIX3 gene), HPE 3 (SHH gene), HPE 4 (TGIF gene), and HPE 5 (ZIC2 gene). Mutation of the SHH gene is the most common cause of syndromic and familial HPE [9].

Interestingly, alobar HPE in a male twin without chromosomal abnormality was identified in the present case. The case reports on HPE occurred in twins are few. Only seven unique cases were searched by the present authors. A case of cyclopia, HPE, and micrognathia in a female twin with normal karyotype was reported before, but whether the twin was dizygotic or monozygotic was unclear [10]. A preterm dizygotic twin baby diagnosed with HPE was also reported without chromosomal abnormality [11].

In present case, the twins were monochorionic diamniotic with normal karyotype, but only one of them had HPE. Similarly, a case of acardius with well-developed brain with lobar HPE and intracerebral retina-like pigmented tissue was reported in only one of the monochorionic diamniotic twin [12]. Only one twin suffered from HPE, but the co-twin was devoid of any major structural anomalies were also reported in the other two cases of monozygotic twins.
however, the subtype of chorion and amnion were not reported by the authors: a male infant with HPE and otocephaly [13, 14]; one twin with semilobar HPE and inv dup(15) marker chromosome and missense SHH gene mutation 1085 C > T (Ser 362 Leu) [14]. HPE and ectopia cordis were found in only one of diamniotic-dichorionic twin, however, whether the twin was monozygotic or dizygotic was not reported [15]. Congenital nasal pyriform aperture stenosis (CNPAS) in one neonate and lobar HPE in the other infant was reported in the monochorionic monoamnionic twin [16]. However, CNPAS is considered to be a mild representation of the HPE spectrum [17]. It is important for doctors to recognize that even with monozygotic twins only one twin may have HPE.

Reference


Address reprint requests to:
H. YU, M.D.
Department of Obstetrics and Gynecology,
West China Second University Hospital,
Sichuan University
No. 20, 3rd Section, Renmin South Rd.
Chengdu 610041 (China)
e-mail: fanjy422@126.com
EXECUTIVE BOARD:
PIERLUIGI BENEDETTI PANICI (Italy)
CARLOS F. DE OLIVEIRA (Portugal)
GIUSEPPE DE PALO (Italy)
SANTIAGO DEXEUS (Spain)
WILLIAM DUNLOP (UK)
STELIOS FOTIOU (Greece)
GERALD GITSCH (Austria)
A. PETER M. HEINTZ (Netherlands)
MICHAEL HOECKEL (Germany)
JAN JACOBS (UK)
JACQUES LANSAC (France)
TIZIANO MAGGINO (Italy)
HARALD MEDEN (Germany)
JOSEPH MONSONEGO (France)
LASZLÓ PÁLFALVI (Hungary)
SERGIO PECORELLI (Italy)
DENIS QUELLEU (France)
STELIO RAKAR (Slovenia)
PIERO SISMONDI (Italy)
CLAES TROPÉ (Norway)
LÁSZLÓ UNGÁR (Hungary)
ANDRÉ VAN ASSCHE (Belgium)
RAIMUND WINTER (Austria)

INTERNATIONAL ADVISORY BOARD
Chairman: Antonio Onnis (Italy)

HUGH ALLEN (Canada)
CURT W. BURGER (Netherlands)
ALBERTO COSTA (Italy)
ANDRÉ GORINS (France)
NEVILLE F. HACKER (Australia)
MARCJA MARCHETTI (Italy)
 STELIO P. MICHALAS (Greece)
MARIA TERESA OSORIO (Portugal)
ULF ULMSTEN (Sweden)
JAN B. VERMORKEN (Belgium)
GEORGE D. WILBANKS (USA)
JAN ZIELINSKI (Poland)

All questions concerning the Academy may be sent to:
PETER BOSZE, M.D. - P.O. Box 46 - Budapest 1301 (Hungary)
Phone: +36 1 4290317 - Fax: +36 1 2752172 - E-mail: eagc@cme.hu

www.cme.hu

Administrative Office:
1301 Budapest, P.O. Box 46 - Hungary
Fax (36 1) 4290318 - E-mail: eagc@cme.hu
**Clinical and Experimental Obstetrics & Gynecology**

*An International Journal*

- **Founding Editor**
  - A. Onnis
  - Montréal (CND)

- **Editors-in-Chief**
  - M. Marchetti
  - J.H. Check
  - Montréal (CND) Camden, NJ (USA)

- **Assistant Editor**
  - A. Sinopoli
  - Toronto (CND)

**European Journal of Gynaecological Oncology**

- **Founding Editor**
  - A. Onnis
  - Montréal (Canada)

- **Editors-in-Chief**
  - M. Marchetti
  - P. Bőszé
  - Montréal (Canada) Budapest (Hungary)

- **Associate Editor**
  - T. Maggino
  - Padua (Italy)

- **Assistant Editor**
  - A. Sinopoli
  - Toronto (CND)

**Subscription Order Card 2015**

**Clinical and Experimental Obstetrics & Gynecology**

*Founded in 1974 (ISSN 0390-6663) - Vol. XLII. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

**Subscriptions** are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

**Discounts:** 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

<table>
<thead>
<tr>
<th></th>
<th>PAPER ISSUE</th>
<th>ONLINE ISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional</td>
<td>600 USD</td>
<td>450 USD</td>
</tr>
<tr>
<td>Individual</td>
<td>400 USD</td>
<td>270 USD</td>
</tr>
<tr>
<td>Single copy</td>
<td>120 USD</td>
<td>100 USD</td>
</tr>
</tbody>
</table>

**Payment:** (USD ONLY)

- for PDF file: online through PayPal (all credit cards)
- for hard copy

Credit Card: [ ] Mastercard [ ] Visa [ ] Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° __________________________ Exp. Date __________________________

Signature __________________________ Date __________________________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net

---

**European Journal of Gynaecological Oncology**

*Founded in 1980 (ISSN 0392-2936) - Vol. XXXVI. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

**Subscriptions** are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

**Discounts:** 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

<table>
<thead>
<tr>
<th></th>
<th>PAPER ISSUE</th>
<th>ONLINE ISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional</td>
<td>600 USD</td>
<td>450 USD</td>
</tr>
<tr>
<td>Individual</td>
<td>400 USD</td>
<td>270 USD</td>
</tr>
<tr>
<td>Single copy</td>
<td>120 USD</td>
<td>100 USD</td>
</tr>
</tbody>
</table>

**Payment:** (USD ONLY)

- for PDF file: online through PayPal (all credit cards)
- for hard copy

Credit Card: [ ] Mastercard [ ] Visa [ ] Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° __________________________ Exp. Date __________________________

Signature __________________________ Date __________________________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net