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Tel. +1-514-4893242 – Fax +1-514-4854513 – e-mail: canlux@mgroup-online.com
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Tel. +39-049-656521 – Fax +39-049-8752018 – e-mail: irog.canada@gmail.com

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The management of a rare case of uterine artery pseudoaneurysm associated with cesarean scar pregnancy is discussed.
Introduction

Repeated implantation failure (RIF) is defined as a series of failed conceptions despite intrauterine transfer of good embryos and/or blastocysts obtained in an in vitro fertilization program [1, 2]. Recent studies demonstrated that local endometrial injury (LEI) improves the pregnancy outcome in infertile patients suffering from RIF with endometrial factors [3-5]. Although the mechanism underlying that LEI increases the endometrial receptivity remains fully undetermined, this procedure was found to upregulate several important genes involved in the embryo implantation [2, 6, 7].

Although LEI is available to the infertile outpatients, the major problem of LEI is vasovagal reflex-associated complications. Diclofenac suppository is often utilized in clinical practice to prevent complications and sequelae during and following tissue biopsy [8, 9]. In this study, the authors investigated if diclofenac suppository pretreatment is feasible to reduce the vasovagal reflex-associated complications.

Materials and Methods

The study was approved by the local ethical committee of the Institutional Review Board. Under informed consent, 86 infertile patients with a history of three failed embryo/blastocyst transfer cycles and who preferred LEI prior to the subsequent embryo/blastocyst transfer cycle were enrolled in the study between July 2012 and June 2013. Diclofenac 25 mg suppository was administered 15 minutes prior to LEI, whereas 51 patients did not. The occurrence of palpitations, bradycardia, hypotension, presyncope, and requirement of bed rest was compared between the two groups.

Results

There were no significant differences in the demographics between the two groups. The prevalence of presyncope and requirement of bed rest was significantly lower in the diclofenac suppository group than in the control group. The pregnancy outcome was similar between the two groups.

Conclusion

The diclofenac suppository administration is a low-cost effective method to reduce the risk of the vasovagal reflex-associated complications in infertile women undergoing LEI.

Key words: Diclofenac suppository; Local endometrial injury; Repeated implantation failure; Single curettage biopsy; Vasovagal reflex.

Diclofenac suppository pretreatment in prevention of vasovagal reflex-associated complications for infertile women undergoing local endometrial injury

K. Kitaya, Y. Tada, T. Hayashi, S. Taguchi, M. Funabiki, Y. Iwaki, M. Karita, Y. Nakamura

IVF Center, Oak Clinic, Nishinari-ku, Osaka (Japan)
Complications when pregnancy occurs in infertile patients [10]. This study demonstrated that diclofenac suppository pretreatment did not affect the reproductive outcome and pregnancy course.

Diclofenac suppository administration is a low-cost and time-saving prescription that has been widely used in pain control for obstetrics gynecologic practice including relief of perineal pain after episiotomy, caesarean section, and laparoscopic sterilization [11]. The present findings also indicate the effectiveness, safety, and feasibility of this method for the pain control in infertile outpatients undergoing office hysteroscopy and LEI.

Table 1. — Demographics and complications in the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Diclofenac suppository group (n = 35)</th>
<th>Non-pretreatment group (n = 51)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), mean ± SD</td>
<td>38.0 ± 2.1</td>
<td>38.2 ± 2.8</td>
<td>0.83</td>
</tr>
<tr>
<td>Body mass index (kg/m²), mean ± SD</td>
<td>20.2 ± 1.7</td>
<td>20.4 ± 1.8</td>
<td>&gt; 0.9</td>
</tr>
<tr>
<td>Category</td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>Primary</td>
<td>24 (66.0%)</td>
<td>38 (74.5%)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>11 (31.4%)</td>
<td>13 (25.5%)</td>
<td></td>
</tr>
<tr>
<td>Uterine pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>19 (54.3%)</td>
<td>28 (54.9%)</td>
<td>0.60</td>
</tr>
<tr>
<td>Fibroids</td>
<td>10 (28.6%)</td>
<td>13 (25.5%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>2 (5.7%)</td>
<td>2 (3.9%)</td>
<td>0.53</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>8 (22.9%)</td>
<td>11 (21.6%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Septa/synechia</td>
<td>1 (2.9%)</td>
<td>1 (2.0%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td>0</td>
<td>1 (2.0%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>1 (2.9%)</td>
<td>3 (5.9%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1 (2.9%)</td>
<td>5 (9.8%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Presyncope</td>
<td>0</td>
<td>6 (11.8%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Requirement of bed rest</td>
<td>1 (2.9%)</td>
<td>9 (17.6%)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*Totals are not 100% due to some patients having more than one diagnosis(s).

Discussion

In this study, the authors demonstrated that diclofenac suppository pretreatment reduces the occurrence of the vasovagal reflex-associated complications, such as presyncope and requirement of bed rest. Adverse effects were not recognized following administration. The authors previously showed that LEI does not increase the obstetric complications when pregnancy occurs in infertile patients [10]. This study demonstrated that diclofenac suppository pretreatment did not affect the reproductive outcome and pregnancy course.

References


Address reprint requests to:
KOTARO KITAYA
IVF Center, Oak Clinic
2-7-9 Tamade-Nishi, Nishinari-ku
Osaka 557-0045 (Japan)
e-mail: kitaya_k@oakclinic-group.com
Oxidative stress markers in uterine fibroids tissue in pre- and postmenopausal women

A. Markowska¹, M. Mardas², E. Gajdzik⁴, P. Zagrodzki⁴, J. Markowska³

¹ Department of Perinatology and Gynecology, Poznań University of Medical Sciences, Poznań
² Department of Human Nutrition and Hygiene, Poznan University of Life Sciences, Poznań
³ Department of Oncology, Poznań University of Medical Sciences, Poznań
⁴ Department of Food Chemistry and Nutrition, Medical College Jagiellonian University, Kraków
⁵ Department of Nuclear Physical Chemistry, Institute of Nuclear Physics, Kraków (Poland)

Summary
Uterine fibroids are common benign tumors of the reproductive organ and occur in approximately 50-80% of women of reproductive age. The pathogenesis of uterine fibroids is multifactorial and includes: sex hormones, genetic factors, cytokines, and oxidative stress. Objectives: The aim of this study was to investigate the oxidative stress markers in tissue samples of women with uterine fibroids, with further analysis on size and menopausal status. Materials and Methods: Fifty-nine patients with the mean age 50.6 (35 premenopausal and 24 postmenopausal) who underwent standard gynecological procedures were recruited in the study. All women had histologically proven uterine leiomyoma. Samples were collected ex vivo immediately after resection. Glutathione peroxidase (GPX), catalase (CAT), and the ferric reducing ability of plasma (FRAP) were measured. Results: The activity of GPX was significantly higher in fibroid samples than in myometrium (0.097 +/- 0.042 vs. 0.057 +/- 0.027 U/mg of protein, p < 0.05), activity of CAT did not differ between samples (1.13 +/- 0.86 vs. 1.23 +/- 0.51 U/mg of protein, p > 0.05), and FRAP presented higher values in fibroid samples than in myometrium (4.58 +/- 6.29 vs. 3.04 +/- 3.81 mM Fe²⁺/mg of protein), but the difference was not statistically significant (p = 0.06). In the subgroups analyses, there were no statistically significant differences when comparing the activity of GPX, CAT, and FRAP in fibroid samples from pre- and postmenopausal women, as well as when comparing fibroid samples of small size (< 50 mm) and large size (≥ 50 mm) tumors. Conclusion: Oxidative stress markers are changed in fibroid tissue samples showing that oxidative stress may play an important role in this tumor formation, although without influencing menopausal status nor tumor size.

Key words: Uterine fibroids; Oxidative stress; Menopause.

Introduction
Uterine fibroids are common benign tumors of the reproductive organ and occur in approximately 50-80% of women of reproductive age, mostly between 30 - 55 years of age. and two to three times more often in Black women [1-5]. Fibroids are monoclonal tumors that develop from myoblasts and ves- sel wall of the myometrium [6, 7]. Depending on the size and location, about 25% of fibroids present clinical signs: abundant, menstrual bleeding, signs of compression of the abdominal organs or discomfort. They cause reproductive failures - infertility or recurrent miscarriage and complications during childbirth [8-10]. The pathogenesis of uterine fibroids is multifactorial and includes: sex hormones (estrogens, estrogen receptors, progesterone) [2, 11-14], genetic factors (mutations, polymorphisms) [3, 7, 15-21], cytokines (insulin-like growth factor 1 - IGF1, transforming growth factor beta 1 - TGFβ1 and cathepsin D) [2, 22-25], and oxidative stress [26].

Free radicals are produced in the normal cell metabolism and are involved in physiological processes such as growth, proliferation, differentiation, and apoptosis of cells. Exces-sive production of free radicals, particularly reactive oxygen species (ROS) are injurious to the organism and is considered to be the cause of many diseases, including those of the female reproductive organs [26-29]. Antioxidant system is complex and includes a number of enzymes such as glutathione peroxidase (GPX) and catalase (CAT). GPX catalyzes, among others, the reaction between glutathione GSH and hydrogen peroxide, resulting in the elimination of excess hydrogen peroxide and blocking the formation of other free radicals, and protects the cell from the damaging effects of lipids [27, 30]. CAT is an enzyme that saves the cells against the toxic effects of hydrogen peroxide and its decreased activity is associated with inflammation. Small CAT activity was found in the connective tissue that forms part of the myoma [27, 30, 31]. Because of the difficulty in measuring each antioxidant component of plasma individually and of the interactions that take place among components, Benzie and Strain described a method to measure the total antioxidant capacity known as the ferric reducing ability of plasma (FRAP) [32].

The aim of this study was to investigate the oxidative stress markers in tissue samples of women with uterine fibroids, with further analysis on size and menopausal status.
Materials and Methods

Participants
Fifty-nine patients with transvaginal ultrasound proven uterine fibroids who underwent standard gynecological procedures in the Department of Oncology at Poznan University of Medical Sciences were recruited in the study after informed consent. The mean age was 50.6 years (48.2-52.9, 95% CI). From 59 woman, 35 were described as premenopausal and 24 as postmenopausal. No woman in this study had a previous history of myomectomy, autoimmune or inflammatory disease. Women with virus C or virus B hepatitis or human immunodeficiency virus infection were not included in this study. None of included women was pregnant or had endometriosis. All women in the study group had histologically proven uterine leiomyoma.

Samples collection
Samples were collected ex vivo immediately after resection in the size of approximately one cm³. In woman who underwent hysterectomy, both fibroid sample as well as macroscopically normal myometrium sample were collected. In woman who underwent conservative surgery (tumor resection) only fibroid sample was collected. Samples were washed with distilled water and frozen (-76°C) until the analysis.

Evaluation of oxidative stress markers
The tissue samples were homogenized in phosphate buffer pH = 7.4. FRAP assay was performed according to Benzie and Strain [32] with some modifications [33]. The reducing ability of the sample was expressed in ferrous ion equivalents (mM Fe²⁺/mg of protein) within the standard incubation period of 30.0 minutes after reagent addition. GPX activity was evaluated with hydrogen peroxide as the substrate, as described previously [34]. CAT activity was estimated according to Aebi [35]. Protein content was determined by Bradford method.

Statistical analysis
Statistical analysis was performed with the use of Statistica 10.0 Software. D’Agostino-Pearson omnibus test was used for normality of the data check. Data were evaluated using the Mann-Whitney U-test for comparison between groups, and the Wilcoxon signed-rank test for comparison within groups. The level of significance was set at the standard level of \( p = 0.05 \).

Results
Comparisons in oxidative stress markers between fibroid tissue and normal myometrium are presented in Figure 1. The activity of GPX shows to be significantly higher in fibroid samples than in myometrium (0.070 +/- 0.042 vs. 0.057 +/- 0.027 U/mg of protein, \( p < 0.05 \)), activity of CAT did not differ between samples (1.13 +/- 0.86 vs. 1.23 +/- 0.51 U/mg of protein, \( p > 0.05 \)) and FRAP presented higher values in fibroid samples than in myometrium (4.58 +/- 6.29 vs. 3.04 +/- 3.81 mM Fe²⁺/mg of protein) but without statistical significance (\( p = 0.06 \)). The subgroups analyses are presented in Figure 2. There were no statistically significant
differences when comparing the activity of GPX, CAT, and FRAP in fibroid samples from pre- and postmenopausal women, as well as when comparing fibroid samples of small size (< 50 mm) and large size (≥ 50 mm) tumors.

**Discussion**

To the present authors’ knowledge, this is the first study which compares tissue oxidative stress markers between fibroid tissue and normal myometrium in the same patients and additionally compares tissue samples in pre- and postmenopausal woman. They found increased GPX activity in fibroid tissue (p < 0.05) as well as a nearly significant increased FRAP. Oxidative stress markers were independent from menopausal status and fibroid size.

Pejić et al. [26] compared endometrial tissue samples in patients with different gynecological disturbances. They found that CAT activity was not altered between polyps...
and myoma, simple and complex hyperplasia, while in adenocarcinoma patients the activity was lower by 43% (p < 0.05). At the same time CAT activity compared with controls, was not altered in blood of subjects with polypus, myoma, hyperplasia simplex, and adenocarcinoma [29]. GPX activity was not different between polyps and myoma in endometrial tissue samples [26] as well as the serum activity was not changed in uterine myoma and controls [29]. Vural et al. [36] showed that ceruloplasmin, CAT, arylesterase, free sulphydryl group, and prolidase activities were significantly higher in fibroid tissue than those in myometrial tissue. It was concluded that the study demonstrated increased antioxidative repair system in the fibroid tissue compared to the myometrium and serum of the same patients. Additionally, higher pathophysiological potential of the submucosal fibroids over intramural and subserosal fibroids were shown with the levels of oxidative stress markers and prolidase activity levels.

The results presented by Ohwada et al. [37] showed that endometrial GPX activity is regulated by sex hormones, being stimulated by estrogen and suppressed by progesterone, that is important especially in premenopausal woman. Foksiński et al. [38] showed a higher level of 8-hydroxy-2'-deoxyguanosine in uterine myoma tissues than in their respective tumor-free tissues what was more elevated in uterine tissues of premenopausal women when compared with postmenopausal ones. They also found the correlation between the size of the tumor and the amount of 8-OH-dG. In another study Chio et al. [31] showed that plasma thiobarbituric acid-reactive substances (TBARS) were on significantly higher level (p < 0.05) in myoma patients than in controls when plasma and erythrocyte superoxide dismutase (SOD) activity was significantly lower than in controls. Erythrocyte GPX activity and CAT activity did not differ in myoma patients and in controls.

Conclusions
Oxidative stress markers are changed in fibroid tissue samples showing that oxidative stress markers may play an important role in this tumor formation, although without influence of menopausal status nor tumor size.

References


Address reprint requests to:
M. MARDAS, M.D., Ph. D.
Department of Oncology
Poznań University of Medical Sciences
Szamarzewskiego Str. 82/84 60-569 Poznan (Poland)
e-mail: marcin.mardas@skpp.edu.pl
Non-association of MMP-9 -1562C/T polymorphism with preeclampsia risk: evidence from a meta-analysis

C.M. Wang¹, S.L. Zhang²

¹ Department of Gynecology and Obstetrics, The Second Affiliated Hospital of the Southeast University, Nanjing
² Department of General Surgery, Zhongda Hospital, Medical College of Southeast University, Nanjing (China)

Summary

Individual genetic association studies examining the relationship between the MMP-9 -1562C/T polymorphism (rs3918242) and preeclampsia risk have yielded inconsistent results. **Objective:** This study aims to evaluate the association between the MMP-9 -1562C/T polymorphism and preeclampsia risk using meta-analysis. **Materials and Methods:** Relevant studies were identified by searching PubMed database. Data were extracted and statistical analysis was performed using STATA 12.0 software. A total of six publications involving 871 cases and 845 controls were included in this meta-analysis. **Results:** Combined analysis revealed no association between the MMP-9 -1562C/T polymorphism and preeclampsia risk (allelic model: OR = 1.10, 95% CI 0.86 - 1.41, P heterogeneity= 0.07; recessive model: OR = 0.38, 95% CI 0.14-1.01, P heterogeneity= 0.64; dominant model: OR = 1.09, 95% CI 0.70 - 1.69, P heterogeneity= 0.01; homozygous model: OR = 0.41, 95% CI 0.15 - 1.09, P heterogeneity= 0.67; heterozygous model: OR = 1.36, 95% CI 0.80 - 2.29, P heterogeneity = 0.01). Similarly, subgroup analysis by ethnicity showed that MMP-9 -1562C/T polymorphism was not associated with preeclampsia risk in Brazilian (allelic model: OR = 1.37, 95% CI 0.92 - 2.05, P heterogeneity= 0.61; recessive model: OR = 0.80, 95% CI 0.18 - 3.57, P heterogeneity= 0.58; dominant model: OR = 1.12, 95%CI 0.60-2.10, P heterogeneity= 0.03; homozygous model: OR = 0.87, 95%CI 0.19-3.94, P heterogeneity = 0.62; heterozygous model: OR = 1.55, 95% CI 0.99 - 1.75, P heterogeneity = 0.32). **Conclusion:** This meta-analysis indicated that MMP-9 -1562C/T polymorphism was not associated with preeclampsia risk. However, large well-designed, multi-center epidemiological studies should be carried out in these and other ethnic populations to confirm our findings.

Key words: MMP-9; Polymorphism; Preeclampsia; Meta-analysis.

Introduction

Preeclampsia is a leading cause of maternal mortality and morbidity worldwide. It is characterized by the new onset of hypertension and proteinuria after the 20th week of gestation and occurs in about 3% to 7% of all pregnancies [1-4]. The pathogenesis of preeclampsia has not been completely elucidated. However, previous studies have suggested that preeclampsia development is multifactorial, resulting from endothelial cell dysfunction, excessive vasoconstriction, inflammation, immunological disorder, etc [5, 6]. Among them, a genetic component in preeclampsia cannot be neglected.

It is well known that abnormal placentation, impairing trophoblast invasion, may play a vital role in the pathophysiology of preeclampsia. MMP-9 is a member of the MMP family which plays a crucial role in restructuring the extracellular matrix, and possesses proteolytic activity against type IV collagen, a major component of the basement membrane. It has been reported that cytotrophoblast MMP-9 activity is increased in human placental tissue [7]. In addition, the inability to produce sufficient matrix metalloproteinases may be an early manifestation of abnormal placentation such as in preeclampsia [8]. Therefore, abnormal MMP-9 expression may be related to preeclampsia development.

The MMP-9 gene, localized to chromosome 20q13.2, encodes zinc-dependent enzyme that breaks down extracellular matrix and promotes cell invasion. MMP-9 gene expression is known to be influenced by functional SNP-1562C/T (rs3918242) in the promoter region [9, 10]. Many epidemiological investigations have investigated the role of MMP-9 -1562C/T polymorphism (rs3918242) in preeclampsia risk. However, the results of these studies remain inconclusive. Therefore, the authors performed a comprehensive meta-analysis to derive a more precise estimation of the relationship between MMP-9 - 1562C/T polymorphism and the risk of preeclampsia.

Materials and Methods

**Publication search and inclusion criteria:** A literature search of the PubMed database was carried out (updated to May 1, 2014) using the following keywords: “polymorphism”, “preeclampsia”, and “MMP-9”. In addition, studies were identified by a manual search of the reference lists of reviews and retrieved studies. Studies were eligible if they met the following criteria: (a) investigating the association between the MMP-9 -1562C/T polymorphism
Table 1. — Characteristics of studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>First author [Ref]</th>
<th>Year</th>
<th>Country</th>
<th>Case/Control</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palei A.C. [15]</td>
<td>2010</td>
<td>Brazil</td>
<td>154/176</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>Fraser R. [16]</td>
<td>2008</td>
<td>UK</td>
<td>117/146</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>Palei A.C. [17]</td>
<td>2012</td>
<td>Brazil</td>
<td>213/181</td>
<td>47</td>
<td>166</td>
</tr>
</tbody>
</table>

Figure 1. — Forest plot of the MMP-9 -1562C/T polymorphism and preeclampsia risk. (A: allelic model; B: recessive model; C: dominant model; D: homozygous model; E: heterozygous model).
and preeclampsia risk, (b) case-control studies, and (c) genotype data for estimating of odds ratio (OR) and 95% confidence interval (CI). Accordingly, the following exclusion criteria were applied: (a) review, abstracts, case reports, and editorials; and (b) studies that reported duplicated results.

Data extraction: The two present reviewers (Wang and Zhang) independently extracted data from included studies. The following data were extracted: the name of the first author, year of publication, country of origin, sample size, and the genotype frequencies in the preeclampsia cases and controls. Disagreements between authors were resolved by consensus.

Statistical analyses: Five genetic models were used, including allelic (T allele vs. C allele), recessive (TT vs. TC+CC), dominant (TT+TC vs. CC), homozygous (TT vs. CC), and heterozygous (TC vs. CC) models. The ORs and their corresponding 95% CIs were used to compare the association between MMP-9 -1562C/T polymorphism and preeclampsia risk. Chi-square-based Q-tests were used to calculate the heterogeneity between the individual studies with significance set at the p < 0.05 level [11]. The random-effect model was used to assess the pooled OR if there was heterogeneity among the individual studies [12]. Otherwise, the fixed-effect model was used. The pooled OR was determined through Z test with significance set at the p < 0.05 level. Then, sensitivity analysis was conducted by excluding each study, one at a time, and recalculating the OR and 95% CI to assess the effects of each study on the pooled risk of preeclampsia. Finally, funnel plot was performed to assess the publication bias of the literatures. All of the statistical tests were performed using STATA version 12.0.

Figure 2. — The sensitivity analysis results of the MMP-9 -1562C/T polymorphism and preeclampsia risk (A: allelic model; B: recessive model; C: dominant model; D: homozygous model; E: heterozygous model).
According to the searching strategy, eight papers were found. The authors reviewed the titles, abstracts and the full texts of all retrieved articles through defined criteria. Finally, six eligible studies concerning the MMP-9 -1562C/T polymorphism and preeclampsia risk were included in the meta-analysis. Among all included studies with 871 cases and 845 controls, three studies were conducted in Brazil, one conducted in Italy, one conducted in UK, and one performed in Netherlands (Table 1).

Table 2 shows the main results of this meta-analysis. Overall, no significant association between the MMP-9 -

<table>
<thead>
<tr>
<th>Poly-morphism</th>
<th>Country</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>Test of heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T vs. C</td>
<td>Overall</td>
<td>1.10</td>
<td>0.86-1.41</td>
<td>0.43</td>
<td>Fixed 0.07</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>1.37</td>
<td>0.92-2.05</td>
<td>0.12</td>
<td>Fixed 0.61</td>
</tr>
<tr>
<td>TT vs. C</td>
<td>Overall</td>
<td>0.38</td>
<td>0.14-1.01</td>
<td>0.05</td>
<td>Fixed 0.64</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>0.80</td>
<td>0.18-3.57</td>
<td>0.77</td>
<td>Fixed 0.58</td>
</tr>
<tr>
<td>TT+TC vs. CC</td>
<td>Overall</td>
<td>1.09</td>
<td>0.70-1.69</td>
<td>0.70</td>
<td>Random 0.01</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>1.12</td>
<td>0.60-2.10</td>
<td>0.73</td>
<td>Random 0.03</td>
</tr>
<tr>
<td>TT vs. CC</td>
<td>Overall</td>
<td>0.41</td>
<td>0.15-1.09</td>
<td>0.07</td>
<td>Fixed 0.67</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>0.87</td>
<td>0.19-3.94</td>
<td>0.86</td>
<td>Fixed 0.62</td>
</tr>
<tr>
<td>TC vs. CC</td>
<td>Overall</td>
<td>1.36</td>
<td>0.80-2.29</td>
<td>0.26</td>
<td>Random 0.01</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>1.55</td>
<td>0.99-1.75</td>
<td>0.06</td>
<td>Fixed 0.32</td>
</tr>
</tbody>
</table>

Figure 3. — Funnel plot analysis to examine publication bias (A: allelic model; B: recessive model; C: dominant model; D: homozygous model; E: heterozygous model).
1562C/T polymorphism and preeclampsia risk was observed (allelic model: OR = 1.10, 95% CI 0.86 - 1.41, \( P_{\text{heterogeneity}} = 0.07 \); recessive model: OR = 0.38, 95% CI 0.14 - 1.01, \( P_{\text{heterogeneity}} = 0.64 \), dominant model: OR = 1.09, 95% CI 0.70 - 1.69, \( P_{\text{heterogeneity}} = 0.01 \); homozygous model: OR = 0.41, 95% CI 0.15 - 0.99, \( P_{\text{heterogeneity}} = 0.67 \); heterozygous model: OR = 1.36, 95% CI 0.80 - 2.29, \( P_{\text{heterogeneity}} = 0.01 \) (Figure 1). Then, the authors performed subgroup analysis by ethnicity. Similarly, no obvious associations were found for all genetic models (allelic model: OR = 1.37, 95% CI 0.92 - 2.05, \( P_{\text{heterogeneity}} = 0.61 \); recessive model: OR = 0.80, 95% CI 0.18 - 3.57, \( P_{\text{heterogeneity}} = 0.58 \); dominant model: OR = 1.12, 95% CI 0.60 - 2.10, \( P_{\text{heterogeneity}} = 0.03 \); homozygous model: OR = 0.87, 95% CI 0.19 - 3.94, \( P_{\text{heterogeneity}} = 0.62 \); heterozygous model: OR = 1.55, 95% CI 0.99 - 1.75, \( P_{\text{heterogeneity}} = 0.32 \). Sensitivity analysis was performed to explore the influence of an individual study on the pooled results by deleting a single study each time from the pooled analysis. The results showed that no individual study significantly affected the pooled OR (Figure 2). Funnel plot was performed to assess the publication bias of the literatures. Symmetrical funnel plots were obtained in the polymorphism tested in all of the models (Figure 3).

**Discussion**

Accumulating number of genetic association studies have focused on the association between gene polymorphisms and preeclampsia risk. However, the findings are generally inconsistent, probably due to some limitation in these studies such as small sample size. Meta-analysis is considered a powerful tool for summarizing the contradicting results from different studies with more statistical power, so that it can obtain more reliable results than a single study [18]. To the best of the present authors’ knowledge, this was the first meta-analysis providing comprehensive insights into the effects of MMP-9 -1562C/T polymorphism on the risk of preeclampsia. The main findings of this meta-analysis are the lack of any significant association between MMP-9 -1562C/T polymorphism and preeclampsia risk in overall populations. Similar results were also found in stratified analysis based on ethnicity. Despite the present authors’ efforts in performing a comprehensive analysis, some limitations exist in this meta-analysis. Firstly, they pooled the data using unadjusted information, whereas a more precise analysis could be conducted if detailed information of original data is available. Secondly, gene-environment interactions should be considered in further studies if individual data of environmental exposure are available. Finally, the pooled sample size was relatively limited in this meta-analysis. Therefore, this meta-analysis could only preliminarily appraise the association of MMP-9 -1562C/T polymorphism with preeclampsia risk.

In conclusion, the present results suggest that MMP-9 -1562C/T polymorphism is not associated with preeclampsia risk. However, large well-designed, multi-center epidemiological studies should be carried out in these and other ethnic populations to confirm the present findings.

**Acknowledgements**

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**References**


Address reprint requests to:
C.M. WANG, M.D.
Department of Gynecology and Obstetrics
The Second Affiliated Hospital of the Southeast University
11 Bengfu Road
Nanjing, Jiangsu (China)
e-mail: wcmwcmlove@163.com
Evaluation of maternal mortality ratio and causes in a university hospital in eastern Turkey

Z. Kurdoglu1, T. Dalbudak2, M. Kurdoglu3, R. Yildizhan4, H.G. Sahin4

1 Ankara Training and Research Hospital, Department of Obstetrics and Gynecology, Ankara
2 Şanmed Hospital, Department of Obstetrics and Gynecology, Sanliurfa
3 Gazi University, Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara
4 Yuzuncu Yil University, Faculty of Medicine, Department of Obstetrics and Gynecology, Van (Turkey)

Summary

Aim: To investigate the maternal mortality ratio (MMR) and causes of maternal death in order to decrease these deaths. Materials and Methods: The number of live births, maternal deaths, and the causes of deaths in Yuzuncu Yil University were recorded between 2004 and 2013. Results: The MMR was 268 per 100,000. Forty-nine maternal deaths were examined in terms of cause. The most frequent cause of death is eclampsia (33%) and associated intracerebral complications. The antenatal follow-up rate was 23.3%. The majority of patients had low income (92.3%), 72.2% were from rural areas, and 95.5% were illiterate. Conclusion: The high MMR may arise from the high incidence of pregnancy complications in eastern Turkey, the rareness of antenatal follow-ups, and the present hospital being a referral hospital. The most frequent cause of maternal mortality is eclampsia and associated complications, followed by bleeding.

Key words: Maternal mortality ratio; Causes of maternal death; Maternal health.

Introduction

Maternal mortality is defined as deaths due to a disease or its treatment during pregnancy or within 42 days of the birth without depending on the period and localization of the pregnancy. In this definition, accidental deaths or deaths due to crashes are not included [1]. Every day approximately 800 women die due to preventable causes related to pregnancy and birth. Almost all (99%) maternal deaths occur in developing countries. The maternal mortality ratio (MMR) is 240 per 100,000 live births, while it is 16 per 100,000 in developed countries. Major complications are responsible for 80% of maternal mortality worldwide. Leading causes of maternal mortality are severe bleeding, infections, high pressure in pregnancy (preeclampsia and eclampsia), and unsafe abortions [2].

In this study, the authors aimed to calculate their MMR and create strategies to decrease maternal deaths by examining the causes.

Materials and Methods

The archive of Yuzuncu Yil University, Faculty of Medicine, Department of Obstetrics and Gynecology was investigated retrospectively. Information about live births and maternal deaths between years 2004 and 2013 was collected. The number of live births, maternal deaths, and the causes of deaths were recorded according to year. The MMR was calculated as maternal deaths per 100,000 live births. In addition, cases were evaluated with regards to age, gravida, parity, educational status, antenatal follow up, status of pregnancy at the time of death, socio-economic status, and literacy.

Results

In the present clinic, during the ten-year period, 18,263 live births and 49 maternal deaths were recorded. The present MMR was 268 per 100,000. Of the 49 deaths, mean age was 32.37 ± 6.5 years, and mean gravidity and parity were 4.09 ± 2.2 and 3.31 ± 1.9, respectively. Mean gestational age at the time of death was 33.34 ± 5.3 weeks. Of the mothers who died, 95.5% were not literate, 92.3% were of low socio-economic status, and 72.2% were living in a rural area. The rate of antenatal care was only 23.3%. The distribution of maternal deaths according to year is shown in Figure 1.

When the causes of death were evaluated, the main causes in 41% of cases were eclampsia and HELLP syndrome, in 39% were bleeding (uterine rupture, placental abruption, uterine atony, placenta percreta), in 12% were cardiac arrest, in 6% were amniotic fluid embolism, and in 2% cases were cerebral venous sinus thrombosis (Figure 2).

Discussion

MMR is an important indicator for evaluating the healthcare system and policy of a country. Although maternal mortality is low in developed countries, it is still an impor-
tant health problem in developing countries. According to the data of the World Health Organization (WHO), the United Nations Children Fund (UNICEF), the United Nations Population Fund (UNFPA), and the World Bank, the global MMR is 170-300 per 100,000 live births. Around 99% of global maternal deaths occur in developing countries, mostly in sub-Saharan Africa and South Asia. While Sub-Saharan Africa has the highest MMR (500/100,000), East Asia has the lowest (37/100,000) [3]. The MMR was reported as 412 in Tanzania, 79 in Egypt, and 8.4 in France per 100,000 live births [4-6].

In Turkey, the MMR was calculated as 208 per 100,000 live births in 1974-1975 [9, 10]. The Ministry of Health began to register maternal mortalities by creating the Ma-
ternal Mortality Monitoring System in 2007. The MMR in this country was reported as 29 per 100,000 according to the National Maternal Mortality Study published in 2009 [11]. The estimation of MMR by the WHO, UNICEF, UNFPA, and the World Bank for Turkey in 2010 was 20 per 100,000, whereas the Ministry of Health reported this rate as 15.5 per 100,000 in 2011 [3, 12].

Between January 1997 and December 2000, the present clinic’s MMR was calculated as 960 per 100,000. When causes of maternal death were examined, it was seen that preeclampsia, severe preeclampsia, and HELLP syndrome ranked first and placental abruption ranked second [13]. In the other study from the present city, between January 1995 and December 2004, MMR was reported as 143.42 per 100,000 live births. Severe bleeding was the most common cause, with 32.28%, followed by pregnancy-induced hypertensive diseases, with 29.92% [14].

In the present study covering a ten-year period, when compared to these two studies in this region, the maternal mortality ratio was calculated as 268 per 100,000 live births. When compared to the first study, the MMR in the present clinic appears lower. However, it is still above the average rate for Turkey. The reason for this may be the excessive fertility rate in the east of Turkey, the lack of observations in antenatal care, and the fact that the present hospital is a tertiary referral hospital. Women with pregnancy and birth complications having high maternal mortality and morbidity like uterine rupture, severe preeclampsia, eclampsia, HELLP syndrome, and uterine atony are mostly referred to the present hospital, and this may explain the present high MMR. The average age of the mothers that died was 32.4 years, were mostly illiterate and came from rural areas, and the rate of antenatal care was low. From the same region but another city, Yalınkaya et al. found an MMR of 1,100 per 100,000 in Dicle University and they reported that the most frequent cause of maternal mortality was postoperative and postpartum bleeding [15].

In a study from the west of Turkey, Aksu et al. reported an MMR of 80 per 100,000 live births. This rate was low compared to the present. The most common cause of maternal death, as in the present study, was pregnancy-induced hypertension [16]. Dolanbay et al. reported MMR as 36.1 per 100,000 live births in Kayseri. They also reported that the most common cause of maternal death was hypertensive diseases, followed by obstetric hemorrhage [17].

In conclusion, maternal mortality is an important measurement associated with the general development and healthcare level of a country. The most frequent cause of maternal mortality in the present region is eclampsia and complications related to it, followed by obstetric hemorrhage. In addition, the lack of antenatal care is also remarkable. In order to reduce maternal mortality, antenatal care should be increased by creating awareness in society and new regulations for urgent cases such as preeclampsia/eclampsia and obstetric hemorrhage should be set up.

References


Address reprint requests to: Z. KURDOGLU, M.D.
Ankara Training and Research Hospital Department of Obstetrics and Gynecology
Ankara (Turkey)
e-mail: zehrakurdoglu@hotmail.com
Analysis of the use of cyclosporin A to treat refractory immune recurrent spontaneous abortion

J.H. Fu

Department of Obstetrics, Shandong Weifang People’s Hospital, Weifang (China)

Summary

Purpose: This study aims to determine the curative effect of cyclosporin A (CsA) in treating refractory immune recurrent spontaneous abortion (RSA). Patients with recurrent abortion caused by dysimmunity were enrolled. Materials and Methods: The patients were given aspirin, prednisone, heparin, immunotherapy with their husband’s leukomonocyte, and intravenous immunoglobulin (IVIG) treatment, but treatment outcomes were unsuccessful. Therefore, CsA was added to treat the women before and after pregnancy. During treatment, CsA concentration was maintained at 80 ng/ml to 150 ng/ml. The clinical effect and pregnancy outcome were observed. Results: Of the 26 patients, 20 cooperated and accomplished complete pregnancy. Twelve cases showed hypertensive disorders during pregnancy but did not exhibit symptoms of preeclampsia. Three cases were lost to follow-up. The success rate was 76.92%. Twenty patients underwent premature labor (34 weeks to 37 weeks). Nevertheless, the mothers and their children were all healthy. Conclusion: An appropriate dose of CsA has good curative effects and pregnancy results in the treatment of RSA.

Key words: Cyclosporine; Recurrent spontaneous abortion; Pregnancy.

Introduction

Reproductive immunology studies recently suggested that approximately 50% to 60% of etiologies for recurrent spontaneous abortion (RSA) are related to immunologic derangement. Successful rate of treatment has exceeded 90% because of the development of mechanism research and treatment of recurrent abortions. Immune abortion is generally classified into two types: autoimmunity and alloimmunity.

Among the RSAs caused by immune factors, applications of prednisone, aspirin, low-molecular-weight heparin, and human gammaglobulin achieved the most successful pregnancy rate in our hospital (>85%). The immune antibody reaction of women before and after pregnancy accounted for the unsuccessful pregnancy rate of 15%. Thus, a question arises as to how immune antibodies can be made negative or how to reduce immune antibody content in the blood of pregnant woman. Du et al. [1] reported that an appropriate dose of cyclosporine A (CsA) contributed to the dual-regulation role in mother-fetus immunoregulation, not only by suppressing immunologic rejection of the mother’s body to the embryonic antigen, which causes failed pregnancies, but also by promoting the growth, movement, and invasiveness of cytrophoblast cells. Therefore, CsA is a potential drug for treating pregnancy diseases, such as the RSA. CsA is a widely used drug for patients receiving organ transplantation and can effectively reduce autoimmune. Many researchers believe that no adverse pregnancy effect can result from using conventional doses of CsA and prednisone for the prevention of immunologic response during organ transplantation. Therefore, these immunosuppressive agents are additionally used before and after pregnancy for women who cannot be successfully treated by four drugs.

Combining CsA with current treatment approaches reduces the immune antibody content in blood and thereby decreases injuries of gestational sac, fetus, placenta, and uterus caused by the antibody for pregnancy success, thus ensuring a normal newborn.

RSA is a medical condition characterized by three or more consecutive pregnancy losses and has an incidence rate of approximately 5%. Since the 1990s, the present hospital has applied prednisone, aspirin, heparin, and intravenous immunoglobulin (IVIG) treatments for RSAs caused by dysimmunity, and the success rate has exceeded 85%. However, treatment for 15% of the patients still failed. This condition is defined as refractory RSA and is the focus of this study. The treatment failures for these patients can mainly be attributed to the immune antibody titers of the currently applied treatment regimens, which cannot reduce pregnancy losses. In this study, CsA was found to be an effective immunosuppressive agent that serves a double-regulation function in maternal-fetal immunoregulation. On one hand, CsA inhibits maternal immunologic rejection against fetal antigen. On the other hand, CsA promotes the growth, migration, and invasive-
ness of Langhans cells. Thus, CsA may become an effective drug for pregnancy-associated diseases, such as RSA. CsA has long been used for treating pregnant women receiving organ transplantation during gestation. These patients are subject to long-term exposure to normal doses of CsA and prednisone in case of immunologic rejection after organ transplantation. No report exists on the influence of CsA transplantation. No report exists on the influence of CsA drug for pregnancy-associated diseases, such as RSA. CsA may become an effective drug for pregnancy-associated diseases, such as RSA. CsA has long been used for treating pregnant women receiving organ transplantation during gestation. These patients are subject to long-term exposure to normal doses of CsA and prednisone in case of immunologic rejection after organ transplantation. No report exists on the influence of CsA transplant.

Materials and Methods

Clinical data

General data: Two hundred fifty-six cases of patients with RSA were selected from February 2008 to June 2011. All patients were examined for etiologies of RSA in the present hospital and other hospitals. Cases with genital malformation and dysfunction, chromosome abnormality, cryptorhea, infection, and hereditary diseases were excluded. Among the immune reasons (ACA, LA, β2-GP1, and ANA), 156 cases exhibited a positive antibody. Conventional systematic treatment was applied to 132 cases (prednisone, aspirin, heparin, and IVIG). However, pregnancy occurred again and 26 newborns did not survive. The general conditions of the 26 cases of pregnant women with treatment failure are shown in Table 1.

Treatment method

Cases with positive APS (ACA; LA; anti-β2-GP1 antibody) or anti-nuclear antibody (ANA) were examined. Some patients had a treatment history with more than two of following treatment regimens, whereas for patients with one treatment before CsA, Regimen (3) was implemented. Four treatment regimens were employed (if the blocking antibody of the patients was negative, immune therapy using the lymphocytes of the husband was additionally performed before gestation until the antibody turned positive).

Results

Among the 26 patients treated with Regimen (4), three lost visits occurred at the medium stage of pregnancy, 18 patients exhibited increased blood pressure at 15 to 34 weeks of pregnancy (gradually rose to 160/100 mmHg), one presented fetus malformation abortion at 26 weeks of pregnancy, and two intrauterine fetal deaths occurred at 13 and 22 weeks, respectively. Between 33 weeks and 37 weeks plus two days of pregnancy, 20 cases became parturient because of the premature rupture of membrane (PROM), and fetuses were prematurely born because of intratruterine distress. Cesarean section was conducted for 12 cases, and parturient parturition was conducted for five cases. Oxytocin odinopoeia was performed for the five cases caused by PROM. Results of the antibodies before and after CsA application are summarized in Table 2. Maternal conditions before and after parturition are shown in Table 3, and the results of newborns are shown in Table 4.

Discussion

The probability of RSA for patients with previous spontaneous abortions ≥ three times and positive anti-phospholipid antibody (ACA, LA, anti-β2-GP1) reaches up to 90%. Decidua and extensive intravascular thrombosis and infarction of placenta are possibly the main pathological bases of abortions caused by anti-phospholipid antibody. Damage to vascular endothelial cells and intravascular thrombosis may result from phospholipid antibody binding with the phospholipid/phospholipid binding protein (β2-GP1) compound. Aggravation of intravascular thrombosis of placenta thereby damages placental function and finally causes abortion [2, 3].
Pregnancy failure, such as RSA, is a problem that distresses gynecology and obstetrics doctors. The existing treatment mechanism is unclear, and the results reported by various researchers are inconsistent [4-6]. Therefore, more effective methods of treating pregnancy failures must be sought. After being treated by prednisone + anticoagulant, heparin combined with aspirin [7, 8] + simple IVIG or IVIG combined with anticoagulant, patients with RSA caused by dysimmunity and with positive anti-phospholipid and antinuclear antibodies achieved a successful pregnancy rate of > 85%. Many studies showed that IVIG was effective for treating RSA patients with positive anti-phospholipid antibody and that the treatment of low-molecular weight heparin combined with aspirin or prednisone was ineffective. Moreover, the above methods still failed in treating RSA caused by dysimmunity, with a resultant pregnancy success rate of approximately 10% to 15%. Although the gestational weeks increased, adverse pregnancy results, such as stillbirth in the early or medium stage of pregnancy or recurrent preeclampsia, still occurred. With the consent of the patients and their families, CsA was added for treatment. In 36 months, a total of 26 patients were involved in this therapy. As a result, 20 cases succeeded, three cases lost visits, and three cases presented stillbirth because of fetus umbilical hernia (one case) and FGA (two cases).

CsA can retard other pathways involved in immunosuppression, as discovered by Kuprash et al. [9]. They found that CsA suppresses the expression of the LTα subunit of TNFα (member of TNF family) and then serves its immunosuppression function.

Immune RSA is caused by immune antibody, and CsA is an immunosuppressive agent that exerts treatment effects by inhibiting the generation or increase of immune antibodies. CsA effectively reduces autoimmunity and is widely used for patients receiving organ transplantation. According to foreign reports [10-12], a conventional dose of CsA and prednisone for long-term therapy to prevent immunologic rejection during organ transplantation can cause no adverse pregnancy result. Moreover, CsA within the effective dose range can stimulate the growth, morphological change, and invasiveness of cytotrophoblast cells during early pregnancy [13]. Among the patients for whom received prednisone, aspirin, heparin, and IVIG treatments were infective, 26 patients were selected to receive CsA in combination with their current treatment. Twenty cases showed successful results. After CsA application, the blood antibody content evidently decreased compared with that in non-CsA patients. Twenty-one cases presented an antibody content <50% beyond the normal maximum value, accounting for 80.76% of the studied group. This percentage also exhibited a significant difference when compared with the group that did not receive CsA (3.8%).

Although all pregnant women presented severe gestational hypertension after 13 weeks of pregnancy during the treatment process, no kidney injury resulted in proteinuria and edema. The fetuses grew normally. Thus, the gestational weeks of the patients were delayed as much as possible. However, 20 patients entered the labor stage in less than 38 weeks of pregnancy and experienced premature birth. The premature newborns, particularly the two cesarean newborns, were transferred into the NICU for treatment. Their neonatal complications were more severe than those of the normal newborns. All 19 cases of newborns left hospital with their mothers five days postpartum, except for one,
who stayed in the hospital for 30 days because of hypoxic ischemic encephalopathy. Nevertheless, this newborn still left the hospital as a healthy child. The newborns did not exhibit any abnormality even at 42 days postpartum. Other conditions returned to the state before pregnancy.

CsA is a better prospect in the treatment of RSAs resulting from dysimmunity and cases with unknown causes because of its immunoregulation in vivo. However, a large-sample randomized controlled study result remains lacking. Thus, more extensive studies must be conducted.

References


Address reprint requests to:
J.H. Fu, M.D.
Department of Obstetrics
Shandong Weifang People’s Hospital
151 Guangwen Street
Weifang 261041 (China)
e-mail: fujinhuacn@163.com
Soy isoflavones, inulin, calcium, and vitamin D3 in post-menopausal hot flushes: an observational study


1 University of Catania, Catania; 2 University of Naples Federico II, Naples 3 University of Cagliari, Cagliari 4 University of Palermo, Palermo; 5 University of Bari Aldo Moro, Bari 6 CNR Pisa, Institute of Clinical Physiology of CNR Pisa, Pisa 7 University of Milan, Department of Clinical and Community Sciences, University of Milan, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan; 8 «D. Camberlingo» Hospital, Francavilla Fontana, Brindisi, A.S.L. Brindisi Presidio Ospedaliero Camberlingo, Francavilla Fontana (BR) (Italy)

Summary

Purpose of investigation: To evaluate the effect of soy isoflavones and inulin (SII) on hot flushes (HF) and quality of life in a clinical setting, the authors conducted an observational study. Materials and Methods: The authors performed an observational, prospective, multicentric study on women in peri-/post-menopause treated or untreated with a product present on the Italian market, consisting in a mixture of calcium (500 mg), vitamin D3 (300 IU), inulin (3 g) and soy isoflavones (40 mg). Results: A total of 135 patients, 75 (55.6%) in the SII group and 60 (44.4%) in the untreated group entered the study. After three months, the mean number of HF declined of 2.8 (SD 3.7) in the SII group and 0.0 in the control group (p = 0.02). Conclusion: This observational trial suggests a possible beneficial effect of a dietary soy supplement containing 40 mg of isoflavone/day plus inulin in the management of menopausal symptoms such as hot flashes.

Key words: Menopause; Hot flushes; Inulin; Isoflavone.

Introduction

Peri-menopausal and post-menopausal women frequently experience vasomotor symptoms (VMS); if severe, they may remarkably worsen the woman’s quality of life. Hormone therapy (HT) is the therapy of choice, but it has potential long term risk [1].

In recent years it has been suggested that phytoestrogens may represent an effective alternative, providing a weak estrogen-like activity sufficient to improve VMS [2], glucose homeostasis [3], and reducing bone turnover [4]. Additionally, inulin has been shown to enhance soy isoflavones absorption [5].

In order to evaluate the effect of soy isoflavones and inulin (SII) on hot flushes (HF) and quality of life in a clinical setting, the authors observed a unselected population of Italian women with VMS, not taking HT, treated with soy isoflavones and inulin, in comparison with an untreated group with similar characteristics.

Materials and Methods

The authors performed an observational, prospective, multicentric study on women in peri-/post-menopause treated or untreated with a product present on the Italian market, consisting in a mixture of calcium (500 mg), vitamin D3 (300 IU), inulin (3 g), and soy isoflavones (40 mg). All peri- and post-menopausal women referring to the participating Menopause Clinics in the study period (beginning of study: January 2012), aged 45-60 years, with at least three hot flushes a day, not requiring HT and/or therapy for osteopenia/osteoporosis, and not on HT in the last month, were eligible for the study. They were asked to give their informed consent to the study. The study was approved by the Institutional Review Committee of IRCCS Policlinico of Milan.

As this is an observational study, the choice of therapy was entirely up to the individual physicians and patients in each center. As by clinical practice, women were proposed soy isoflavones and inulin and calcium therapy. Those who accepted were included in the treatment group, those who did not in the untreated group. General characteristics and anamnesis were collected and patients fulfilled the MenQOL questionnaire [6]. Follow-up visits were scheduled after three and six months and information about HF frequency and adverse events was recorded. The MenQOL questionnaire was administered at each visit.

Statistical analysis

Descriptive statistics (mean±standard deviation, SD), median (interquartile range, IQR), and frequency (%) were used to describe the study population. Women were evaluated at study entry and after three and six months. Primary endpoint was the mean change in the number of HF’s/day; secondary endpoints were the mean change in the domains of the MenQOL validated question-
Sample size

The authors foresaw to include into the study 200 subjects. They interrupted the trial when the recruitment reached 135 women due to the low recruitment rate during the last period of the study. With this sample size, they were able to identify a decrease of two HF per day (SD 4). This decrease may be considered the minimum clinically important change from baseline in the treated group as compared to the not treated one.

Results

A total of 135 patients, 75 (55.6%) in the SII group and 60 (44.4%) in the untreated group entered the study. General characteristics are shown in Table 1. Baseline characteristics were comparable by group: no statistically significant difference was present, in particular the two groups were comparable in terms of baseline values of mean number of HF and of the considered domains of the MenQOL questionnaire.

A total of 19 women in the SII were lost to follow up (16 in the first three months of treatment and three in the three to six months period). The corresponding numbers in the no treatment group were six and 22.

Out of the 69 women in the SII group who were seen at three- or six- month visit, nine (13.0%) stopped the treatment: they were not considered in the HF and MenQOL analysis. After three months, the mean number of HF declined of 2.8 (SD 3.7) in the SII group, but in the control group it was unchanged \((p = 0.0009)\). The corresponding values after six months were –3.7 (SD 2.7) and –0.9 (SD 5.3) \((p = 0.02, \text{Table 2})\).

Considering the MenQOL questionnaire, after three-month follow up, the mean changes from baseline showed a favourable effect of SII as compared to no treatment in the vasomotor and sexual domain. At six-month follow-up, these improvements were statistically significant in all the domains.

Adverse events are considered in Table 3. Ten patients in the soy isoflavones and inulin and three in the no treatment group did not attend the three-month visit but were seen at six-month visit: accordingly, overall period adverse events recorded were from 69 and 57 subjects, respectively.

Discussion

In this study, three-month supplementation with soy isoflavones and inulin was effective in reducing the mean number of hot flushes per day in peri- and post-menopausal women. Further significant changes in the quality of life score were observed in all the MENQOL domains after six months of treatment.

The results of this study are consistent with those reported in several studies \([7-12]\) but not in others \([13-15]\). This inconsistency has been, although not entirely explained due to different and inadequate isoflavone doses \([7]\).

In this study the authors used 40 mg of soy isoflavones plus inulin, that has been shown to enhance soy isoflavones absorption \([5]\) and, potentially, the therapeutic effect.
To evaluate the quality of life at baseline and during the sixth-month follow-up, the 29-item MenQOL was used [6] It is a self-administered, health-related quality of life scale for menopausal women. It indicates the well being as regards four domains (vasomotor, psychological, physical, and sexual).

In the present study; after three months of treatment, the differences were not significant in the psychological and physical domains, whereas vasomotor and sexual improvement emerging. At the six-month follow-up, women still on treatment showed a consistent relief of discomfort in all the domains.

In a double-blind, randomized, controlled, intention-to-treat trial a treatment with 50 mg of lignans, 25 g of soy and 42 mg of isoflavones showed no significant effect of treatment on all quality-of-life domains measured with the MenQOL questionnaire [13]. It is difficult to interpret these different results. The observational design versus the randomized one should be considered, however it is conceivable that the association of isoflavones with inulin may enhance the clinical effect of isoflavones.

In the present study, gastrointestinal adverse events were more frequent, but in a non-statistically significant way, in the SII group. Nausea and vomiting were reported in about 25% of treated women and 15% of untreated ones, whereas feeling bloated was more frequent among women not receiving the treatment. This may be at least partially explained by a bias toward reporting adverse effects in women aware of taking an active treatment. Along this line, in several randomized studies reporting adverse effects in women taking soy isoflavones [8, 16, 17], the frequency of gastrointestinal complaints was much lower. In fact, for example, the higher figure was 7.3% in the active treatment and 8.6% in the placebo group, reported by in Ferrari [16] and similar by treatment arm, when patients were blind to the assigned treatment.

The main limitation of this study was the observational design. Being aware of the treatment can affect both the response to supplementation and the adverse event reporting. In fact, the proportion of women reporting nausea and vomiting was remarkably higher than in randomised, placebo controlled trial using similar interventions [8, 17].

In conclusion, this observational trial suggests a possible beneficial effect of a dietary soy supplement containing 40 mg of isoflavone/day plus inulin in the management of hot flushes. As alternative treatment, soy isoflavones plus inulin combination might represent an interesting and safe, at least in the medium term, alternative in women who do not accept conventional HRT, but are seeking relief for menopausal vasomotor symptoms.

References

Address reprint requests to: F. PARAZZINI, M.D.
Department of Clinical and Community Sciences
University of Milano, Fondazione IRCCS
Ca’ Granda Ospedale Maggiore Policlinico
Via Commenda 12, 20122 Milan (Italy)
e-mail: Fabio.Parazzini@unimi.it
Introduction

Low density lipoprotein (LDL)-cholesterol elevation is closely related to endothelial damage and increased inflammatory response. After infiltrating the arterial endothelium LDL enters into intima, where it undergoes oxidative modification that leads to the accumulation of cholesterol and migration of macrophages [1, 2]. Macrophages that phagocytose ox-LDL, are transformed into foam cells and contribute to the maintenance of this process. Oxidation of LDL lipids is associated with many clinical conditions [3-6]. In particular, the study of atherosclerosis has gained importance in recent years [7]. It was determined that inflammation in atherosclerosis plays an important role in tissue damage and oxidative stress, and in these patients increased LDL oxidation is associated with end products of lipid peroxidation [2, 7].

Preeclampsia is a hypertensive disorder leading to abnormalities like proteinuria, coagulation disorders, and various systemic symptoms [8-10]. Although preeclampsia is the most common complication of pregnancy that causes maternal and perinatal morbidity and mortality, very little information is available about its etiology [10]. Increased oxidative stress and endothelial disfunction have been suggested as the reason of poor placentation [11] and the presence of oxidative stress are shown in both of maternal circulation and placenta [12, 13]. Villar et al. [14] have reported high levels of oxidatively modified forms of proteins and lipoprotein particles in the serum of pregnant women with preeclampsia.

Ox-LDL plays a key role in the development of atherosclerotic cardiovascular disease [15]. Because of the similarities between the pathogenesis of atherosclerosis and preeclampsia, the interest of researchers has concentrated on this issue in recent years; however, conflicting results have been found [16-18]. The aim of this study was to measure and compare serum Ox-LDL and other lipid parameters in preeclamptic and normotensive pregnant women. The present results suggest that the levels of serum Ox-LDL and other lipid parameters rise as a result of pregnancy rather than as a result of preeclampsia.

Materials and Methods

Twenty-seven preeclamptic and 26 normotensive pregnant women in their third trimester, were included in the study. The aim of the study was explained to the pregnant women and those who accepted were enrolled into the study. Blood pressure measurements were taken on the left arm at the heart level in a seated position, after a ten-minute rest period. Those with blood pressures under 140/90 mmHg were considered normotensive, and...
those with blood pressures equal to or higher than 140/90 were considered as hypertensive. Following criteria were used in the diagnosis of preeclampsia: two blood pressure values measured at least six hours being 140/90 or higher; > 75 mg/dL urine protein levels; > +1 edema after 24 hours bed rest. The blood samples were taken into ten-ml vacuum glass tubes from antecubital vein for Ox-LDL and lipid profiles. Samples were centrifuged at 4,000 rpm for 15 minutes and sera were stored at -80 °C for a maximum six months until the time of the study. Ox-LDL levels were determined by enzyme linked immunosorbent assay (ELISA) (solid phase bidirectional enzyme immunoassay). Total-cholesterol, HDL-cholesterol, and TG levels were measured by colorimetric method in an autoanalyzer. LDL-cholesterol levels were calculated by dividing the TG values by 5. Statistical evaluation was performed by SPSS, and comparisons were made using Student’s t test. A p<0.05 was considered significant.

Results
The ages and gestational weeks of normotensive and preeclamptic women were 27.9±5.7 (minimum 19, maximum 39) and 28.7±5.5 years (minimum 18, maximum 40); 30.9±3.0 (minimum 26, maximum 36), and 32.3±2.7 weeks (minimum 27, maximum 36), respectively. Age and gestational week distributions did not differ significantly between the two groups. Diastolic and systolic blood pressures of normotensive and preeclamptic pregnant women were 71±5 mmHg and 96±8 mmHg; 110±8 mmHg and 147±8 mmHg, respectively. Statistically significant difference was observed between diastolic and systolic blood pressures of the two groups (p<0.01). Ox-LDL levels of normotensive and preeclamptic women were 130±60 U/L and 133±69 U/L, respectively, and there was no significant difference between these values. LDL-cholesterol, HDL-cholesterol and total cholesterol levels measured in normotensive and preeclamptic pregnant women were 147±61 mg/dL and 136±59 mg/dL; 68±14 mg/dL and 61±16 mg/dL; 248±49 mg/dL and 248±82 mg/dL, respectively. There was no significant difference between two groups’ total cholesterol, LDL-cholesterol, and HDL-cholesterol levels (Table 1). TG and VLDL-cholesterol levels of normotensive and preeclamptic women were 207±77 mg/dL and 257±88 mg/dL; 41±15 mg/dL and 51±18 mg/dL, respectively. TG and VLDL-cholesterol levels were significantly higher in the preeclamptic pregnant group (p<0.05) (Table 1).

Discussion
Hyperlipidemia predisposes to the development of atherosclerotic disease by impairing endothelial function [19]. Physiological hyperlipidemia in pregnancy have been reported [20]. Sattar et al. reported that high levels of TG in normal pregnancy is accompanied by an increase in small dense LDL [21]. According to a study made by Belo et al. [22], TG, VLDL-cholesterol, and small dense LDL levels were high in normal pregnant women. In this study, 77% of normal pregnant women’s total cholesterol, TG and VLDL-cholesterol, 53% of their Ox-LDL, 58% of their LDL-cholesterol, and 69% of their HDL-cholesterol levels were higher than upper reference values, and these findings are supportive of the results of Belo et al. [22].

In preeclampsia, contradictory results have been found regarding the levels of serum lipid parameters. Although there is a study which has reported that abnormal lipid profiles in preeclampsia play a role in the progression of vascular dysfunction and oxidative stress [23]. Kim et al. [16] reported low serum total and HDL-cholesterol levels in preeclamptic women when compared to normotensive ones. Uzun et al. [17] have found higher serum total cholesterol, LDL-cholesterol and VLDL-cholesterol, and TG levels in preeclamptic women than in normotensive pregnant women. According to Roberts et al. [24], impairment of antioxidant-antioxidant balance against antioxidants causes a ‘defense’ dysfunction of vascular endothelial vasodilator and antiplatelet activity by increasing the lipid peroxidation and lipid peroxides show a higher degree of circulation in preeclamptic women than in normal pregnant women. Oxidative degradation products of modified polyunsaturated fatty acids, malondialdehyde (MDA), and 4-hydroxynoneval (4-HNE), are covalently bound to apoB lysine residue [25]. In the present study, total cholesterol levels in 74%, TG and VLDL-cholesterol levels in 85%, Ox-LDL levels in 48%, and LDL-cholesterol and HDL-cholesterol levels in 59% of preeclamptic women were higher than upper reference values, unlike some of the results of some researches [16, 17, 23-25], no significant difference was observed between the values of total, LDL-cholesterol and HDL-cholesterol in preeclamptic and normotensive pregnant women. However, compatible with the study of Uzun et al. [17], TG and VLDL-cholesterol levels were significantly higher in the preeclamptic group than in the normotensive group.

Different and conflicting results have been reported concerning Ox-LDL levels in preeclamptic women. While Raijmakers et al. [18] have reported that in preeclamptic women ox-LDL levels are lower than the Ox-LDL levels in the sim-

Table 1. Comparison of Ox-LDL and lipid parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean ± standard deviation (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox-LDL (U/L)</td>
<td>Control</td>
<td>130.08 ± 60.04</td>
<td>0.878</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>132.81 ± 68.81</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>Control</td>
<td>147.00 ± 61.25</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>135.81 ± 59.03</td>
<td></td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dL)</td>
<td>Control</td>
<td>41.42 ± 15.26</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>50.81 ± 17.55</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>Control</td>
<td>67.46 ± 14.30</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>61.41 ± 16.69</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>Control</td>
<td>248.19 ± 49.37</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>248.04 ± 81.50</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>Control</td>
<td>207.08 ± 76.90</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>256.74 ± 87.86</td>
<td></td>
</tr>
</tbody>
</table>
ilar gestational age control group and low ox-LDL levels could be an indicator of preeclampsia; in contrast, Kim et al. [16] reported that high serum ox-LDL levels in preeclamptic women is important in endothelial dysfunction, and Uzun et al. [17] emphasized that pre-eclampsia is associated with high ox-LDL, leading to vascular endothelial damage by contributing to the pathogenesis of preeclampsia. Pavan et al. [11] have reported that in-vivo human trophoblast invasion could be modulated by ox-LDL and this defective trophoblast invasion could shed light on the pathogenesis of preeclampsia. According to Hörkkö et al. [26], antibodies against MDA-LDL are very important in pathogenesis of ox-LDL. In a study conducted by Branch et al. [27], MDA-LDL auto-antibody titer remained quite high in preeclampsia compared to healthy pregnant women. Pecks et al. [15] found no difference between preeclamptic and normotensive pregnant women’s placental ox-LDL levels, whereas Açıkgöz et al. [28] reported that they found lower placental ox-LDL levels in preeclamptic women than in normotensive ones. In the present study, although the mean serum ox-LDL levels were found above the upper reference range, no significant difference was found between the two groups.

As a result, high VLDL-cholesterol and TG levels in preeclamptic women suggesting dyslipidemia associated with high TG, may contribute to the pathogenesis of preeclampsia despite the lack of significant difference between ox-LDL, total cholesterol, LDL-cholesterol, and HDL-cholesterol levels in normotensive and preeclamptic women, who have similar distribution of age and gestational week. Even though the ox-LDL levels were above the upper reference limit in preeclamptic and normotensive women, still there was no significant difference between the levels of these two groups. The present results show that these parameters are not suitable for use as diagnostic markers.

References

Address reprint requests to: S. TURKMEN YILDIRMAK, M.D.
Department of Clinical Biochemistry
SB Okmeydani Educational and Research Hospital
Darülceze Cad, 34384 Sisli, Istanbul (Turkey)
e-mail: yildirmaksembolu@gmail.com
Introduction

Prolactin (PRL) is a 23 kDa protein secreted by the pituitary gland and has many actions in the reproductive system and metabolism [1]. PRL secretion depends on various neurotransmitters and its serum concentration differs in various somatic and psychological conditions [1].

The most common medical problem of climacteric women are vasomotor and depressive symptoms [2]. Etiology of these symptoms still is not clear with hormonal background suggested.

This study was conducted to elucidate the problem of serum PRL concentration in menopausal women and its possible relations with climacteric and depressive symptoms.

Material and Methods

The study included 202 women aged 40-65 years admitted to the Department of Gynecological Endocrinology, Poznań University of Medical Sciences, because of climacteric symptoms. The authors assessed the intensity of climacteric and depressive symptoms with the Kupperman index and the Hamilton depression scale, measured BMI index, serum PRL, FSH, LH, 17β-estradiol, total testosterone, and dehydroepiandrosterone sulfate (DHEAS) levels in all studied women. Results: They found a correlation between serum PRL concentration and result of M. Hamilton depression scale (R=0.21; p = 0.005) and a between serum PRL concentration and serum 17β-estradiol concentration (R=0.21; p = 0.003). Conclusion: The authors concluded that serum PRL concentration is related to severity of depressive symptoms in menopausal women.

Key words: Prolactin; Menopause; Climacterium; Depression.
correlation between serum PRL concentration and serum 17β-estradiol concentration (R=0.21; p = 0.003). There was no other correlation between serum PRL concentration and other hormonal parameters of the study group.

Discussion

Mean serum PRL concentration in menopausal women in the present study (13.8 ng/ml ± 10.2 ng/ml) was in the normal range (4.8 - 23.3 ng/ml). Serum prolactin concentration is reported to be lower by about 40% in postmenopausal women than in premenopausal women [5]. This is probably not an effect of aging but menopause as in men after fifty there is an 18% increase of PRL serum concentration in comparison with younger men [5]. In the present study there was no effect of age and time since last menstruation on serum PRL concentration.

The present authors did not observe any correlation between serum PRL concentration and BMI. Such a correlation is reported by other authors [5, 6]. The explanation for this correlation is a positive impact of leptin on PRL secretion [7].

There was no correlation between serum PRL concentration and climacteric symptoms severity. There are no studies on possible relationship between serum PRL concentration and severity of climacteric symptoms. Studies referring to impact of hormonal replacement therapy on the severity of climacteric symptoms revealed that improvement in hot flashes was connected with increase of PRL serum concentration [8].

In the present study there was a correlation between serum PRL concentration and depressive symptoms severity. Similar results were obtained in the study referring to the patients with clinic hypothyroidism and subclinical hypothyroidism. These patients had hyperprolactinemia and depression together with sexual dysfunction [9]. There are also studies which reveal normal serum PRL concentration in depression [10]. Patient with improvement of depressive symptoms during treatment had higher serum PRL concentration [8] and increase of PRL serum concentration during antidepressant therapy was suggested to be a positive prognostic factor of beneficial effect of such a therapy [11].

There was a correlation between serum PRL concentration and serum 17β-estradiol concentration. Such a correlation is reported also by other authors [8]. 17β-estradiol acts on the level of pituitary stimulating synthesis and secretion of PRL [12], proliferation of lactotrophs [13], and sensitizing lactotrophs to respond to neuropeptides and growth factors [14].

Conclusion

Serum PRL concentration is related to severity of depressive symptoms in menopausal women.

References

Serum prolactin concentration and severity of depression symptoms in climacteric women


Address reprint requests to:
R. SLOPIEŃ, M.D.
Department of Gynecological Endocrinology
Polna 33, 60-535
Poznań (Poland)
e-mail: asrs@wp.pl
Adverse perinatal outcomes of adolescent pregnancies in one center in Istanbul, Turkey

E.C. Eren1, A. Ekiz2, S. Mumusoglu3, D. Yildirim2, B. Aydiner2, M. Bestel2, H.C. Ark2

1 Medipol Hospital, Istanbul; 2 Kanuni Sultan Suleyman Education and Research Hospital, Istanbul; 3 Istanbul Zeynep Kamil Maternity and Children Training and Research Hospital, Istanbul (Turkey)

Summary

Purpose: The objective of this study was to evaluate fetal and perinatal outcomes of pregnancies of adolescents and compare them with adult pregnancies. Materials and Methods: This retrospective case-control study was carried out at Bakirkoy Maternity and Children’s Diseases Education and Research Hospital in Istanbul, Turkey. It enrolled 2,491 pregnancies who delivered between 2005-2010, of which 998 were adolescent pregnancies and 1,493 were adults as controls. Results: The mean age of the adolescent group was 17.10 years and in the control group the mean age was found to be 26.73 years. Intermarriage, vaginal delivery, preterm rupture of membranes, preterm birth, and preeclampsia were significantly higher in adolescent pregnancies than the control group. Gestational diabetes was more common with increasing age. There was no statistically meaningful difference between the groups in terms of intrauterine growth restriction (IUGR), low birth weight, anemia, 5-minute APGAR score, and intrauterine fetal demise. Conclusions: Young maternal age is a risk factor for preterm birth, preterm rupture of membranes, and preeclampsia. According to this study, adolescent pregnancies are more risky and more likely to have adverse fetal outcomes.

Key words: Perinatal outcome; Adolescent pregnancies; Maternal age.

Introduction

In many countries, adolescent pregnancy and its results are one of the crucial public health problems with major social implications. In 2008, 16 million infants were born from mothers aged between 15-19 years. About 95% of these births were in the countries with low socio-economic level [1] and 11% of all the births were given by adolescent mothers. According to the latest estimates, even if there are relatively few births under the age of 16 years, every year, one million girls between the ages of 12 to 15 years are giving birth [2].

Adolescent pregnancies are associated with adverse pregnancy outcomes such as low birth weight, preterm birth, and perinatal and maternal mortality [3-5]. In addition, it is reported that there is an increased rate of pregnancy-induced hypertension, anemia, low weight gain during pregnancy, preterm birth, low birth weight infant, and perinatal mortality [6, 7]. Prenatal malnutrition, emotional stress, and suboptimal maintenance are more often among adolescents. Maternal lifestyle disadvantages and biological immaturity can introduce many problems for both intrauterine fetus and newborn during postnatal life [1]. The aim of the present study was to research, as adolescent pregnancies are compared with adult pregnancies, if there is more complications with the adolescents and what type of complications often occur.

Materials and Methods

The pregnant women who gave birth under the age of 18 years in Istanbul Bakirkoy Maternity and Child Diseases Education and Research Hospital between years 2005 to 2010 were studied retrospectively. The authors began running this study after approval from ethical board of Istanbul Bakirkoy Maternity and Child Diseases Education and Research Hospital (dated June 12, 2009 and 239 numbered ethical committee approval). The study included 2,628 maternal birth files that were retrieved from the hospital archive. The cases excluded were 126 that were over the actual age of 18 years and 11 cases with twin pregnancies. In addition, pregnancies that were less than 22 weeks and under 500 grams of fetal weight were not taken into account.

A number of 998 adolescents were included in the study. The control group was selected by random sampling method and 1,493 files of mothers between the ages 20-35 years who gave birth in the present hospital were studied retrospectively. A total of 2,491 files were studied throughout the trial. The age distribution of adolescent pregnancies is shown in Table 1. The records were reviewed by the same observer in terms of demographic and clinical results. With regards to the mother, maternal age, parity, gravidity, intermarriage, parity, gestational age, birth type, cesarean indications, complete blood count and hematocrit values, and obstetric complications were noted.

In addition, fetal results were assessed in terms of live births, stillbirths, birth weight, APGAR score, and requirement of neonatal intensive care unit. Obstetric complications as anemia, preeclampsia, eclampsia, HELLP syndrome, premature birth, premature rupture of membranes, intrauterine growth restriction (IUGR), and gestational diabetes were screened.
All the statistical calculations were performed with the Statistical Package for Social Sciences (SPSS version 15.0) statistical software package.

Results

The demographic characteristics of both the control and adolescent groups are shown in Table 2. It was calculated that the average age of the adolescent group was 17.10 years and in the control group it was 26.73 years; 188 of the adolescent pregnancies were intermarriage. There was a statistically significant difference compared to the control group. The adolescent group was significantly less in number of gravidity, parity, and abortion (Table 2). The number of parity of six adolescents were two and in 69 of them, it was one. The average gestational age was 38.3 weeks in the adolescent group and 38.4 weeks in the control group. There was no significant difference in terms of gestational age.

The rate of vaginal delivery was 79.8% for the adolescent group and 61.4% for the control group. The type of birth was statistically different between the groups (Table 2). There was a statistical differences when the nulliparous patients of the groups were compared in terms of birth type.

In the present study, it is found that the reasons why adolescents undergo cesarean delivery were as follows: cephalopelvic disproportion (CPD), breech presentation, and fetal distress; however in the control group, CPD was the second reason after previous cesareans. As the previous cesareans were excluded, primary cesarean rate was 19.6% in the adolescent group and 23.4% in the control group. This difference was statistically meaningful (p = 0.033). The incidence of premature rupture of membranes (PROM) in the adolescent group was 5.4% and in the control group it was 2.3%, and this was a statistically significant outcome. Ninety-five of the patients in the adolescent age group had premature delivery but in the control group the number of patients with premature delivery was only 75. The young age was found to be a risk factor for premature delivery; 4.7% of the adolescent patients developed preeclampsia, but in the control group the ratio of preeclampsia was 2.9%. The incidence of preeclampsia between these two groups was statistically significant. When the nulliparous patients in both groups were compared in terms of preeclampsia, there was no statistical difference. For preeclampsia, the nulliparity was found to be a more important risk factor than the age. In the adolescent group, six preeclamptic patients had an eclamptic seizure. No eclampsia case was seen in the control group. This difference was statistically significant (p < 0.001).

There was no significant difference in terms of IUlGR and HELLP syndrome incidence. There were 68 patients with gestational diabetes in the control group and only nine in the adolescent group. This statistically significant difference supports the outcome that the incidence of gestational diabetes increases with the advancing age. There was no statistical difference with respect to placental disorders.

The ratio of low birth weight babies (< 2,500 grams) was 13.5% in the adolescent group and 10.7 in the control group. Babies with low birth weight were more in the adolescent group, but no significant differences were found. The average of 1st minute and 5th minute APGAR scores of live births in the adolescent group were 7.22 and 9.16 and in the control group were 6.93 and 8.94, respectively; 1.8% (n=18) of the babies by adolescent mothers had a 5-minute APGAR < 7 and this ratio was found to be 1.13% (n:17) in the control group members. Between the groups, there was no significant difference with regards to the neonatal outcomes.

### Table 1 — The age distribution of adolescent pregnancies.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>3.50</td>
</tr>
<tr>
<td>16</td>
<td>191</td>
<td>19.13</td>
</tr>
<tr>
<td>17</td>
<td>369</td>
<td>36.97</td>
</tr>
<tr>
<td>18</td>
<td>392</td>
<td>39.27</td>
</tr>
<tr>
<td>Total</td>
<td>998</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 2 — The demographic characteristics of the groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>≤ 18 age (n=998)</th>
<th>20-35 age (n=1493)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>17.10</td>
<td>26.73</td>
<td></td>
</tr>
<tr>
<td>Intermarriage</td>
<td>6%</td>
<td>18%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.16±0.44</td>
<td>2.26±0.38</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parity</td>
<td>0.08±0.29</td>
<td>0.96±0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Abortion</td>
<td>0.07±0.30</td>
<td>0.23±0.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Week of birth</td>
<td>38.3</td>
<td>38.4</td>
<td>NS</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>31.6%</td>
<td>38.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3087.18</td>
<td>3134.61</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Not significant

### Table 3 — Comparison of obstetric complications and neonatal results.

<table>
<thead>
<tr>
<th>Obstetric complications and neonatal results</th>
<th>≤ 18 age</th>
<th>20-35 age</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPROM</td>
<td>54 (5.41%)</td>
<td>35 (2.34%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Preterm delivery (&lt;37 weeks)</td>
<td>95 (9.51%)</td>
<td>75 (5.02%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>47 (4.70%)</td>
<td>44 (2.94%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HELLP</td>
<td>1 (0.10%)</td>
<td>3 (0.20%)</td>
<td>NS</td>
</tr>
<tr>
<td>IUGR</td>
<td>39 (3.90%)</td>
<td>52 (3.48%)</td>
<td>NS</td>
</tr>
<tr>
<td>Intrauterine demise</td>
<td>8 (0.80%)</td>
<td>23 (1.54%)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>9 (0.90%)</td>
<td>68 (4.55%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPROM</td>
<td>10 (1.00%)</td>
<td>9 (0.60%)</td>
<td>NS</td>
</tr>
<tr>
<td>5-minute APGAR &lt; 7</td>
<td>18 (1.80%)</td>
<td>17 (1.13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Small for gestational age (&lt; 2,500 gr)</td>
<td>135 (13.52%)</td>
<td>160 (10.71%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Not significant
Discussion

Adolescent pregnancy is a social problem in the countries at all levels including developed ones. However, the rate of childbearing in adolescent women varies depending on factors such as regional, cultural, religious, political, economic, and many others. Based on the results of Turkey Demographic and Health Survey in 2008, in Turkey 12.1% of women under the age of 18 give birth [8, 9]. In the present study, the average age calculated was 17.1 years in the adolescent group and the consanguinity rate was 18.8%. According to the results of 2008 survey, it is clear that the consanguinity rate was determined as 34.9% amongst the adolescent pregnancies in Turkey [9]. This difference occurs because the patients enrolled in the study are living in urban areas rather than rural ones.

Adolescent pregnancy is often considered as a high-risk pregnancy. The main reason responsible for the increased risk is not known but immaturity of the mother, low level of education, and low socio-economic status may be factors contributing to this situation. In the present study, there was no difference in terms of gestational week during delivery between adolescent and control groups. Preterm birth rate in the adolescent group was statistically and significantly higher (Table 3). In many other studies in the literature on adolescent pregnancies, the preterm birth rate was found to be higher depending on sociodemographic properties [4, 10, 11]. Similarly, in some studies there was no difference between the mean gestational age values, but preterm delivery rates were higher [12]. In addition, in another study similar to the present hospital-based study, preterm birth rate in adolescent age was found to be 27.7% and an increased risk of preterm birth amongst adolescents, when compared to the control group, was reported [13, 14]. In another study conducted in Turkey likewise showed an increased risk of preterm delivery [13, 15].

Recently, studies in Turkey and Cameroon, adolescent pregnancies had not been shown as a risk factor for PROM [5, 16]. In the present study, the frequency of PROM in the adolescent age group was found to be increased (2.3% to 5.4%). In the literature, being adolescent has been reported as a risk factor for PROM [14]. The contradicting results in the literature may be due to the variety of factors that influence PROM or the studies were generally arranged on referral hospitals in home countries.

In a study conducted on 403 patients, there was no difference in terms of preeclampsia among adolescents compared with the control group [17]. Similarly, in other studies conducted in Iran and in Turkey, preeclampsia did not show a higher incidence in adolescent group compared with the control group [5, 18]; 4.7% of the present patients in adolescent group (n = 47) developed preeclampsia. In the control group the ratio was 2.9% (n = 44). The incidence of preeclampsia between these two groups was statistically significant.

Many studies support the present research in terms of preeclampsia [15, 19]. However, studies conducted in France showed that as the maternal age decreased, the number of cases with preeclampsia decreased [20]. A large-scale cohort study conducted in Canada showed in adolescent pregnancies that the hypertensive problems are less common [14]. In the present study, comparing the nulliparous patients in the control and adolescent groups, there was a statistically significant difference in the incidence of preeclampsia. The different outcomes with the studies may be caused by the study design and the fact that these studies did not assess the entire community.

Between the two groups, no significant difference was observed in terms of HELLP syndrome and IUGR. Similarly, in another study in Turkey, there was no increased risk in terms of HELLP syndrome and IUGR for adolescents; on the other hand, as being adolescent was shown to be a risk factor for eclampsia [21]. In contrast, Turkey and Iran conducted studies that showed an increased risk for IUGR [18, 22]. In utero mort fetalis (IUMF) was seen in 1.54% of the patients in the reproductive age group (n=23) and in 0.80% of adolescents (n=8); this difference was not statistically significant. In the present study, similar results were found [21, 23]. In a study by Mukhopadhyay et al., stillbirth occurred in 5.1% of the patients and this result was significantly higher than the control group. Adolescent pregnancies are considered as a risk factor for stillbirth [13]. Although 4.55% of the patients in the reproductive age group had gestational diabetes (n=68), in the adolescent group the ratio was 0.90% (n=9) (p < 0.001). As other large-scale studies indicate, the incidence of gestational diabetes increases with the increase in age: similar to the present study [14]. Ihab et al. showed in their study that gestational diabetes was at a similar rate in both groups, however in Turkey, Karabulut et al. in adolescent group and in the older age group showed that gestational diabetes was observed more often than in the control group [15, 23].

Unlike many studies in the literature, the present authors observed no increase in the incidence of anemia for adolescent pregnancies when compared with the control group [5, 20]. In a study on pregnant women by Karaoglu et al., anemia rate was 27.1%, and 24.3% in both groups, respectively and they found no differences between the two groups [24]. In addition, adolescent pregnancies frequently result as a risk factor for low birth weight and very low birth weight [4, 5, 11, 12, 19, 25]. In many studies conducted in last year, low birth weight risk increased with adolescents [5, 15, 26]. Khash et al. in their study examined nearly 50,000 pregnancies and did not show an increased risk for low birth weight in adolescents [27]. As a matter of fact, it has been a topic of debate whether adolescent mothers are at higher risk of these complications or not. Among adolescents who have easy access to health services are reported to be generally at less risk of complications [28]. Harville et al. reported in their study that preterm birth and low weight birth was distributed


equally among adolescent and young adult mothers [29]. In the present study there were no significant differences in terms of having low birth weight babies. In terms of having 5-minute APGAR score below 7 and other neonatal outcomes, there was no significant difference between the two groups. In the literature there are studies showing similar results [23]. Another study conducted in Turkey showed that there were no significant differences between the two groups [21]; another study, though difficult to explain, reported higher 5-minute APGAR scores in adult women [5]. In studies from Canada and conducted in Turkey the infants of adolescent mothers had lower 5 minute APGAR scores [12, 26]. Differences in the results of studies may depend on receiving inadequate antenatal follow-up and different socio-demographic characteristics of the study groups.

Mahavark et al. did not report a difference between the type of delivery in adolescents and control group [19]. However, strongly parallel to literature, the present study showed that the adolescent age group had a higher rate of normal birth than the control group [5, 9, 11, 14, 15, 20]. In this study, the cesarean rate was 21.1% for the adolescent group and 31.6% for the control group. Similarly, in a study by Bildircin et al., the rate of cesarean section was found to be 17.1% and 28.8% for the adolescent and control groups, respectively. Higher cesarean birth rates in older age groups may be attributed to several cesarean reasons previously performed. In the present clinic, vaginal birth after cesarean is not common, patients with sections routinely undergo surgery. In parallel to other studies CPD was found to be the most common indication for cesarean section in the adolescent group [11, 30]. Teenage pregnancies in Western countries are unwanted pregnancies by those living with a very irregular life. Considering the conditions of the present country, adolescent pregnancies are more common both in rural places as well as in urban migrations that have subsequently broken socioeconomic status. Large proportion of these pregnant women are married and have a settled life. Drug, alcohol, and smoking habits are virtually nonexistent. Most of these young couples are influenced by family elders, although these young parents are unwilling to accept ].

Conclusion

In the present study as the authors compared adolescent pregnancies with normal reproductive age pregnancies in terms of obstetric and fetal outcomes, adolescent pregnancies were identified as a risk factor for preterm delivery, PROM, preeclampsia, and eclampsia. It would not be reasonable to compare the teenage pregnancies in Western society with pregnancies in the present country to expect similar results. Because when only the adolescent age group is viewed, according to the data of Turkey Demographic and Health Survey in 2008, out of wedlock pregnancies remained below 1%; this ratio was 79.4% in USA for adolescents. Sixteen adolescent mothers in the present study, were removed from their families due to pregnancy and were housed in social service agencies. In the general population, sexual intercourse without marriage among young people is increasingly acceptable. Therefore, future problems with teenage pregnancies can vary and may be similar to developed countries. In adolescence, pregnancies within marriage are caused by lack of education and unemployed parents; on the other hand, pregnancies without marriage are caused by ignorance and not knowing the risks of pregnancy. In this regard, improving the girls’ participation in education, making high school education mandatory, and encouraging women to take place in working life more often, will help increasing the status of women, as well as protect teens from early marriage and consequently from early pregnancies and various complications. To close the knowledge gap regarding safe sex life, the curriculum should be enriched with sexual health information and mass media education campaigns should be carried out in order to educate adolescents remaining out of school. Young people should have easy access to health units to receive counseling services without any judgement.

References

Adverse perinatal outcomes of adolescent pregnancies in one center in Istanbul, Turkey


Address reprint requests to: A. EKIZ M.D.
Fevzi Cakmak mah. Barbaros cd. 775
Sk Validesuyu Konutları C2 blok D:34
Gaziosmanpasa, Istanbul (Turkey)
e-mail: draekiz@gmail.com
Efficacy of fetal thigh volumetry in predicting birth weight using the virtual organ computer-aided analysis (VOCAL) technique

J. Park, T.H. Kim, H.H. Lee

Department of Obstetrics and Gynecology, Soonchunhyang University College of Medicine, Bucheon (Republic of Korea)

Summary
Objectives: The present study was conducted to compare the accuracy of formulas used to calculate fetal thigh volume (FTV) using the virtual organ computer-aided analysis (VOCAL) technique with two-dimensional (2D) in formulas predicting birth weight. Materials and Methods: This was a prospective, cross-sectional study of 84 pregnant women with 72 hours of delivery evaluated at a university hospital between May, 2008 and April, 2010. After 2D ultrasounds (US) measurement, 3DUS was also used to determine FTV with estimates computed using the VOCAL program. Results: The correlation between fetal weight predicted by the 3D equation of FTV and the actual birth weight was significant. While FTV and the Hadlock II equation exhibited a low sensitivity for detection of low-birth-weight infants, FTV was a more sensitive method of detecting high-birth-weight infants than the Hadlock II equation. Conclusions: It is clear that using 3DUS-VOCAL to measure FTV provides more accurate estimation of fetal birth weight.

Key words: Birth weight; Fetus; Thigh; Ultrasonography; Prenatal.

Introduction
Birth weight is an important variable affecting perinatal mortality [1] and an accurate estimation of birth weight is critical to making an informed obstetric decision. The estimated fetal weight (EFW) calculation has been reported to be the most accurate model of birth weight [2], and is based on head size, abdominal circumference (AC), and femur length (FL). A fetal weight estimating formula that uses two-dimensional (2D) ultrasound (US) to measure common fetal growth indices such as biparietal diameter (BPD), AC, and FL is a part of a routine perinatal examination. However, EFW by 2DUS is relatively inaccurate because of the presence of soft tissue and the fact that it is based on mathematical equations that are operator-dependent [3].

Limb volume is strongly correlated with gestational age, fetal growth, and nutrition [4], and several studies have attempted to use limb circumference and volume to predict birth weight using three-dimensional ultrasound (3DUS). This technique has shown promise as an alternative to 2DUS for birth weight estimation [5, 6]. However, all available data on fetal thigh volumetry (FTV) by 3DUS are based on the conventional multiplanar method, as initially described by Chang et al. [5]. There have been limited studies of the usefulness of a new rotational technique, virtual organ computer-aided analysis (VOCAL), for this purpose.

The present study was conducted to assess the accuracy of FTV for prediction of birth weight using the vocal technique compared to the commonly used 2DUS formulae. This study also examined additional maternal parameters, including body weight, body mass index (BMI), and thigh circumference (TC), together with infant body weight and TC.

Materials and Methods
Patients
This was a prospective, cross-sectional study of 84 pregnant women between May 2008 and April 2010 and was approved by Soonchunhyang University Bucheon Hospital Institutional Review Board. All patients provided informed signed consent. Inclusion criteria consisted of a single live fetus with a planned delivery or cesarean section within 72 hours of ultrasonography. Exclusion criteria were multiple pregnancies, intrauterine fetal death at presentation, fetuses with structural malformations detected by ultrasonography or chromosomal anomalies, pregnant women who smoke or suffer from chronic illnesses (diabetes mellitus, hypertension, chronic kidney disease, amongst others), poorly visualized fetal limbs due to technical factors, and oligohydramnios (amniotic fluid index [AFI] below the 5th percentile) or polyhydramnios (AFI above the 90th percentile) [7, 8]. Gestational age was based on the first day of the last menstrual period and confirmed by a first trimester dating scan. Each fetus was included only once. Maternal age, gravidity, BMI, and the presence of obstetric complications were also documented.

2DUS measurements
Before delivery, maternal weight and TC were recorded. All infants were delivered within 72 hours after the ultrasound examinations, and TC was measured within 72 hours of birth. 2DUS
was also used to measure BPD, AC, and FL. BPD was measured from the outer edge of the proximal fetal skull bone to the outer edge of the distal bone. No correction was made for different shapes of the fetal head in non-vertex presentations. AC was measured in standard transverse planes at the levels of the stomach and umbilical vein-ductus venosus complex. FL was measured from the proximal end of the greater trochanter to the distal metaphysis. The side of the thigh closest to the transducer was used to assess the FL, since no significant difference between the right and left thigh volumes has been reported [9].

3DUS measurements
After 2DUS measurement, 3DUS was also used to acquire FTV using multifrequency (4-7 MHz) convex volumetric transducers (3D4-7EK) on an Accuvix XQ scanner. When the fetus was at rest, the transducer was placed to display the femur diaphysis in the traditional plane for assessing the FL. The 3D volume box was adjusted for the size of the thigh and the sweep angle was set 60°. The slowest sweep velocity at four frames per second was chosen in order to guarantee the best resolution. Several thigh volumes were obtained using automatic sweeps, but only two to four were saved for further analysis. The criteria for further analysis included the absence of motion artifacts and the presence of the entire thigh within the 3D image. Thereafter, the VOCAL program was activated. Fetal thigh images were initially displayed on the ultrasound screen in three orthogonal planes (Figure 1). The sagittal view of the femur in plane A was chosen (top left image) and rotated so that the orientation of the thigh and whole diaphysis was coincident with the y-axis. Two demarcating arrows were positioned at the proximal and distal femoral extremities (echogenic femoral diaphysis), similar to the femoral length method. Plane A was selected in the multiplanar display and used as a reference image. Volumes were calculated utilizing the VOCAL program with a manual trace at 30° of rotation. At the end of the 180° rotation, the computer automatically calculated the volume. Before this calculation was accepted, contouring imperfections were modified in the corresponding axial section exposed in plane C (bottom left image) by repeatedly scrolling up and down. The volume displaying the best quality, in terms of contrast and definition, was selected for estimating fetal weight. All measurements were obtained by a single ultrasound operator.

Calculations
EFW by each formula was calculated separately using the fetal parameters for BPD, AC, FL, and TV, as described in Table 1 [2, 5, 10-12]. All data were collected in a spreadsheet and further analyzed using SPSS for Windows. Maternal age was reported as means ± standard deviation (SD). Other maternal features, such as number of previous gestations, BMI, and TC were documented. Correlation between all measured parameters and actual birth weight was determined by Pearson’s correlation coefficient. Regression analysis with correlation coefficients was used to determine the relation between the parameters. The authors compared the sensitivity and specificity of fetal thigh volume formula for prediction of infants ≤ 2.5 and ≥ 3.5 kg with those of the Hadlock II equation. Finally, the significance of differences between EFW and actual birth weight were assessed by paired t-tests with a value of $p<0.05$ considered to indicate statistical significance.

Results
The demographic and clinical data of the 84 patients are shown in Table 2. The mean maternal age was 31.2 ± 4.3 years. In the present study, 61.9% of the participants were in their thirties. The actual birth weight ranged from 1.05 to 4.76 kg (mean, 3.03 ± 0.519). Sixty-two infants (73.8%) were considered to be normal weight at delivery. Low-birth-weight infants (< 2,500 grams) were nine (10.7%) and large-birth-weight (> 3,500 grams) infants were 13

### Table 1. Formulas used for fetal weight estimation.

<table>
<thead>
<tr>
<th>Fetal parameter</th>
<th>Author</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC, BPD (cm)</td>
<td>Shepard et al. [10]</td>
<td>$\log_{10} BW (g) = -1.599 + 0.144 X AC - 0.000111 X BPD$</td>
</tr>
<tr>
<td></td>
<td>Warsof et al. [11]</td>
<td>$BPD + 0.032 X AC - 0.000111 X BPD$</td>
</tr>
<tr>
<td>AC, BPD (cm)</td>
<td>Mancuso et al. [12]</td>
<td>$BW (g) = (BPD X AC X 9.337) - 299$</td>
</tr>
<tr>
<td>BPD, AC, FL</td>
<td>Hadlock et al. [2]</td>
<td>$\log_{10} BW (g) = 1.335 - 0.0034 X AC + 0.1623 X FL$</td>
</tr>
<tr>
<td></td>
<td>Chang et al. [5]</td>
<td>$BW (g) = 1080.87350 + 22.43701 X TV$</td>
</tr>
</tbody>
</table>

**BW** = birth weight, **AC** = abdominal circumference, **BPD** = biparietal diameter, **FL** = femur length, **FC** = fetal thigh circumference, **TV** = thigh volume.

### Table 2. Demographic data of the study group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.2 ± 4.3</td>
</tr>
<tr>
<td>Gravity</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>Height of mother (m)</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Weight of mother (kg)</td>
<td>67.9 ± 10.8</td>
</tr>
<tr>
<td>BMI of mother (kg/m²)</td>
<td>51.9 ± 23.0</td>
</tr>
<tr>
<td>Delivery type</td>
<td></td>
</tr>
<tr>
<td>Normal spontaneous (n)</td>
<td>34</td>
</tr>
<tr>
<td>Cesarean (n)</td>
<td>34</td>
</tr>
<tr>
<td>Mother’s thigh circumference (cm)</td>
<td>51.7 ± 5.4</td>
</tr>
<tr>
<td>Body weight of newborn (g)</td>
<td>3,025 ± 519</td>
</tr>
<tr>
<td>FC (cm)</td>
<td>14.7 ± 1.9</td>
</tr>
<tr>
<td>Infants TC (cm)</td>
<td>15.5 ± 1.7</td>
</tr>
<tr>
<td>TV (l)</td>
<td>122.9 ± 35.6</td>
</tr>
</tbody>
</table>

FC = fetal thigh circumference, **TC** = thigh circumference, **TV** = thigh volume.
Efficacy of fetal thigh volumetry in predicting birth weight using the virtual organ computer-aided analysis (VOCAL) technique

(15.5%). As shown in Table 3, the correlation between fetal weight of thigh volume and the actual birth weight predicted by 3DUS was significant (R=0.662, n=84, \(p<0.001\)). Material BMI (51.9 ± 23.0 kg/m², R=0.439, \(p<0.001\)) and infant TC (15.5 ± 1.7 cm, R=0.561, \(p<0.001\)) were correlated with actual birth weight. However, 2DUS using the Hadlock II equation predicted actual birth weight more accurately (2,928.7 ± 489.8, R=0.920, \(p<0.001\)). As shown in Figure 2, the scatter plot of actual and estimated birth weight by 3D FTV (A, \(R^2=0.438\)) and the Hadlock II equation (B, \(R^2=0.846\)) indicates a positive correlation. Although FTV and the Hadlock II equation showed low sensitivity for prediction of low birth weight, FTV is a more sensitive method for detection of large-birth-weight infants (92.3% vs. 46.2%). However, both methods exhibited high specificities for detection of low-birth-weight infants (100% vs. 93.3%).

Discussion

The aim of the present study was to determine the accuracy of formulae used to calculate FTV in predicting birth weight compared that of other commonly used formulas composed of BPD, AC, and FL. The authors calculated fetal weight by inputting FTV into the Hadlock II formula developed by Chang et al. [5], which uses the multiplanar technique [13]. They also used to the VOCAL technique to demonstrate that FTV is highly correlated with birth weight. They did not record the time necessary to measure fetal biophysics with these techniques but did note that the actual time required to measure FTV by 3DUS was three to five minutes. These findings correspond with those reported previously [14, 15]. When the authors compared the sensitivity and specificity of the 2DUS Hadlock II equation with those of 3DUS FTV for detecting low- or high-birth-weight babies, FTV exhibited a higher sensitivity in detecting high-birth-weight babies than the Hadlock II equation. However, in the birth weight ≤ 2.5 kg category, the Hadlock II equation was more sensitive. After the third trimester it was difficult for both the examiner and the mother to evaluate the whole fetus at one time by ultrasound. Thus, the present data support the superior efficacy of 3DUS in estimating fetal weight close to delivery and suggest that FTV using the VOCAL technique is useful for estimating fetal weight. This is in agreement with another study in which 3D volumetric measurements were performed together with conventional 2D biometry and proved to provide superior results [16].

The authors reviewed the published literature from 2000–2012 in the PubMed database using the keywords ‘3D ultrasound’ and/or ‘fetal weight’. A total of 51 papers in English related to 3D ultrasound were retrieved. When studies of solid organs such as the heart, brain, and lung were excluded, only articles related to fetal weight or birth weight remained. The remaining 13 studies are summarized in Table 4. Most examined primarily third-trimester

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Pearson’s correlation coefficient (R)</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hadlock II</td>
<td>2,928.7 ± 489.8</td>
<td>0.920</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>FTV</td>
<td>3,841.4 ± 799.8</td>
<td>0.662</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Mother’s weight</td>
<td>67.9 ± 10.8</td>
<td>0.443</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Mother’s BMI</td>
<td>51.9 ± 23.0</td>
<td>0.439</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Fetus TC</td>
<td>15.5 ± 1.8</td>
<td>0.561</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

FTV = fetal thigh volume, BMI = body mass index, TC = thigh circumference.
Table 4. — Summary of the medical literatures about 3DUS and fetal weight.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Design</th>
<th>Subjects</th>
<th>Major inclusion criteria</th>
<th>Parameter</th>
<th>Results</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yousef et al. [17] (2012)</td>
<td>Prospective, cross-sectional</td>
<td>137</td>
<td>Between 6 - 13 wks pregnancies, GA = 77.1 ± 14.3 days, CRL = 45.7 ± 21.8 mm</td>
<td>CRL (2DUS, 3DUS)</td>
<td>GA = 77.1 ± 14.3 days, CRL = 45.7 ± 21.8 mm</td>
<td>3DUS is a highly accurate and reproducible tool for fetal CRL measurement</td>
</tr>
<tr>
<td>Lee et al. [18] (2001)</td>
<td>Prospective, cross-sectional</td>
<td>164</td>
<td>Within 4 days of delivery</td>
<td>BPD, AC, FDL, Avol, and Tvol</td>
<td>21.7 - 42.0 wks, Tvol results improved precision of EFW</td>
<td>Tvol can be added to 2DUS measurement to improve precision of EFW</td>
</tr>
<tr>
<td>Lima et al. [19] (2012)</td>
<td>Prospective</td>
<td>102</td>
<td></td>
<td>BPD, FL, AC, EFW (Headlock)</td>
<td>No significant systematic error between 2DUS and 3DUS</td>
<td>To confirm the agreement for fetal biometry obtained by 2DUS and 3DUS, 3DUS improved the reliability and agreement of fetal biometry and EFW compared with 2DUS,</td>
</tr>
<tr>
<td>Yang et al. [20] (2011)</td>
<td>Prospective, cross-sectional</td>
<td>290 (developmental group: n=100, validation group: n=190)</td>
<td>Within 5 days of delivery Hong Kong Chinese women</td>
<td>Tvol, FL, AC, BPD</td>
<td>38.9 ± 3.1 wks, 3346 ± 452 g (n=190), M5 = 2978.107 + 18.708 × BPD + 1764.2 + 13.906 × FL + 13.906 × AC</td>
<td>A new birth-weight prediction model allows better prediction than does the Hadlock models.</td>
</tr>
<tr>
<td>Antsaklis et al. [21] (2011)</td>
<td>Pilot</td>
<td>156</td>
<td>Between 11 and 13+6 wks GA, CRL = 64.7 ± 8.2 mm (45 - 84 mm), women delivered before 37 or after 42 weeks were excluded</td>
<td>Embryo volume, CRL, Gestational sac volume, placenta volume, birth weight</td>
<td>The embryo volume appears the strongest association with the birth weight at delivery, followed by the CRL, and the gestational sac volume. The placenta volume reveals the smallest weak association.</td>
<td>Embryonic volume measured by 3DUS during first trimester of pregnancy had superior correlation than CRL with the birth weight.</td>
</tr>
<tr>
<td>Bemmi et al. [22] (2010)</td>
<td>Two-stage prospective, cross-sectional</td>
<td>210 (formula-generating group: n=150, prospective-validation group: n=60)</td>
<td>Delivery within 49 hours after USG measurement</td>
<td>BPD, HC, AC, FL, ThiM, ThiV</td>
<td>38.5 ± 5.8 (29.4 - 42.4) wks, 3247.4 ± 698.3 g (1475 - 4750), EFW = -562.824 + 11.962 × AC × FDL + 0.009 × BPD + 0.0534 × Abdvol</td>
<td>There were no significant differences between the accuracies of the new 2D and 3D models in the prediction of birth weight.</td>
</tr>
<tr>
<td>Srisantiroj et al. [23] (2009)</td>
<td>Prospective, cross-sectional</td>
<td>176 (Intra-observer reliability test: 20, Formula finding group: 100, Formula evaluating group: 56)</td>
<td>Delivery within 7 days after USG measurement</td>
<td>BPD, HC, AC, FL, Tvol</td>
<td>38.96 ± 2.13 wks, 3,159.64 ± 589.20 gm (n=56), EFW (g) = 774.744 + 32.658 × Tvol (ml)</td>
<td>Compared and validated with the Hadlock's and the Tongson formula. New formula shows the smallest absolute percentage error (APE), and can improve the accuracy of fetal weight prediction.</td>
</tr>
<tr>
<td>Srisantiroj et al. [24] (2009)</td>
<td>Prospective, comparative</td>
<td>176</td>
<td>GA ≥ A 287 days, within 4 days of delivery</td>
<td>BPD, HC, AC, FL, ThiM, ThiV</td>
<td>New formula: FW = 2088.4904 + 81.0519 × HC − 0.1214 × HC2 − 69.0966 × AC + 0.4741 × AC2 + 6.4944 × Tvol + 0.0534 × Abdvol</td>
<td>The formula of Persson and Weldner (2D) was compared with two 3D formulae (Lee and colleagues). In prolonged pregnancy, new formula is relatively easy to use regardless of weight percentiles or fetal lie.</td>
</tr>
<tr>
<td>Srisantiroj and Maršál [24] (2009)</td>
<td>Prospective, comparative</td>
<td>176</td>
<td>Within 4 days of delivery</td>
<td>FW = 2088.4904 + 81.0519 × HC − 0.1214 × HC2 − 69.0966 × AC + 0.4741 × AC2 + 6.4944 × Tvol + 0.0534 × Abdvol</td>
<td>It does not seem reasonable to abandon the 2D ultrasound methods. Because 2D US show same accuracy with 3D US.</td>
<td></td>
</tr>
<tr>
<td>Schidler et al. [25] (2008)</td>
<td>Prospective, cohort</td>
<td>150</td>
<td>EFW of ≥2000 gm using the Hadlock formula, within 7 days before delivery, BW &lt; 1600 gm</td>
<td>28.3 ± 3.6 wks (18.6 - 36.4 wks), 960 ± 357.1 (230 - 1550 gm), EFW = 656.41 ± 18321 × volAIDO + 31.1981 × HC + 57.8787 × volEFM + 75.2141 × FL + 8.3099 × AC - 449.8863 × BPD + 32.5340 × BPD3</td>
<td>New formula is relative easy to use regardless of weight percentiles or fetal lie.</td>
<td></td>
</tr>
<tr>
<td>Bromley et al. [26] (2007)</td>
<td></td>
<td>27</td>
<td>Only 6 fetus were delivered within 7 days</td>
<td>Fetal presentation, placental location, amniotic fluid volume, fetal biometry, Trend analysis were not possible.</td>
<td>The evaluation of the 3rd trimester fetus via 3DUS is a reliable screening method in fetuses with IUGR.</td>
<td></td>
</tr>
<tr>
<td>Chang et al. [27] (2005)</td>
<td>Prospective, cross-sectional</td>
<td>82 (with IUGR: n=40, without IUGR: n=44)</td>
<td>Taiwanese population, 20 - 40 wks IUGR classified when their birth weights &lt;10th percentile for GA, all the fetuses were all followed up to delivery</td>
<td>upper arm volume, common fetal growth parameters (BPD, OFD, HC, AC, and FL), EFW (Hissch's equation)</td>
<td>The author compared the sensitivity and specificity with upper arm volume and the common fetal biometric indices.</td>
<td>Fetal upper arm volume assessment by 3DUS would be a reliable screening method in fetuses with IUGR.</td>
</tr>
<tr>
<td>Schidler et al. [16] (2000)</td>
<td>Prospective, cross-sectional</td>
<td>190 (formula - finding groups: n=125, formula evaluation group: n=65)</td>
<td>within 7 days of delivery</td>
<td>Formula-evaluation groups : 36.4 ± 4.1 (23.7 - 41.3) wks, EFW = -1478.557 + 5.282 x upper arm volume + 852.998 x log abdominal volume + 0.526 × BPD3</td>
<td>The correlation between actual birth weight and fetal weight estimation was highly significant. 3DUS allows superior EFW by including soft tissue volume.</td>
<td></td>
</tr>
</tbody>
</table>

wks = Weeks, GA = gestational age, IUGR = intra-uterine growth retardation, EBW = estimated birth weight, BW = actual birth weight, Avol = arm volume, Tvol = thigh volume, 2DUS = twodimensional ultrasound, 3DUS = three dimensional ultrasound, BPD = biparietal diameter, AC = abdominal circumference, HC = head circumference, FL = femur length, FDL = femur diaphysis length, EFW = estimated fetal weight, VOCAL = Virtual Organ Computer-aided Analysis, ThiM = thigh volume measured by multiplanar method, ThiV = thigh volume measured by VOCAL method, AD = abdominal diameter, Abdvol = abdominal volume.
Efficacy of fetal thigh volumetry in predicting birth weight using the virtual organ computer-aided analysis (VOCAL) technique

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fetuses and showed 3D measurements to have acceptable reproducibility. Like the present study, most manually traced TV measurements, and only one [28] evaluated arm volume measurement and reported greater sensitivity and specificity for a fetus with intrauterine growth retardation (IUGR) than TV. Nine of these 13 studies reported a better result with 3DUS than 2DUS in terms of estimation of fetal weight, while two studies reported no significant difference between 3DUS and 2DUS. One study was not compared due to the small number of cases. Thus, it appears that as the 3DUS technique has advanced, the use of 3DUS in prenatal evaluations is becoming more prevalent [29, 30].

The present study had a number of limitations. For example, the sample size was small and the study was conducted in an obstetric unit of a tertiary care center, which may not be representative of the Korean population. Thus, a large, prospective and multicenter study is necessary to confirm the conclusions of this report.

Conclusions

The authors validated the 3DUS-VOCAL method and reviewed the literature regarding use of 3DUS to estimate fetal birth weight. Based on these results, it is clear that the use of 3DUS-VOCAL to measure FTV will provide more accurate estimations of fetal birth weight and that it has the potential to become an essential prenatal reassessment tool.

Acknowledgments

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References


Address reprint requests to:
T.H. KIM, M.D., Ph.D.
Department of Obstetrics and Gynecology,
Soonchunhyang University Bucheon Hospital
170 Jomaru-ro, Wonmi-gu, Bucheon-si,
Gyeonggi-do, 420-767 (Republic of Korea)
e-mail: heeobgy@schmc.ac.kr
Echogenic intracardiac focus in fetus and association with maternal respiratory tract infection in Shanghai, China

Q. Huang1, W. Cheng1, Y. Huang2

1 The International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai
2 Department of Anatomy and Neurobiology, Tongji University School of Medicine, Shanghai (China)

Summary
Purpose of investigation: To evaluate the association between fetal echogenic intracardiac focus (EIF) and maternal respiratory tract infections (RTI) during gestation. Materials and Methods: Fetal echocardiography was performed on 1,720 pregnant women (1,723 fetuses), and 205 of them showed evidence of fetal EIF. Other 245 pregnancies without fetal EIF were selected randomly as a control group. Comparison was undertaken to evaluate the difference between these two groups, and the possible association between EIF and maternal RTI. Results: EIF was present in 11.9% of the examined fetuses, and the presence of fetal EIF with maternal RTI was significantly higher (p < 0.05) than those without RTI. Multivariate logistic regression correction of RTI showed that the likelihood of EIF was 49 fold higher in pregnant women with RTI than without it (odds ratio [OR] = 49.958, 95% confidence interval [CI], 25.973–96.092, p < 0.05). Conclusions: Fetal EIF is suggested to be associated with maternal RTI during gestation in the present study population.

Key words: Ultrasound; Fetus; Echogenic intracardiac focus; Prenatal diagnosis; Respiratory tract infection.

Introduction
Fetal Intracardiac echogenic foci (EIF) are defined as discrete areas of echogenicity in the ventricles first described in 1987 [1]. They are mostly observed in the papillary muscles and the chordate tendineae of the fetus hearts, and appear to be caused by mineralization or small deposits of calcium in heart muscle histologically [2]. Investigations had been conducted to explore the clinical relevance of EIF in fetal hearts at different aspects. It has been reported that fetal EIF is associated with ethnicity, [3, 4] chromosomal abnormalities [5, 6], and maternal body mass index. [7]

Respiratory tract infections (RTI) are acute infections of the respiratory tract [8, 9] caused by over 200 different bacteria and viruses [10]. Among them, certain pathogens such as Streptococcus pyogenes has been found to result in rheumatic heart disease [11], which can be detected by echocardiographic screening [12]. Other pathogens, for example, those causing maternal community-acquired pneumonia are found to seriously affect their fetuses, and then increasing the risk of preterm delivery and abortion [13]. The present authors thus hypothesized that maternal RTI might also lead to transient or permanent changes in developing fetal heart, which may be reflected by the presence of EIF.

Materials and Methods
Between May 2009 and April 2010, 1,720 pregnant women with a gestation period of six to 41 weeks underwent prenatal ultrasound examinations in the clinic of the International Peace Maternity and Child Health Hospital, Shanghai, China. Among them, 205 pregnancies were detected to carry babies with EIF. Meanwhile, 245 pregnancies without fetal EIF in the similar gestation period were selected randomly as control. Comparison was done between the maternal RTI before the diagnosis of EIF in EIF group and maternal RTI during the gestation in control group. The differences in the maternal age, gestational period, maternal BMI [maternal weight (kg)/maternal height (m²)] of the first gestation period, and medication treatment for maternal RTI were also compared between the two groups. All participants were explained the purpose of the study and gave informed consent. The study protocol was approved by the Ethics Committee of the International Peace Maternity and Child Health Hospital.

Fetal ultrasonography was performed using a color Doppler ultrasonic diagnostic apparatus, equipped with a one- to five-MHz transabdominal transducer. During prenatal ultrasound examination, the location of the fetal heart was determined by the appearance of fetal cardiac four-chamber and left/right ventricular outflow tract sections. The atriocavitentricular connection with the vessels and the internal structure of the fetal heart (including the size of the atriocavitentricular chamber, valve morphology, and continuity of interval and foramen ovale) were examined.

Fetuses were diagnosed with EIF if a > two mm enhanced echo appeared in the fetal heart, with an echo strength similar to the strength of bone structures, and if the EIF ranged in size from two to five mm. [14] Pregnant women were diagnosed with a RTI if they had a sore throat, running nose, coughing, or fever (< 38.5°C), with most recovering within a few days. [8, 9, 15] Patients with RTI were recorded only after the diagnosis was confirmed.
Statistical analysis was performed using SAS 8.2. Groups of fetuses with and without EIF were compared by the chi square test or Fisher’s exact test, as appropriate. Multivariate logistic regression analysis was performed to evaluate the association between clinical factors and fetal EIF. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. A $p < 0.05$ was considered statistically significant.

Results

The study population was Chinese Han ethnic pregnant women underwent routine prenatal ultrasound examinations in the Hospital. Their general health conditions were good during pregnancy.

The present study showed that of the 1,720 pregnancies, 205, including three sets of twins, were diagnosed with fetal EIF, and the incidence of fetal EIF was approximately 11.9% (205/1723). The mothers aged 21 to 42 years (mean 32.4 ± SD4.3), and gestation periods varied from 17 to 38 weeks (mean 22.1 ± SD1.6). No fetal chromosomal abnormality was found in the EIF cases. The control group contained 245 pregnancies without fetal EIF that was randomly selected. The maternal age was from 21 to 43 years (mean 31.1 ± SD 4.2), and the gestation period varied from 17 to 38 weeks (mean 22.4 ± SD1.9). The BMIs of the first gestation period of the two groups varied from 15.08 to 23.93 (mean 18.6 ± SD 1.56) and 15.08 to 34.16 (mean 21.11 ± SD 2.83), respectively. The median of the BMIs between the two groups was 19.48.

Of these 205 fetuses with EIF, 91.7% EIF was detected in the left ventricle, 2.9% EIF in the right ventricle, and 5.4% EIF was detected in both ventricles. All fetal hearts had the typical four-chamber structure, with no obvious abnormalities in the inner diameters and courses of the aorta and pulmonary artery, the size of the atrioventricular cavities, in the activities of the atrioventricular valves and the continuity of the ventricular septa, and in the normality of ovale foramen.

EIF was detected as early as 17 weeks of gestation by prenatal ultrasound examination in six fetuses (2.9%). Of the 205 fetuses in which EIF was detected in early gestation, the signal disappeared in 172 cases (83.9%) by 34 weeks of gestation and 199 cases (97.1%) by 38 weeks of gestation. The peak of monthly EIF cases distribution was found in January (Table 1).

The ages, gestational periods, and maternal BMI were all significantly different in the groups of pregnant women with fetal EIF and without fetal EIF ($p < 0.05$ each; Table 2). A trend test for gestational period incidence of EIF revealed a significantly higher occurrence of EIF in the first 20 weeks of pregnancy, compared to 20-24 weeks or over 24 weeks gestation ($p < 0.05$; Table 2).

Moreover, of the pregnant women, 179 out of 205 (87.3%) in the EIF group experienced RTI before the diagnosis of EIF, whereas only 50 out of 245 (20.4%) in the control group had RTI during gestation, with a statistically significant difference ($p < 0.05$; Table 2). Meanwhile, the number of pregnant women who took medication for RTI treatment was only six out of 179 (3.35%) in the EIF group, and 29 out of 50 (58%) in the control group. The difference was also significant between these two groups ($p < 0.05$; Table 2).

Multivariate logistic regression analysis showed that the risk of carrying a fetus with EIF, compared to the gestational period of less than 20 weeks, declined during the gestational period of 20 - 24 weeks (OR = 0.334, 95% CI 0.202–0.553, $p < 0.05$; Table 3), as well as the period over 24 weeks (OR = 0.258, 95% CI 0.080–0.836, $p < 0.05$; Table 3). Meanwhile, compared to maternal BMI of less

### Table 1. Association between fetal EIF and time of prenatal ultrasound screening.

<table>
<thead>
<tr>
<th>Month and year</th>
<th>Number of fetuses with EIF</th>
<th>Number of fetuses examined</th>
<th>EIF rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009 May</td>
<td>16</td>
<td>172</td>
<td>9.3</td>
</tr>
<tr>
<td>June</td>
<td>18</td>
<td>148</td>
<td>12.2</td>
</tr>
<tr>
<td>July</td>
<td>10</td>
<td>137</td>
<td>7.3</td>
</tr>
<tr>
<td>Aug</td>
<td>10</td>
<td>129</td>
<td>7.8</td>
</tr>
<tr>
<td>Sept</td>
<td>9</td>
<td>135</td>
<td>6.7</td>
</tr>
<tr>
<td>Oct</td>
<td>14</td>
<td>127</td>
<td>11.0</td>
</tr>
<tr>
<td>Nov</td>
<td>10</td>
<td>101</td>
<td>9.9</td>
</tr>
<tr>
<td>Dec</td>
<td>16</td>
<td>127</td>
<td>12.6</td>
</tr>
<tr>
<td>2010 Jan</td>
<td>26</td>
<td>117</td>
<td>22.2</td>
</tr>
<tr>
<td>Feb</td>
<td>19</td>
<td>145</td>
<td>13.1</td>
</tr>
<tr>
<td>Mar</td>
<td>30</td>
<td>185</td>
<td>16.2</td>
</tr>
<tr>
<td>Apr</td>
<td>27</td>
<td>197</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Note: The EIF rate was defined as the number of fetuses with EIF divided by the total number of fetuses examined.

### Table 2. Demographic and clinical characteristics of the EIF and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EIF Group (total n = 205)</th>
<th>Control Group (total n = 245)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td>0.0021</td>
</tr>
<tr>
<td>≥ 35</td>
<td>70 (34.1)</td>
<td>52 (21.2)</td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>135 (65.9)</td>
<td>193 (78.8)</td>
<td></td>
</tr>
<tr>
<td>Gestational period (week)*</td>
<td></td>
<td></td>
<td>0.0155</td>
</tr>
<tr>
<td>&gt; 24</td>
<td>12 (5.9)</td>
<td>22 (9.0)</td>
<td></td>
</tr>
<tr>
<td>20 - 24</td>
<td>165 (80.4)</td>
<td>209 (85.3)</td>
<td></td>
</tr>
<tr>
<td>≤ 20</td>
<td>28 (13.7)</td>
<td>14 (5.7)</td>
<td></td>
</tr>
<tr>
<td>Maternal BMI</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>&gt; 19.48</td>
<td>57 (27.8)</td>
<td>167 (68.2)</td>
<td></td>
</tr>
<tr>
<td>≤ 19.48</td>
<td>148 (72.2)</td>
<td>78 (31.8)</td>
<td></td>
</tr>
<tr>
<td>Maternal RTI</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>yes</td>
<td>179 (87.3)</td>
<td>50 (20.4)</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>26 (12.7)</td>
<td>195 (79.6)</td>
<td></td>
</tr>
<tr>
<td>Medication treatment for maternal RTI</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>yes</td>
<td>6 (3.35)</td>
<td>29 (58.0)</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>173 (96.65)</td>
<td>21 (42.0)</td>
<td></td>
</tr>
</tbody>
</table>

* $p$ value of trend test for gestational period is 0.0157.
EIF on prenatal ultrasound represents a strong echo reflection caused by thickened ventricle chordae. A previous histological study of six terminated fetuses with EIF confirmed that the strong echoes in the ventricle in their study were caused by myocardial fibrosis or calcified endocardium and papillary muscle [2].

Several mechanisms were considered to cause vascular calcification, such as lipid metabolism, mineral and hormonal balance, or inflammatory cytokines [16, 17]. Some of the inflammatory signalings were also considered to be involved in respiratory diseases [18]. When the pregnant women suffered RTI during pregnancy, although many of them were able to recover from RTI, the responsible pathogens may have affected their fetus [13]. If the certain pathogens were able to attack the fetal hearts [11, 12], along with the maternal body changing in various aspects because of pregnancy, including mineral need and hormone levels [9], the fetus with maternal RTI were possibly more susceptible to form calcification in their circuits. On the cardiac sonographic examination, these calcifications would appear as the strong echo inflections in the fetus heart. In this study the authors found strong association between EIF and maternal RTI. There were more than half of the cases (94.2%) were detected before the second trimester, and then the occurrence of EIF declined with the progress of the pregnancies in this study. Statistically the results suggested the strong risk for fetal EIF with maternal EIF.

Two previously studies found the incidence of fetal EIF was higher in Asian ethnicity [3, 4], though others reported no link between fetal EIF and ethnicity [19]. The EIF rate was 11.9% in the present study, similar to previously reported that ranged from 1% to 30% [3, 4, 5, 6, 19, 20]. Since many EIF reported rates were less than 10% excluding Asian ethnicity, and the population of the present study was all Han Chinese, this result also suggests that Asians of Chinese origin may have a relatively high prevalence of echogenic intracardiac foci.

There are studies that showed that low maternal BMI would be an independent risk factor for the development of fetal EIF [7]. The present study also indicated that the risk of fetal EIF declined along with the increasing of maternal BMI, however the present author did not find the incidence of EIF related to chromosomal abnormality, which was considered controversially as the possible risk factor in some previous studies [5, 6].

Furthermore, 91.5% of the cases had EIFs only in the left ventricle, which is in agreement with previous findings [19, 20, 21]. This imbalance in EIF incidence may be due to the characteristics of fetal blood circulation. In fetuses, blood circulation may depend primarily on the right side of the heart, reducing the likelihood of mineral deposits and calcification on that side because of strong blood flow. No correlation was observed between the presence of EIF and congenital structural abnormalities of the fetal heart in many reports [1, 21]. Similarly, none of the fetuses with EIF the present authors assessed had any obvious structural abnormalities in the heart. Moreover, they were able to detect fetal EIF on prenatal ultrasound as early as week 17 of pregnancy. In fact, most of cases (94.2%) were detected before the second trimester, and then the occurrence of EIF declined with the progress of the pregnancies in this study. Statistically the results suggest that gestational period itself may protect fetuses from EIF formation. This finding was similar to previous evidence that the 7.4% prevalence of EIF at 13-16 weeks’ gestation decreased to 3% at 20-22 weeks’ gestation [20]. The present authors also observed seasonal variations in the occurrence of fetal EIF, with 22.2% occurring in January. This was consistent with flu infection peak in China [22], as well as with the outbreak of H1N1 influenza A virus infection at the end of 2009 [23].

Conclusion

In a specific Chinese population, the present authors found an association between fetal EIF and maternal RTI during pregnancy. The pitfall of this study is that the authors could not follow these children to measure their heart functions during childhood development, so as to gain insights of the EIF pathophysiology.
References


Address reprint request to:
W. CHENG, M.D.
The International Peace Maternity and Child Health Hospital
Shanghai Jiao Tong University School of Medicine
110 Hengshau Road
Shanghai 200030 (China)
e-mail: weiwicheng@gmail.com
Environmental pollution due to cadmium: measure of semen quality as a marker of exposure and correlation with reproductive potential


Department of Woman, Child and of General and Specialized Surgery, Second University of Studies of Naples, Naples (Italy)

Summary

Purpose: Contradictory reports exist in the literature regarding an association of cadmium with parameters of semen quality. The aim of the study was to assess cadmium levels in both blood and seminal plasma and to analyze the relationships between cadmium concentrations and lifestyle and semen parameters. Material and Methods: Fifty healthy male volunteers were recruited to provide semen and blood samples. Each patient completed an extensive questionnaire regarding his occupation, residence, social status, diet, water source, smoking habits, and medical and surgical history. Semen analysis was carried out according to WHO guidelines. Detection of cadmium in both semen and blood samples was carried out by means of atomic absorption spectrophotometer. Results: Mean concentrations of cadmium were 8.18 ± 1.6 ng/ml in blood samples and 2.56 ± 0.9 ng/ml in semen samples. Cadmium blood levels were significantly higher in men from industrialized areas and in current smokers, but were not correlated with semen levels. A significant positive correlation was found between cadmium blood levels, number of immotile spermatozoa, and teratozoospermia index (TZI). Significant inverse relationships between cadmium blood concentration and type-a and type a + b motility were found. Conclusions: The present data show a significant correlation between blood cadmium concentrations, cigarette smoking, occupational exposure, and parameters of semen quality. Such a reduction in spermiogenetic function could be an early marker of a toxic effect by cadmium pollution.

Key words: Cadmium; Seminal plasma; Male infertility; Environmental pollution.

Introduction

Many studies carried out on infertile men have linked environmental factors and remarkable impairment of semen parameters (concentration, motility, and cell morphology). It has been suggested that testicles are among the most vulnerable organs to the action of environmental, chemical, and physical agents. Heavy metals are partially high-density chemical elements and are toxic even at low concentration. They are generally ubiquitous and represent a continuous and uncontrollable source of environmental pollution: penetrating human organism through food, air, and water, they sediment in various tissues or organs and are united to the cell structures which they sediment in, thus preventing the development of some vital functions [1-3].

Traces of some metals (mercury, cadmium, lead, and aluminum) were found in gonadal tissues, in oocytes, in sperm, in biological fluids, and even in embryos, at variable concentrations, likely influenced by residence, occupation, and lifestyle [4, 5]. In gonadal tissues they may be responsible for ultrastructural alterations, both genetic and somatic, as well as for functional modifications affecting cell metabolism with interactive and lasting toxic effects [6-8].

Cadmium is a trace toxic mineral similar to zinc in its structure, with no biological function in human organism. It is found in the environment because it derives from ecodispersion phenomena due to both productive activities and to public utility services – such as landfills for urban solid waste, incinerators, thermoelectrical stations – potentially polluting air, water, food, and also to cigarette smoke. Cadmium values between 20% and 50% are absorbed through respiratory system into blood (95% of it is found in erythrocytes, the rest in plasma): partly attached to a protein – metallothionein – cadmium finally reaches the testicles. Exposure to cadmium has been related to adverse effects on reproductive functions in mammals: in experimental animals, toxic effects on testicles led to necrosis, atrophy, and edema, presumably because of specific vascular damages [9-11]. Occupational exposure has been associated to a decrease in sperm quality and to an increase of spontaneous miscarriages, by reason of a hypothesized – direct or hormone-mediated – effect on testicular function [12-14].

To date, contradictory data exist in the literature regarding an association of cadmium and parameters of semen quality [15-18]. According to Amad et al. there is a nega-
tive correlations between seminal lead and cadmium levels on semen parameters (sperm motility and vitality) [19]. Moreover, many studies showed a correlation between exposure to cadmium and semen features, and a strictly correlation with age, diet, and smoking [20]. The aim of this study was to evaluate the presence of cadmium in biological fluids (blood and semen) at toxic levels, and to examine the relationship between cadmium concentration and lifestyle, geographical area of residence, occupational exposure, age, and semen parameters.

Materials and Methods

Fifty healthy patients attending the Outpatient Infertility Center at Second University of Naples were recruited to provide semen samples. All the patients were between 24- and 42-years-old (average 32.5 ± 5.5 years) and childless, signed an informed consent form, and completed an ad hoc questionnaire. Every patient was requested to provide a semen sample after at least three (not more than five) days of abstinence from intercourse; a blood sample was taken from each patient.

Each semen sample was preliminarily analyzed to register chemical and physical parameters (such as volume, pH, viscosity, colour, liquefaction), sperm concentration (registered in millions of spermatozoa per milliliter), morphology (morphology of 100 cells – divided into forms with head, mid-piece, and tail defects and normal forms – was evaluated per each slide); teratozoospermia index (TZI) was obtained dividing the total number of abnormalities (head, mid-piece, and tail abnormalities) added together by the number of abnormal spermatozoa, while the sperm deformity index (SDI) was calculated dividing the number of defects by the number of counted cells. These indices are predictors of fertilization potential of spermatozoa, both in vivo and in vitro. TZI value varies from 1.00 (every abnormal spermatozoon shows one anomaly only) and 3.00 (every abnormal spermatozoon shows head, midpiece and tail abnormalities). Previous studies have shown that a TZI >1.6 is associated to a low fertilization rate in infertile couples who have not received medical treatment; it has also been proven that SDI 1.6 is the highest value to be compatible with in vitro fertilization. The evaluation of sperm motility was performed considering the number of: fast straight motile cells, slow straight motile cells, in situ motile cells, and immotile cells.

Cadmium evaluation was performed on sperm and blood samples by graphite furnace atomic absorption spectroscopy an highly efficient technique designed to perform the qualitative and quantitative analysis of heavy metals in a wide variety of samples. The sample, properly treated for matrix separation and for atomization, was then dried, incinerated, and atomized at a temperature which allows the atomic absorption. The atoms of the element under observation absorb energy only at their own wavelength and according to their number. During cadmium analysis, drying, incinerating, and atomizing temperatures were respectively 120°C, 500°C, and 2,200°C, in conditions of transportation gas (Argon) flow interruption. A 228.8 nm wavelength was adopted.

Data are presented as averages ± DS. The statistical analysis was performed using Student’s t-test and Pearson for parametric data. Groups comparing analysis and correlation analysis were carried out using, respectively, Mann-Whitney test and Spearman rank-test, for non-parametric data. P values < 0.05 were considered as statistically significant.

Results

Out of the selected 50 metals exposed patients observed, 28 were current smokers, the other 22 were non-smokers from at least three years. Twenty-four patients lived in areas close to chemical or metal industries, but none of them had a proven occupational exposure to heavy metals. Mean values of cadmium concentration in blood and in semen were 8.18 ± 1.6 ng/ml and 2.56 ± 0.9 ng/ml. Cadmium blood levels were significantly higher in men from industrialized areas (Figure 1) and in current smokers, but were not correlated with semen levels, though the authors observed a positive correlation trend (p = 0.251 and p = 0.079) (Table 1). An inverse correlation trend was found between the highest metal values and sperm quality, especially in motility, which was inversely proportional to blood cadmium levels, but not correlated to sperm cadmium levels (Table 1).
Discussion

Semen can be used as an indicator of exposure and effect because structural and functional alterations specifically and precociously (especially with regards to spermatogenetic function) occur in the gonads. Such alterations are secondary to toxics of various nature, even when exposure corresponds to very low chronic doses. The results of the present study registered both significant positive correlations between total presence of cadmium in blood, number of immotile spermatozoa, and TZI, as well as relevant inverse correlations between total cadmium concentration, a-type and a+b motility, and number of morphological normal spermatozoa. On this basis, it can be inferred that sperm damages occur previously because of an adverse effect of cadmium on germinative epithelium.

The use of semen parameters as indicators of toxic effect of environmental contaminants seems to be not fully reliable because seminal characteristics should be evaluated together with clinical and hormonal data. A further difficulty is the high inter- and intra-individual variability of sperm count among different subjects at ordinary conditions, as well as in the same subject, in relation to several factors, such as sexual habits, seasonal changes, and lifestyle. Quantitative and qualitative alterations in semen can be produced by various conditions, not depending on individual features: this may cause further problems in interpretation. Furthermore, biological factors such as infections, ionizing radiations, and heat may have a great influence, especially when sperm canals are concerned. On the other hand, semen analysis is a simple and sensitive test which can be used as an early indicator of germinative epithelium damage due to exposure to environmental toxics, even at low concentrations of contaminants.

Data from literature regarding the relationship between cadmium and sperm quality are contradictory. Some authors [11, 21, 22] did not register any association between cadmium concentrations and sperm concentration and motility in a general population, as well as in battery workers; others observed an inverse relationship between cadmium concentration and semen volume [13] and motility [23-24], inferring adverse effects on prostatic function. Some studies showed a significant negative correlation between cadmium concentration in semen and sperm concentration in oligoasthenospermic subjects in the general population. Pant N. et al. showed that lead and cadmium induce DNA damage, particularly influencing testosterone level [21].

Our data show a correlation between blood cadmium concentration, some risk factors (cigarette smoke, occupation, age, area of residence) and sperm function parameters: noteworthy was a decrease in spermatozoa number and vitality observed mostly in smokers and in industrial areas residents. Nonetheless, a relationship between cadmium concentration in blood and in semen was not observed, while a relationship was shown between semen cadmium concentration, TZI, and SDI. It can be hypothesized that cadmium penetrating into cells through voltage-dependent calcium channels does not accumulate because of competing with zinc (normally present in sperm) at intracellular bond site [25-26]. It can also be hypothesized that low concentrations of toxic may cause worsen morphologic characteristics of abnormal spermatozoa, as suggested by positive correlations between seminal cadmium levels and SDI-TZI. Such observation could find wide application in assisted reproduction. In conclusion, the impairment of seminal parameters in subjects exposed to cadmium may represent a precocious indicator of the toxic effect of cadmium exposition.

References


Address reprint requests to:
G. MAININI, M.D., Ph.D.
Via Armando Diaz, 77
80055 Portici, Naples (Italy)
e-mail: giampaolomainini@libero.it
Expression and significance of CD133 and ABCG2 in endometriosis

S. Liu1, J. Zhou2, J. Wen1

1 Department of Obstetrics and Gynecology, The Fourth Affiliated Hospital of Jiangsu University, Zhenjiang City
2 Department of Oncology, Chinese Medicine Hospital of Changzhou, Changzhou City (China)

Summary

Background: Endometriosis is a common gynecological disease and exact pathogenesis is still unclear. Recently, an increasing interest has been given to the potential role of stem cells in the development of endometriosis. The aim of this study was to test the expression of stemness-related markers CD133 and ABCG2 in endometriosis. Materials and Methods: CD133 and ABCG2 protein expression in eutopic and ectopic endometrial tissue with endometriosis and endometrium tissue without endometriosis were examined by Western blot. Results: Eutopic endometrium showed high level of CD133 and ABCG2 protein when compared with ectopic endometrium (p = 0.042, p = 0.038) and control endometrium (p = 0.000, p = 0.000). The expression of CD133 protein in ectopic endometrium was positively correlated with R-AFS score of endometriosis (p = 0.000, r = 0.793) and no significant relation was noted between ABCG2 and R-AFS score (p = 0.563). Two of three patients with recurrence had much higher expression of ABCG2 protein than the patients without recurrence. Conclusion: Aberrant expression of CD133 and ABCG2 in eutopic and ectopic endometrial tissue with endometriosis suggests that they are probably associated with the pathogenesis of endometriosis and stem cells play a possible role in its development.

Key words: Endometriosis; Stem cells; CD133; ABCG2.

Introduction

Endometriosis is a chronic, progressive, and complex gynecological disease. It affects about 5% to 10% of women of reproductive age and has had a significant increase in case number and an earlier onset in recent years [1]. Endometriosis is characterized by the presence of functional endometrium-like tissue in ectopic sites and typically causes infertility and pain. Meuleman et al. reported that in a subset of infertile women, the prevalence of endometriosis was 47% and was also comparable to patients with (54%) and without (40%) pelvic pain [2]. At present the exact pathogenesis of endometriosis is still unclear. Many studies have focused on elucidating the immunological, endocrinological, environmental, and genetic factors involved in endometriosis, but none of them can completely explain the cause of all its types.

Recently, an increasing interest has been given to the potential role of stem cells in endometriosis development [3, 4] and growing evidences have suggested that it may arise from stem cells [4-8].

CD133, also known as Prominin-1, is a pentaspan, highly glycosylated, membrane glycoprotein that is associated with cholesterol in the plasma membrane. It is expressed in a wide range of somatic stem and progenitor cells [9-11]. ABCG2 is a member of the ATP binding cassette (ABC) transporters, which is widely expressed in stem cells, and also found to confer the side population phenotype, which is recognized as a universal marker of stem cells [12, 13]. Studies have shown that CD133 was expressed by epithelial cells of the endometrium [14] and ABCG2 positive cells were diffusely distributed in the stroma compartment of endometrium [15], but there are less data on CD133 and ABCG2 expression in endometriosis.

The present study aimed to determine whether CD133 and ABCG2 expression is altered in the human ectopic, eutopic endometrium, and to evaluate whether the expression of these two molecules correlates with the formation and progression of endometriosis.

Materials and Methods

Ethics statement

The research was approved by the Institutional Review Boards of the present hospital and the consent form was obtained before surgical procedure.

Tissue collection

Samples were obtained from endometrium in uterine cavity and ovarian endometriomas. A total of 22 eutopic endometrial and 22 ovarian endometriotic specimens were collected from patients with endometriosis, who underwent laparoscopic or laparotomy surgery at the present hospital between January 2011 and December 2011. Endometrial tissues from 20 patients without endometriosis, obtained during surgeries for other gynecol-
logical disease at the same period, served as controls. All of the patients had regular menstrual cycles and no other hormone-dependent diseases, did not receive hormone therapy during the three months before surgery, and were not pregnant or lactating during the study. The surgeries were performed at three to seven days after menstruations ended. The specimens were washed with saline to remove blood and immediately frozen in liquid nitrogen for Western blot analysis. All samples were histologically confirmed.

Western blot analysis

Tissues were grinded in RIPA buffer (150 mM NaCl, 1mM EGTA, 0.1%SDS, 1mM NaF, 1mM Na₃VO₄, one mg/ml apro tinin, and one mg/ml leupeptin in ten mM Tris, pH 7.4) containing one mM phenylmethanesulfonyl fluoride. Then the tissue homogenate was centrifugated at 1,200 r/min for 15 minutes at 4°C and the supernatant was removed for protein analysis. Protein concentration was determined by the BCA assay kit (CWBO). Total protein was fractionated on 10% SDS–polyacrylamide gel, then was transferred to polyvinylidene fluoride membranes, blocked with 5% skim milk, and blotted against primary antibodies and secondary antibody sequential y. Primary antibodies were follows: GAPDH (CWBO), CD133 (PTG), and ABCG2 (PTG).

Table 1. — The relative gray scale value of CD133 and ABCG2 in eutopic, ectopic, and control endometrium.

<table>
<thead>
<tr>
<th></th>
<th>Eutopic endometrium</th>
<th>Ectopic endometrium</th>
<th>Control endometrium</th>
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</thead>
<tbody>
<tr>
<td>CD133</td>
<td>830.2±257.8ᵃ</td>
<td>662.0±275.0</td>
<td>327.0±179.0</td>
</tr>
<tr>
<td>ABCG2</td>
<td>884.9±221.6ᵇ</td>
<td>727.6±264.7</td>
<td>397.5±187.5</td>
</tr>
</tbody>
</table>

ᵃ: p = 0.042 compared with ectopic endometrium
ᵇ: p = 0.000 compared with control endometrium
ᶜ: p = 0.038 compared with ectopic endometrium
ᵈ: p = 0.000 compared with control endometrium

Western blot analysis was performed by a crescendo Western HRP substrate.

Statistical analysis

The relative gray scale value of the target protein band was shown as the mean ± SD. Statistical analysis was plotted by using SPSS 17.0. The results were analyzed using t-test and Pearson correlation coefficient was used for correlation analysis. The values of p < 0.05 were considered statistically significant.
Results

There were no statistically significant differences between age and body mass index groups ($p > 0.05$). To determine the expression level of CD133 and ABCG2 protein in endometriosis, the authors performed Western blot assay with GAPDH as an internal control. The results showed CD133 and ABCG2 protein were expressed in eutopic, ectopic endometrium of endometriosis group, and endometrium of control group. The relative gray scale value of CD133 in eutopic, ectopic, and control endometrium was $830.2 \pm 257.8$, $662.0 \pm 275.0$, and $327.0 \pm 179.0$, respectively, and the relative gray scale value of ABCG2 in eutopic, ectopic, and control endometrium was $884.9 \pm 221.6$, $727.6 \pm 264.7$, and $397.5 \pm 187.5$, respectively. As shown in Table 1, Figure 1, and Figure 2, the eutopic endometrium showed a high level of CD133 and ABCG2 protein when compared with ectopic ($p = 0.042$, $p = 0.038$), and control endometrium ($p = 0.000$, $p = 0.000$). In the endometriosis group, the extent of disease was staged according to the Revised American Fertility Society (R-AFS) classification (renamed later American Society for Reproductive Medicine’s classification—ASRM classification) [16]. The expression of CD133 protein in ectopic endometrium was positively correlated with R-AFS score of endometriosis ($p = 0.000$, $r = 0.793$) (Figure 3), no significant relation was noted between ABCG2 and R-AFS score ($p = 0.563$).

Each patient received intensive follow-up for two years and there were three patients with recurrence, two of which had much higher expression of ABCG2 protein than the patients without recurrence and the gray scale values were $1,298$ and $1,358$, respectively.

Discussion

In the present study, the authors have shown that the ectopic endometrium expressed the proteins CD133 and ABCG2, which are generally considered markers of stem cells. The advanced stage patients had increased expression of CD133 in ectopic endometrium compared with the early stage patients and the level of CD133 was positively correlated with R-AFS score of endometriosis. It is known that self-renewal, differentiation, and high proliferative capacity are key functional properties of adult stem cells [17]. Therefore, there are probably a subset of stem cells inside the endometriosis lesions, which may be the reservoir cells that allow the ectopic endometrial cells to remain active and promote the lesions’ progression. The presence of stem cell markers in endometriotic lesions suggests the possible involvement of stem cells in the pathogenesis and the ectopic stem cells might lead to the establishment and progression of endometriosis. In addition, the monoclonal origin and the long-term culturing properties of cell clones established from endometriotic lesions, also support the stem cell hypothesis of endometriosis [18]. At present, many study groups have identified, isolated, and characterized endometrial stem/progenitor cells through a variety of methods such as clonogenicity, label-retaining cells, “side-population” cells, undifferentiation markers, and cellular differentiation [19-22]. The concurrent expressions of CD133 and ABCG2 in both eutopic and ectopic endometrium are circumstantial evidence that ectopic endometrial stem cells probably source from eutopic endometrial stem cells. Stem cells that are inappropriately shed during retrograde menstruation may contribute to the pathogenetic process, because their immense regenerative capacity promotes rapid clonal expansion [23, 24]. However, Du and Taylor used murine models to investigate the contribution of non-endometrial stem cells to endometriosis found that ectopic lesions also probably derived from ectopic differentiation of bone marrow stem cells [25].

Liu and Lang demonstrated that there are fundamental abnormal changes within the eutopic endometrium of women with endometriosis compared to normal endometrium of women without and thought that the character of eutopic endometrium determines the fate of the backward-flowing endometrial tissue [26]. The present study showed the expression of CD133 and ABCG2 in eutopic endometrium was significantly higher than in the endometrium of the control, hence they suppose that the endometrial stem cells with endometriosis have probably some specialities that are distinct from the endometrial stem cells without endometriosis, which cause them to shed more easily from uterine cavity and have increasing abilities to spread and attach in an ectopic site resulting in endometriotic lesions. In other words, aberrant expressions of CD133 and ABCG2 probably cause aberrant stem cell function, which may contribute to the pathogenesis of endometriosis.
Endometriosis is a benign disease but has a high recurrence rate even after excision and prevention by medical management [27]. In the present study two of three patients with recurrence after surgery combined with the administration of drugs for six months had significant stronger expression of ABCG2 in ectopic lesions than the patients without recurrence. Many investigations indicated that the stem cells resistant to chemotherapy or radiation is often characterized by an elevated expression of the stem cell surface marker ABCG2 [28, 29]. The present authors proposed that the ABCG2 positive ectopic endometrial cells are also probably resistant to medication and can still remain active and grow, form lesions again after treatment is concluded, which can explain the higher recurrence rate of endometriosis. Because the present sample size was too small, further study is needed to prove the relationship between ABCG2 and the recurrence of endometriosis.

In addition, endometriosis is considered a neoplastic disease with malignant potential [30, 31] and the stem cell-like ability is one of the features shared by endometriosis and cancer [32]. Hence the present authors suggest that CD133+ and ABCG2 positive cells transformation may be the underlying mechanism leading to the progression from endometriosis to ovarian cancer.

To conclude, the present results demonstrated that the expression of CD133 and ABCG2 in ectopic lesions indicates the existence of putative stem cells, which are probably sourced from the eutopic endometrial stem cells with aberrant functions, and can induce the development of endometriosis and also have relationship with the recurrence and the progression to ovarian cancer. CD133 and ABCG2 have the potential to be utilized as novel markers for predicting recurrence and malignancy and CD133 and ABCG2 positive cells have the possibility of becoming new therapeutic targets of endometriosis. In the future the present authors will isolate cells CD133+/ABCG2+ to show their biological characteristics and abilities associated with endometriosis, in order to check their expressions from the other stemness-related markers, to identify their colony-forming potential, self-renewal capacity, and multipotency, and to examine their functions in an animal model of endometriosis initiation or progression, and so on.

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References


Address reprint requests to:
S. LIU, M.D.
Department of Obstetrics and Gynecology
The Fourth Affiliated Hospital of Jiangsu University
20 Zhengdong Street
212001 Zhenjiang City (China)
e-mail: leo423923@163.com
Umbilical cord blood endocan levels according to the delivery mode

M. Aksoy¹, A.N. Aksoy², E. Laloglu³, A. Dostbil¹, M. Gursac Celik¹

¹ Department of Anesthesiology and Reanimation, Faculty of Medicine, Ataturk University, Erzurum
² Department of Obstetrics and Gynecology, Nenehatun Hospital, Erzurum
³ Department of Biochemistry, Faculty of Medicine, Ataturk University, Erzurum (Turkey)

Summary

Purpose of investigation: The authors aimed to evaluate the endocan levels in the umbilical cord blood regarding the delivery mode.

Materials and Methods: One hundred six women aged between 20 to 35 years, undergoing delivery at term were studied. Three groups were formed: 37 neonates born by spontaneous vaginal delivery (group 1), 34 neonates born by an elective cesarean section with the general anesthesia (group 2), and 35 neonates born by an elective cesarean section with spinal anesthesia (group 3). In delivery, umbilical cord blood samples were collected and endocan levels were measured. Results: The endocan levels of cord blood (mean ± standard deviation, ng/ml) were found to be lower in group 2 (1.21 ± 0.46) compared to group 1 (1.52 ± 0.52) (p = 0.011). Cord blood endocan levels were not different in group 1 than those of group 3 (p = 0.49). Conclusion: It may be concluded that cord blood endocan levels are affected by the delivery mode.

Key words: Vaginal delivery; Cesarean section; Anesthesia; Neonate; Endocan.

Introduction

Endocan, a chondroitin-dermatan sulphate proteoglycan is called an endothelial cell-specific molecule-1 and has an important role in the control of cell proliferation [1]. It is synthesised by endothelial cells and regulated by tumor cell-derived factors, including vascular endothelial growth factor (VEGF) [2]. Anti-VEGF antibody was reported to be has an inhibitory effect on pregnancy development and placental angiogenesis in a rodent model [3]. Also, various cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor alpha (TNF-α) play a role in the regulation of endocan synthesis [4]. IL-1β and TNF-α upregulate the endocan messenger RNA and increases the secretion of endocan by the endothelium. Endocan regulates leukocyte functions via inhibiting leukocyte function-associated antigen and intercellular adhesion molecule [5].

Angiogenesis is the formation of new vessels from pre-existing vessels and it plays an important role in the development and wound healing [6]. Zhang et al. [7] demonstrated that endocan is expressed in neogenically active tissue and cell types. They reported that endocan may be an important marker in angiogenesis and neogenesis. Scherpereel et al. [8] demonstrated higher serum endocan levels in septic shock patients compared to patients with sepsis. They suggested that endocan may be a marker to determine the severity of sepsis and a useful marker of endothelial cell activation. On the other hand, increased secretion of endocan has been observed in several malignant diseases such as hepatocellular carcinoma, bladder cancer, and clear cell renal cell carcinoma [9-11].

Both spontaneous vaginal and surgical delivery stress may cause the local inflammatory response in the fetus [12-14]. It has been shown that fetal well-being and maternal comfort are affected by the delivery mode, type of anesthesia, and different anaesthetic drugs [15-17]. The secretion of cytokines increases during perioperative period due to surgical stress and endocan secretion is regulated by cytokines [4, 12]. The present authors believe that cord blood endocan levels might be a marker to demonstrate fetal endothelial cell activation and neoangiogenesis. In this prospective observational trial, they aimed to evaluate the endocan levels in the umbilical cord blood regarding the delivery mode.

Materials and Methods

This prospective study was approved by the Ethics Committee of the Medical Faculty, Ataturk University and was performed over a ten-month period in two institutions (a university hospital and other state hospital). One hundred six women without antepartum complications, aged between 20 to 35 years, with full-term fetuses (≥ 37 weeks’ gestation) in a vertex presentation, with no congenital malformation, Apgar score ≥7 at five minutes after birth were selected for this study. Written informed consent was obtained from all parents before participation. At first gestational week, amniotic fluid volume, presentation of fetus, and possible...
fetal malformation were detected via obstetric ultrasound. Smokers, alcohol consumers, mothers with complicated pregnancies (such as pre eclampsia, placenta previa, oligohydramnios and intrauterine growth retardation), hypertension, diabetes mellitus, and multiple pregnancies were excluded from the study.

Patients undergoing elective cesarean section were informed about advantages and disadvantages of the anesthesia methods by an anesthetist. Spinal or general anesthesia was decided according to medical considerations and the patient’s decision without randomization. The number of patients in each group was planned as 40 at the beginning of the study. Consecutive patients who preferred each anesthesia technique were included in this study. Before entering the operating room, all patients were premedicated with 0.02 mg/kg intravenous (iv) midazolam to provide synchronization and received 500-750 ml of Ringer lactate solution via iv cannula. Standard monitoring included non-invasive arterial pressure, electrocardiography, and pulse oximetry was established. Eventually, three groups were formed according to the delivery modes:

Group 1: Neonates, born by spontaneous vaginal delivery. All women had spontaneous contractions of adequate frequency. Induction of labour was done only with artificial rupture of fetal membranes when the cervix was three-cm dilated in all women. No other method was used for induction of labour such as syntocin infusion. No analgesic method was used for pain relief such as opioids, nitrous oxide, and epidural analgesia. Continuous external fetal monitoring and maternal blood pressure monitoring were applied.

Group 2: Neonates, born by an elective cesarean section with general anesthesia. General anesthesia was induced with iv propofol two mg/kg. Then iv rocuronium (0.6 mg/kg) was given to patients for facilitating endotracheal intubation. Anesthesia was maintained with nitrous oxide 50%, oxygen 50%, and sevoflurane 1-2%. After delivery of the baby, fentanyl one µg/kg was given by iv.

Group 3: Neonates, born by an elective cesarean section with spinal anesthesia. Ringer lactate solution (12 ml/kg) was given by iv in 15 minutes. After skin infiltration with 2% lidocain, 26-gauge Quincke’s needle was inserted through the L2-3/ L3-4 intervertebral space of sitting position patient. Once free flow of cerebrospinal fluid was obtained, hyperbaric bupivacaine 0.5%, nine mg (1.8 ml) was injected intrathecally. Then, the patient was positioned with a wedge under their right hip to prevent aortocaval compression. Oxygen was administered three to four L/minute with a face mask until delivery. In the case of hypotension (a 30% decrease in systolic blood pressure compared with preoperative values), ephedrine was administered. When the sensory block reached the T4 dermatome, surgery was initiated.

All mothers had a normal complete blood count, negative for CRP and normal appearance of placenta. The operation indication was previous cesarean section in elective cesarean section groups in this study. All operations were performed between the hours of 08:00 and 12:00.

During delivery, the umbilical cord was doubly clamped and cord blood was drawn from the umbilical artery. Blood was collected into tubes and centrifuged at 3,000 g for ten minutes after coagulation. Then the obtained serum samples were kept at -80°C until measurements were conducted.

Endocan levels in cord blood samples were detected via a sandwich-based enzyme-linked immunosorbent assay (ELISA) according to recommendations of the manufacturer. It was determined by the use of immunoassay kit and expressed in ng/ml. The detection limit of this measurement was 0.3 ng/ml. Results less than 0.3 ng/ml were accepted as ‘0’.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.82±3.43</td>
<td>28.27±3.73</td>
<td>28.86±3.73</td>
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<tr>
<td>Maternal BMI (kg/m²)</td>
<td>24.05±1.47</td>
<td>24.21±1.47</td>
<td>23.66±1.51</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.85±0.74</td>
<td>38.76±0.48</td>
<td>38.66±0.48</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.24±0.96</td>
<td>2.44±0.50</td>
<td>2.31±0.42</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3530±208</td>
<td>3512±168</td>
<td>3449±184</td>
</tr>
<tr>
<td>Apgar scores One minute</td>
<td>7.46±0.56</td>
<td>7.61±0.49</td>
<td>7.51±0.50</td>
</tr>
<tr>
<td>Apgar scores Five minutes</td>
<td>9.27±0.45</td>
<td>9.38±0.51</td>
<td>9.49±0.51</td>
</tr>
<tr>
<td>Duration of surgery (minutes)</td>
<td>49.12±3.79</td>
<td>42.75±2.54</td>
<td>42.75±2.54</td>
</tr>
</tbody>
</table>

Sociodemographic information (age, body mass index, parity, the week of gestation), the duration of surgery, duration of labor, birth weight, Apgar scores at minutes one and five, and endocan levels in cord blood samples were recorded.

The authors tested for associations among cord blood endocan levels, delivery modes, and socio-demographic characteristics. The Kolmogorov-Smirnov test was used to assess the normal distribution of data. If data was not normally distributed, comparisons were determined using the Kruskal-Wallis and Mann-Whitney U-test. Comparisons were determined using the ANOVA test when data was normally distributed. SPSS software 12.0 was used for the statistical analysis. The data was calculated as mean ± standard deviation and p = < 0.05 was considered significant. By performing a power calculation, when α error and β error were considered, respectively, as 0.05 and 0.04, with 85% power, the patient number for each group was determined as minimum 35. The common standard deviation within a group was assumed to be 0.50.

**Results**

Data from 14 neonates with Apgar scores less than 7 at five minutes after birth (three in group 1, six in group 2, and five in group 3) was excluded from the study. In the final analysis, data of 37 patients in the group 1, 34 patients in the group 2 and 35 patients in the group 3 were included.

There were no significant differences among the groups in terms of age, body mass index, parity, the week of gestation, birth weight, and Apgar scores (Table 1). The duration of surgery was significantly lower in group 3 (42.57 ± 2.54 minutes) compared with group 2 (49.12 ± 3.79 minutes) (p < 0.0001). There were no differences among patients in terms of duration of labor (p > 0.05). In group 3, hypotension developed in five patients just before the operation and was treated with iv ephedrine five mg. Hyper-
Umbilical cord blood endocan levels according to the delivery mode

tension (systolic/diastolic tension > 160/95 mmHg) and tachycardia (heartbeat of over 100 beats per minute) occurred in four patients of group 2 and this situation was corrected with hyperventilation.

Cord blood endocan levels in group 2 (1.21 ± 0.46 ng/ml) were found to be significantly lower than those of group 1 (1.52 ± 0.52 ng/ml, \( p = 0.011 \)). Cord blood endocan levels in group 1 were similar to group 3 (1.44 ± 0.53 ng/ml, \( p = 0.49 \)). Mean endocan levels were lower in group 2 compared with group 3, although the difference was not statistically significant (\( p = 0.064 \)) (Figure 1). When all groups were evaluated together, no correlation was found between clinical characteristics and cord blood endocan levels (Spearman’s rank correlation).

Discussion

This is the first study examining the association between endocan levels in cord blood and delivery modes. The authors found that cord blood endocan levels in healthy neonates were higher in spontaneously vaginally delivered, compared to cesarean section using general anesthesia. In neonates born by spontaneous vaginal delivery cord blood endocan levels were similar to those in neonates born by cesarean section with spinal anesthesia.

Endocan is secreted by endothelial cells and regulated by VEGF and various cytokines, especially IL-1\( \beta \) and TNF-\( \alpha \) [4]. VEGF has an important role in fetal development and placental angiogenesis [3]. Zhang et al. [7] suggested that endocan may be a marker in the tissue damage, angiogenesis, oncogenesis, and the process of inflammatory reactions. Scherpereel et al. [8] found the relationship between endocan levels and severity of sepsis and endocan was reported as a useful marker of endothelial cell activation in their study. In another study [18] researchers found negative correlation between serum endocan levels and the development of acute lung injury in patients exposed to major trauma. Additionally, an increase in the content of endocan of endothelial cells has been detected in the lung, kidney, liver, colon, ovary, and brain tumours [19].

Surgical stress causes an increase in the levels of cytokines and this increase varies depending on severity of surgical injury [20]. The expression of endocan is regulated by cytokines [1]. The effects of different anaesthetic techniques on cytokine levels have been investigated in surgical patients [21-22]. Zura et al. [23] investigated the effect of spinal and general anesthesia on serum concentration of cytokines in patients undergoing transurethral resection of the prostate. They found low IL-2 levels on postoperative days 1 and 3 in general anesthesia group compared to spinal anesthesia group. It may be suggested that general anesthesia may induce cytokine release less than spinal anesthesia. In another study in contrast, the general anesthesia group had higher pro-inflammatory cytokines compared to the regional anesthesia group in patients undergoing laparoscopic cholecystectomy [24].

Researchers have reported variable levels of cytokines according to different delivery modes [25-27]. A study by Dermitzaki et al. [26] found no changes in maternal serum IL-6 and TNF-\( \alpha \) concentrations between two different anesthetic techniques for cesarean section. Buyukkokcak et al. [27] reported decreased acute phase protein levels in patients undergoing vaginal delivery with epidural analgesia compared to cesarean section and these alterations were not influenced by anesthetic techniques (spinal, epidural, and general anesthesia). Consistent with these studies [26, 27], the present authors found similar cord blood endocan levels in patients undergoing elective cesarean section with general and spinal anesthesia. However, they observed higher cord blood endocan levels in patients undergoing spontaneous vaginal delivery compared to the patients undergoing elective cesarean section with general anesthesia. The use of different general anesthetic agents (propofol and thiopental) in studies may be a cause in obtaining these different results.

In the present institute, regional anesthesia is the most common method to provide anesthesia for cesarean section. However, general anesthesia is used in case of maternal refusal of regional techniques, failed regional attempts, and in the presence of contraindications to regional anesthesia such as coagulation disorders or spinal abnormalities. In this current study, randomization was not done and anesthesia technique was selected in consideration of the pa-
tient’s request and medical condition. Because the present authors thought that selection of anesthesia technique without randomization may be more ethical. Also, they thought that the results of this study would not be affected by this situation.

After intravenous bolus administration of propofol which was used in the present study, it rapidly distributes to tissues (half-life of two to four minutes) and passes through the placenta. Propofol is known to suppress pro-inflammatory cytokine response in rats [28]. In the present study, lower endocan levels in neonates born by cesarean section with general anesthesia may be due to the anti-inflammatory effect of propofol. Conversely, spinal anesthesia may cause a reduction in maternal and uteroplacental blood flow/presence due to maternal hypotension [29]. This situation may be a cause of endothelial activation and higher endocan levels in neonates born by cesarean section with spinal anesthesia than in general anesthesia. On the other hand, regional anesthesia was reported to be associated with a decrease in the stress-inducing hormones adrenaline, noradrenaline, and cortisol [30]. In this instance, the exact mechanisms for low cord blood endocan levels in spinal anesthesia group remains unclear.

The cause of a high level of cord blood endocan in spontaneous vaginal delivery group may be increasing stress caused by pain during uterine contractions. In this study, there was no CRP elevation in any of the patients. These results may show that elevation of endocan might be due to endothelial activation rather than acute inflammation. On the other hand, the present authors suggested that an increase in cord blood endocan levels may play a role in the beginning of uterine contractions in pregnant women. The current findings raise the interesting question of whether elevated cord blood endocan levels are useful for neonates.

Endocan is a new marker of endothelial cell activation and may play critical roles in many physiological and pathological events associated with endothelium [4]. Also, endocan is a biomarker of neoangiogenesis and some cellular activities such as migration, adhesion, and proliferation were regulated by endocan [2]. On the other hand, the range of cord blood endocan levels in spontaneous vaginal delivery group in the present study was 0.5 - 2.87 ng/ml, while the range of serum endocan levels was 0.48 - 1.21 ng/ml in healthy adults [31]. Whereas significantly increased levels of serum endocan were reported in patients with severe sepsis [8] and cancer, such as invasive bladder cancer [10] and hepatocellular carcinoma [9]. For these reasons, the present authors suggested that slightly higher levels of cord blood endocan may be beneficial for neoangiogenesis and neonatal development.

The present authors could not measure cytokines that may lead to elevation of endocan and this is a weakness of the present study.

Conclusion

Cord blood endocan levels are affected by the delivery mode. The present authors reported higher cord blood endocan levels in healthy neonates born by spontaneous vaginal delivery compared to the neonates born by cesarean section with general anesthesia. They suggested that slightly higher levels of cord blood endocan may be beneficial for neoangiogenesis of neonates. They also suggested that an increase in cord blood endocan levels may be a physiological process in the beginning of labor. Future studies are needed to identify the effect of the delivery mode on the endocan levels of fetal tissues such as placenta and amniotic fluid.

References

Umbilical cord blood endocan levels according to the delivery mode


Address reprint requests to:
M. AKSOY, M.D.
Department of Anesthesiology and Reanimation,
Faculty of Medicine, Ataturk University,
Cemal Gursel Street, Ataturk District
Erzurum 25240 (Turkey)
e-mail: drmaksoy@hotmail.com
The safety and acceptability of intravenous fentanyl versus intramuscular pethidine for pain relief during labour

M. Rezk¹, E.S. El-Shamy¹, A. Massod¹, R. Dawood¹, R. Habeeb²
¹ Department of Obstetrics and Gynecology, ² Department of Anesthesiology, Faculty of Medicine, Menoufia University, Menoufia (Egypt)

Summary
Objectives: This trial aimed to ascertain the relative efficacy, adverse effects, and acceptability of fentanyl versus pethidine for pain relief during labour. Materials and Methods: Parturients (n=80) in the active phase who requested analgesia were randomly assigned to receive either intravenous fentanyl (n=40) or intramuscular pethidine (n=40). Pain scores hourly, maternal and fetal adverse effects, neonatal outcome, and maternal acceptability were assessed. Results: Pain scores decreased in both groups, the decrease varying from mild to moderate, average pain scores remaining above 3.5 in both groups. Pain scores returned towards baseline over time; three hours after the initiation of treatment in the fentanyl group. Pethidine was associated with more maternal nausea and vomiting (p < 0.05) while fentanyl was associated with more neonates with low Apgar scores at one minute and more need for neonatal resuscitation and naloxone administration when compared to pethidine (p < 0.05). Both drugs were acceptable for pain relief during labour. Conclusion: Fentanyl is comparable to pethidine for pain relief during labour regarding efficacy and acceptability, but with more neonates with low Apgar scores at one minute and higher need for neonatal resuscitation and naloxone administration. Further larger trials are needed to confirm its safety.

Key words: Fentanyl; Pethidine; Pain relief during labour.

Introduction
Labour is a painful experience and analgesia is often required. It is emphasized that request for pain relief be considered as a sufficient medical indication for the use of labour pain relief methods [1].

Pain intensity is influenced by numerous factors such as anxiety, environmental factors, culture, support from caregivers, focus of attention, and previous experiences [1, 2].
Pethidine, otherwise known as meperidine, is a widely used analgesic for labour pain worldwide. Research has demonstrated that pethidine provides variable pain relief in labour; much of its effect is sedation rather than analgesia [3]. Pethidine also has adverse effects in both the mother and neonate. It crosses the placenta and may cause reduced fetal heart rate variability and fewer heart rate accelerations. Neonatal adverse effects include respiratory depression, impaired breastfeeding, and altered crying [4, 5]. Systematic reviews comparing parenteral opioids in labour have suggested the need for well-designed and adequately powered trials of pethidine versus other opioids [6, 7].

Fentanyl, a phenyl piperidine derivative, is a short-acting and potent synthetic narcotic. Several comparative studies have shown that analgesic effects of intravenous fentanyl are better than pethidine [8-10].
The aim of this study was to assess the efficacy, safety, and acceptability of intravenous fentanyl versus intramuscular pethidine for pain relief during labour.

Materials and Methods
This was a single center balanced randomized parallel group study carried out at the Department of Obstetrics and Gynecology, Menoufia University Hospital, Egypt between April 2013 and April 2014. The institutional review board approved the study protocol and an informed consent was obtained from all participants prior to commencing the study.

Based on previous trials, power was set at 0.8, alpha level at 0.05, and the confidence interval (CI) at 95%. A total sample size 80 subjects was needed for this trial (40 subjects in each group), after adding a 10% to compensate for possible dropout of cases.

Participants
The study was conducted on 80 healthy nulliparous women who requested analgesia for labour pains. Inclusion criteria included women who were in the active labour (defined as regular uterine contractions of at least two in ten minutes), with a singleton pregnancy, cervical dilatation of at least four cm, with gestation of 37-41 weeks, and reactive non-stress test.

Exclusion criteria included allergy or previous adverse reaction to opioids or opioid dependency, use of parenteral opioids within the previous 24 hours, presence of severe systemic or mental disease, maternal respiratory rate ≤ 8 or maternal bradycardia (pulse rate less than 60), and women requesting additional dosage of analgesia. The authors excluded women requesting additional analgesia in order to test the specific single dosing for a particular opioid.

Randomization
Enrolled women were randomly assigned into two groups according to the method of treatment. Randomization in 1:1 ratio was carried out using computer-generated simple random tables.

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It was not possible to blind the study participants from knowledge of which drug was received because methods of administration were clearly different.

Intervention

**Group 1 (Fentanyl group):** 40 pregnant women in whom 50 micrograms fentanyl was given after being diluted in 18 ml normal saline (total volume 20 ml - 50 μg /20 ml) during ten minutes intravenous infusion according to the present hospital policy.

**Group 2 (Pethidine group):** 40 pregnant women in whom 100 mg of pethidine was given by intramuscular injection. Vital signs were monitored every ten minutes. Artificial rupture of membranes (AROM) was performed for all women with intact membranes and intravenous oxytocin infusion was started if there was no efficient uterine contractions. An oxytocin infusion was started at two mU/min and increased in increments of one to two mU/min at 15-30 minutes intervals as needed to achieve adequate uterine contraction pattern (≥ 200 MVU). Continuous cardiotocography (CTG) was done during delivery and the modified WHO partograph was followed up for the labour management. An anesthesiologist and resuscitation equipments were available at all times.

Outcome measures

Primary outcome measure included changes in pain scores. Pain severity during the last contraction was assessed using a Visual Analogue Scale [VAS] (with anchor points of 0 = no pain at all and 10 = the most excruciating pain) every 60 minutes during the three-hour period after administration of the trial drug. Pain severity was estimated four times (before and one, two, and three hours after drug intake). This information was used to derive measures of pain relief at each time-point using absolute change in pain intensity (on a 10-cm VAS) from pre-analgesia (score 0).

Maternal adverse effects [fainting, nausea and vomiting, respiratory depression, hypotension (blood pressure < 90 mmHg), bradycardia (heart rate < 60 beats min)], post-delivery maternal acceptability and neonatal outcome (Apgar scores at one and five minutes, need for resuscitation, and admission to neonatal intensive care unit) were recorded as secondary outcomes (Figure 1).

Statistical analysis

Data entry and analysis was carried out using SPSS version 16.  
1) Descriptive statistics: quantitative data were expressed to measure the central tendency of data and diversion around the mean, mean (x) and standard deviation (SD). Qualitative data expressed in number and percentage.  
2) Analytic statistics: t-test was used for comparison of two groups of normally distributed variables. Fisher exact test was used to compare categorical outcomes when expected cell or more in 2x2 tables was less than 5.

All these tests were used as tests of significance at:
The safety and acceptability of intravenous fentanyl versus intramuscular pethidine for pain relief during labour

Table 1. — Maternal characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl group (n=40)</th>
<th>Pethidine group (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.72±2.63</td>
<td>21.70±1.69</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.00±1.43</td>
<td>39.22±1.25</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.40±5.68</td>
<td>70.00±5.52</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Duration of active phase (hours)</td>
<td>5.25±0.86</td>
<td>5.31±0.85</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Need for oxytocin</td>
<td>26 (55%)</td>
<td>23(57.5%)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. — Maternal pain scores using visual analogue scale (VAS).

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl group (n=40)</th>
<th>Pethidine group (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score at inclusion (0)</td>
<td>8.50±1.13</td>
<td>8.25±0.84</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Score at 1 hour</td>
<td>4.50±1.13</td>
<td>4.80±1.09</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Score 1 vs Score 0</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Score at 2 hours</td>
<td>5.10±1.13</td>
<td>4.70±1.32</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Score 2 vs Score 0</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Score at 3 hours</td>
<td>6.5±1.13</td>
<td>4.85±0.86</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>p Score 3 vs Score 0</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 3. — Maternal adverse effects.

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl group (n=40)</th>
<th>Pethidine group (n=40)</th>
<th>Chi square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension</td>
<td>4 (10%)</td>
<td>5 (12.5%)</td>
<td>0.125</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>1 (2.5%)</td>
<td>0 (0 %)</td>
<td>*1.013</td>
</tr>
<tr>
<td>Fainting</td>
<td>2 (5%)</td>
<td>3 (7.5%)</td>
<td>*0.213</td>
</tr>
<tr>
<td>Headache</td>
<td>2(5%)</td>
<td>5 (12.5%)</td>
<td>*1.41</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>4(10%)</td>
<td>11 (27.5%)</td>
<td>4.02</td>
</tr>
<tr>
<td>Need for anti-emetics</td>
<td>4 (10%)</td>
<td>5 (12.5%)</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 4. — Fetal-neonatal outcome.

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl group (n=40)</th>
<th>Pethidine group (n=40)</th>
<th>Chi square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal FHR</td>
<td>6 (15%)</td>
<td>4 (10%)</td>
<td>0.457</td>
</tr>
<tr>
<td>Appgar score at 1 minute</td>
<td>5.5±1.13</td>
<td>6.35±1.37</td>
<td>3.026</td>
</tr>
<tr>
<td>Appgar score at 5 minutes</td>
<td>8.00±1.43</td>
<td>8.20±1.18</td>
<td>0.681</td>
</tr>
<tr>
<td>Need for resuscitation</td>
<td>11 (27.5%)</td>
<td>4 (10%)</td>
<td>*0.346</td>
</tr>
<tr>
<td>NICU admission</td>
<td>2 (5%)</td>
<td>1 (2.5%)</td>
<td>*0.293</td>
</tr>
<tr>
<td>Need for Naloxone</td>
<td>10</td>
<td>3</td>
<td>*4.501</td>
</tr>
</tbody>
</table>

Table 5. — Post-delivery maternal acceptability.

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl group (n=40)</th>
<th>Pethidine group (n=40)</th>
<th>Chi square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall satisfaction with analgesia: 1. Good</td>
<td>26 (55%)</td>
<td>23 (57.5%)</td>
<td>0.493</td>
</tr>
<tr>
<td>2. Neutral or somewhat satisfied</td>
<td>11 (27.5%)</td>
<td>13 (32.5%)</td>
<td>*0.392</td>
</tr>
<tr>
<td>3. Not satisfied</td>
<td>3 (7.5%)</td>
<td>4 (10%)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Would choose the same analgesia in future labour and/or would recommend the analgesic to other women: 1. Highly or somewhat agree</td>
<td>35 (87.5%)</td>
<td>33 (82.5%)</td>
<td>0.392</td>
</tr>
<tr>
<td>2. Neutral or somewhat disagree</td>
<td>5 (12.5%)</td>
<td>7 (17.5%)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

• p value > 0.05 was considered statistically non significant.
• p value ≤ 0.05 was considered statistically significant.
• p value ≤ 0.001 was considered statistically highly significant. [p values in bold in the tables are significant].

Results

Table 1 displays the maternal characteristics and the duration of the active phase of labour in the two groups. Table 2 reveals the maternal pain scores. There was a significant reduction of pain scores at one and two hours after administration of the analgesic drugs in both groups with return of pain intensity after three hours in the fentanyl group. Table 3 shows the maternal adverse effects. There was a significant number of women suffering from nausea and vomiting in the pethidine group (p < 0.05). Table 4 reveals the fetal-neonatal outcome. There was a significant low Apgar score at one minute and the need for neonatal resuscitation and naloxone administration in the fentanyl group (p < 0.05). Table 5 displays the post-delivery maternal acceptability. There was no significant difference between the two groups.

Discussion

The present study showed that intravenous administration of fentanyl decreased pain intensity similar to pethidine; the authors used the VAS for pain intensity [11]. Overall, the decrease in pain scores varied from mild to moderate, average pain scores remaining above 3.5 cm in both groups in the present study, which is comparable to previous studies concluding that intravenous patient-controlled analgesia with either remifentanil or fentanyl provides a moderate degree of labour analgesia [12-14]. Some have suggested that the minimum difference in pain that can be subjectively measured by women using the VAS is 1.3 cm, 1.4 cm or 1.8 cm [15-17]. In the fentanyl group, pain scores no longer differed significantly from baseline three hours after treatment was
started. Similar results of analgesia have been shown in other studies [10, 12, 14]. The short half-life of fentanyl (30-60 minutes) may explain the increasing pain score in third hour. Also, pain scores tended to increase with the progress of labour [12].

In the present study, more women suffering from nausea and vomiting in the pethidine group which is familiar with previous studies [9, 10].

There were a significant differences in the neonatal primary outcomes of the need for resuscitation and Apgar scores < 7 at one minute between the two groups as fentanyl was associated with lower Apgar scores at one minute and higher need for neonatal resuscitation which was confirmed in previous results [9, 10]. Also, fentanyl administered by subcutaneous route for pain relief during labour in a previous trial [18] was associated with significant need for naloxone during neonatal resuscitation which was confirmed in the present study.

Overall satisfaction scores were similar in both groups and approximately 85% of women in both groups would choose the same analgesia in a future labour and would recommend it to another women, when questioned within 24 hours of delivery.

Inability to design a double blinded trial and the small number of the present patients are the main limitations of our study. Further larger trials are needed to examine the safety of intravenous fentanyl on the neonatal outcome over longer periods of time.

In conclusion, under the conditions of the present study, intravenous fentanyl was effective as pethidine in providing pain relief during labour, but it was associated with significantly more depressed Apgar scores at one minute and a higher need for neonatal resuscitation and naloxone administration. Its effectiveness was time-limited (two hours); therefore, the authors would recommend the use of fentanyl only in the last phase of cervical dilation with the availability of neonatologists during delivery.

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References


Address reprint requests to:
M. REZK, M.D.
Kafir Manawhla, El-Bajour
20 Al-Takwa Mosque Street
Menoufia (Egypt)
e-mail: m_rezk9207@yahoo.com
Prevalence of endometriosis at a university hospital in Jeddah, Saudi Arabia

A.A. Rouzi, N. Sahly, S. Kafy, D. Sawan, H. Abduljabbar

Department of Obstetrics and Gynecology, King Abdulaziz University, Jeddah (Saudi Arabia)

Summary

Purpose: To determine the prevalence of endometriosis in women who had gynecologic laparoscopy at a university hospital in Saudi Arabia. Materials and Methods: The hospital records were reviewed to identify all women who had undergone gynecological laparoscopy between January 2008 and December 2013. Results: A total of 190 gynecologic laparoscopies were performed. The indications for laparoscopy were infertility (n = 76; 40%), chronic pelvic pain (n = 34; 17.9%), infertility and chronic pelvic pain (n = 7; 3.7%), ectopic pregnancy (n = 30; 15.8%), pelvic mass (n = 12; 6.3%), removal of a missing intrauterine contraceptive device (n = 6; 3.2%); other indications were documented in 25 cases (13.1%). Endometriosis was diagnosed in 21 women (11.1%). The presenting complaints in women with endometriosis were pelvic pain (n = 7; 33.3%), infertility (n = 5; 23.8%), pelvic pain and infertility (n = 6; 28.6%), and pelvic mass (n = 2; 9.5%); the complaint was unknown in one patient (4.8%). Conclusion: Endometriosis was uncommon in women who had undergone gynecologic laparoscopy.

Key words: Endometriosis; Infertility; Laparoscopy; Pelvic pain.

Introduction

Endometriosis is an estrogen-dependent inflammatory disease that causes intractable pelvic pain and infertility in millions of women in their reproductive years. Approximately 10% of women in the United States are estimated to have endometriosis, [1] and about 30% of infertile women have endometriosis [2, 3]. However, the basic epidemiology of endometriosis has been difficult to evaluate owing to the fact that a large proportion of women with the disease may be asymptomatic [4, 5]. Endometriosis may be consequently underreported, as diagnosis can only be made definitively by the clinical expertise of the surgeon. Furthermore, because women are often taught to consider severe pain during menses to be normal, they do not seek medical care. As a result, many cases of endometriosis remain undiagnosed, also contributing to the underreporting of this disease. In Saudi Arabia, only a few isolated cases of endometriosis have been reported, [6-9] and the prevalence of this disease is unknown. The objective of this study was to determine the prevalence of endometriosis in women who had gynecologic laparoscopy at a university hospital in Jeddah, Saudi Arabia.

Materials and Methods

A retrospective chart review was performed of the medical records of all women who had undergone gynecological laparoscopy in the Obstetrics and Gynecology Department of King Abdulaziz University Hospital, Jeddah, Saudi Arabia from January 2008 through December 2013. Permission to conduct the study was granted by the Biomedical Ethics Research Committee of King Abdulaziz University. For all cases included in this study, the authors collected demographic data, indications for laparoscopy, diagnosis, and gynecologic operations performed.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences, version 22.0. Descriptive statistics were computed for all variables. Results are expressed as frequency (percent) and as mean ± standard deviation (SD).

Results

During the study period, 190 gynecologic laparoscopies were performed. The age of the patients was 33.8 ± 8.9 (mean ± SD) years. The indications for laparoscopy were: infertility, 76 (40%); chronic pelvic pain, 34 (17.9%); infertility and chronic pelvic pain, seven (3.7%); ectopic pregnancy, 30 (15.8%); pelvic mass, 12 (6.3%); and removal of a missing intrauterine contraceptive device, six (3.2%). Other indications were documented in 25 cases (13.1%). Endometriosis was diagnosed in 21 women (11.1%). The presenting complaints in these women were pelvic pain (n = 7; 33.3%), infertility (n = 5; 23.8%), pelvic pain and infertility (n = 6; 28.6%), and pelvic mass (n = 2; 9.5%). The presenting complaint was unknown in one patient (4.8%). Diagnostic laparoscopy was done in 93 (48.9%) women, while operative laparoscopy was performed in 97 (51.1%) cases.
Discussion

This study is, to the best of the authors’ knowledge, the first to assess the prevalence of endometriosis in a cohort of women at a university hospital in Jeddah, Saudi Arabia. The present analysis shows that the prevalence of endometriosis in women who had undergone laparoscopic surgery was 11.1%. Previous studies have reported varying prevalence rates for endometriosis, ranging from 0.7% to 45% in surveys of asymptomatic women [10], 20% to 40% in fertile women [11], 6% to 18% in women undergoing sterilization [12], and 15% to 70% in cases with chronic abdominal pain [13-15].

Previous studies [16, 17] showed that dysmenorrhea is frequent among women with endometriosis. Moreover, other authors found that chronic pelvic pain was reported as the main complaint for which patients with endometriosis seek medical attention [18, 19]. Although pelvic pain was the most common presenting complaint in women with endometriosis in the present study, it was not, unfortunately, specifically mentioned in the patients’ records whether pelvic pain was due to menstruation. Infertility was a relatively common symptom in the present cohort, diagnosed in 23.8% of the women. In the medical literature, approximately 30% of women with endometriosis have primary or secondary infertility. Although it is reported as one of the main clinical features of endometriosis, [17] some studies [2, 3] showed that a significant proportion of women may not present symptoms of endometriosis at all, and the disease will remain undiagnosed until the patient complains or requires surgery for infertility.

The findings of this study should be interpreted with caution because of its limitations. First, it is limited by its retrospective nature. Second, because it was hospital-based, the results cannot be generalized to the population of Jeddah. Third, cases of endometriosis may have been missed given that a significant number of patients may not present any symptoms [2, 3]. In addition, women may be precluded from seeking medical attention for socio-cultural reasons, as gynecologic issues are generally considered taboo in an ultraconservative society such as Saudi Arabia.

Conclusion

The prevalence of endometriosis among women who underwent gynecologic laparoscopy at King Abdulaziz University Hospital was low. While it is possible that the prevalence may be underreported due to socio-cultural barriers and challenges in making an accurate diagnosis, patients with endometriosis most commonly present with pelvic pain and / or infertility. Future prospective studies with a larger patient group are warranted to explore the prevalence of endometriosis in women who undergo gynecologic laparoscopy at the present institution and to determine the most common presentation mode.

References


Address reprint requests to:
A.A. ROUZI, M.D.
PO Box 80215
Jeddah, 21589 (Saudi Arabia)
e-mail: aarouzi@gmail.com
Introduction

Preeclampsia is characterized with hypertension and proteinuria after 20 weeks’ gestation. It is a disease which is specific to pregnancy and affects approximately 3% – 5% of pregnancies [1]. It is the leading cause of maternal deaths in developing countries [2]. Etiopathogenesis of preeclampsia is not yet well understood, but is thought to arise in the abnormal interaction of maternal and fetal tissue at the uteroplacental interface. Reduced maternal blood flow and oxygenization causing placental ischemia due to insufficient cytotrophoblast invasion to the myometrium, and abnormal remodeling of the uterine spiral arteries, are the pathologic changes in the uteroplacental unit [3]. Pathologic changes in uteroplacental unit and abnormal placental morphologic changes however are associated with the early-onset preeclampsia (before 34 gestational weeks) more than the late-onset preeclampsia [4].

Fetal-derived DNA has been found in the maternal circulation firstly in 1997 [5]. Real-time quantitative PCR assay for measuring the concentration of cell free fetal DNA in maternal plasma and serum has been described [6]. Several investigations have marked an increase in the concentration of cell free fetal DNA in the maternal circulation of women with preeclampsia [7,8]. However, generally these studies were not performed for early or late preeclampsia subgroups individually. The present authors planned to determine the levels of the cell free both fetal and maternal DNA in the maternal circulation in early preeclampsia subgroup and compare it with normotensive control cohort.

Materials and Methods

Pregnant women attending the Ankara University Medical Faculty, Department of Obstetrics-Gynecology, were recruited for the study. The study was approved by the Ankara University Medical Faculty Ethics Committee and all subjects provided written informed consent prior to data collection. Women with singleton male pregnancy between 28 and 32 gestational weeks were included in the study. Real-time PCR analysis was performed for determining the circulating cell free DNA levels. Results: Cell free fetal DNA concentrations were higher in early preeclamptic women than control subjects. The authors found no statistically significant difference in each levels of maternal and total DNA between hypertensive and normotensive groups. Conclusions: The present findings suggest that the levels of cell free fetal DNA in maternal circulation were higher in pregnancies which are complicated with early preeclampsia than normotensive controls.

Key words: Fetal DNA; Maternal DNA; Early-onset preeclampsia; Real-time PCR.
Table 1. — Primers and probes for SRY and β-globin real-time PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td></td>
</tr>
<tr>
<td>SRY-109F</td>
<td>5’TGGCGATTAAGCTAAAATTCGC-3’</td>
</tr>
<tr>
<td>SRY-245R</td>
<td>5’CCCCCTAGTTACACCTGACAATTT-3’</td>
</tr>
<tr>
<td>SRY-142T</td>
<td>5’-FAM)AGCAGTAGGAGCTGAGCGAGCAGA (TAMRA)-3’</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b-globin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>b-globin-354F</td>
<td>5’-GTGCACTGACTCCTGAGGAG-3’</td>
</tr>
<tr>
<td>b-globin-455R</td>
<td>5’-CCTTGATACCAACCTGCCCAG-3’</td>
</tr>
<tr>
<td>b-globin-402T</td>
<td>5’-FAM)AAAGGTGAACGTGATGAAGTTGG (TAMRA)-3’</td>
</tr>
</tbody>
</table>

Mann-Whitney rank-sum test performed.

Table 2. — Maternal age, gestational age, blood pressures, and cell free DNA levels in maternal plasma of the groups.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia (n=8)</th>
<th>Normal (n=8)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years) - Median [IQR]</td>
<td>28.5 [24.5 - 31.75]</td>
<td>32.5 [25.5 - 36.5]</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (weeks) - Median [IQR]</td>
<td>31.0 [30.0 - 32]</td>
<td>30.0 [28.2 - 31.0]</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) - Median [IQR]</td>
<td>90 [90 - 90]</td>
<td>70 [70 - 70]</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg) - Median [IQR]</td>
<td>150 [145 - 157.5]</td>
<td>110 [110 - 117.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg) - Median [IQR]</td>
<td>110 [107 - 111.2]</td>
<td>83.3 [80.8 - 88.3]</td>
<td>0.001</td>
</tr>
<tr>
<td>Fetal DNA (genomoequivalents/ml) - Median [IQR]</td>
<td>604 [413.0 - 821.0]</td>
<td>310.0 [100.0 - 423.0]</td>
<td>0.018</td>
</tr>
<tr>
<td>Maternal DNA (genomoequivalents/ml) - Median [IQR]</td>
<td>5,848.5 [847.7 - 12,992.5]</td>
<td>2,362.0 [1,378.0 - 40,223.0]</td>
<td>0.834</td>
</tr>
<tr>
<td>Total DNA (genomoequivalents/ml) - Median [IQR]</td>
<td>6,700.0 [1,280.2 - 13,605.7]</td>
<td>2,710.0 [1,479.2 - 40,227.0]</td>
<td>0.916</td>
</tr>
</tbody>
</table>

* Mann-Whitney rank-sum test performed.
the threshold cycle was plotted against the input target quantity, with the latter plotted on a common logarithmic scale. The linearity of the plot indicates that the threshold cycle value could be used to quantify the starting copy number of unknown samples over a wide dynamic range. There was a good correlation between the cycle number and the gene concentration in every assay \( (r \geq 0.99) \). After confirming the absence of dimmer formation in PCR, the authors calculated the number of SRY copies in each sample using Light Cycler System software. The system was able to detect the DNA equivalent from a single target cell. Positive amplification signals were seen in all tested samples subjected to a TaqMan assay for the beta-globin gene, thus confirming the quality of the DNA samples. This is accepted the control for the ability of serum-extracted DNA to be amplified.

Table 2 shows median cell free fetal, maternal, and total DNA concentrations in preeclamptic and control pregnancies. Cell free fetal DNA concentrations were higher in preeclamptic than control subjects \( (604 \ [413–821] \ vs \ 310 \ [100-423], p = 0.018) \). There was no statistically significant difference in each levels of maternal and total DNA between hypertensive and normotensive groups.

**Discussion**

The present authors are able to confirm that the level of cell free fetal DNA was significantly higher in pregnancies complicated by early preeclampsia than normotensive control group. This study also confirms that real-time quantitative PCR is a reliable and reproducible method for the detection and quantification of cell free fetal and maternal DNA [9]. The authors did not detect any false-positive or negative results. Obtaining quantitative information at the threshold cycle before the plateau phase is one of the main advantage of the real-time PCR against the conventional PCR [10]. This feature enables to determine target quantity and accurate and efficient method to PCR.

The definite mechanism of the releasing free extracellular DNA into the circulation is not clear, yet. However it has been hypothesized that fragments from apoptosis and some other form of cell deaths are the most suspected causes of the releasing free extracellular DNA into circulation [11]. Even release of DNA may be a part of physiological process; it would also be increased pathologically with increase cell death or necrosis. Obviously the amount of circulating DNA was generally found to be higher in patients with malignant disorders or injuries, who have higher rates of cell death or damage, than in healthy control subjects [12, 13].

Destruction of circulating fetal cells and liberation from placenta are the suspected sources of the cell free fetal DNA in maternal circulation. Current consensus, major component of cell free fetal DNA in maternal circulation is placental origin [14, 15]. Evidences like very rapid clearance of cell free fetal DNA against fetal cells after birth [16], postpartum presence of cell free fetal DNA in cases with placenta in crete [17] suggest theory of placental origin. The classical way of liberation of trophoblast material into maternal circulation is apoptotic release of syncyhtial knots [18].
Elevation in cell free fetal DNA in preeclampsia may help to increase understanding of enigmatic disorder. Although pathogenesis of preeclampsia is not clear yet, low placental perfusion, hypoxia, and uteroplacental ischemia by the impaired trophoblast invasion are focused mostly on the pathogenesis. It is also indicated that especially early-onset preeclampsia (before 34 gestational weeks) was associated with impaired placental invasion and abnormal placental morphology by studies which investigate placentas of preeclamptic cases morphologically [4]. Association between preeclampsia and increased apoptosis of cytotrophoblasts among placental bed has been reported in recent studies [19]. It is also well understood that placental hypoxia favours necrotic rather than apoptotic shedding of syncytiotrophoblasts into the maternal circulation [18]. In addition, cell free DNA has been accepted an indicator of hypoperfusion, tissue hypoxia and cell death [20, 21]. Thus it is suggested that the increased amounts of cell free fetal DNA is liberated from necrotic or apoptotic areas in the placenta [10]. The present authors though increased amounts of cell free fetal DNA in the maternal circulation in preeclampsia is an evidence of increased cell damage in placental bed caused by hypoxia and low perfusion which is very important for the pathogenesis of early preeclampsia.

The present authors could not detect statistically significant difference in cell free maternal DNA between preeclamptic and normotensive groups. In contrast to this study, several investigators indicated increase in the concentration of circulating cell free fetal and maternal DNA together in the plasma of women with preeclampsia, compared with normotensive women [7, 8, 22]. The present authors thought that they could not prove statistically significant difference probably because of the low number of patients in this study. Actually an important limitation of this study is the insufficient number of pregnant included.

The present findings suggest that the levels of cell free fetal DNA in maternal circulation were higher in pregnancies which are complicated with preeclampsia than normotensive controls. The present authors had poor number of patient inclusion and because of this they did not had a chance to compare their results with late preeclampsia cases. This was the major limitation to this study. However they believe that this study is valuable for future reviews with the content of fetal DNA PCRing in a subgroup of early preeclampsia population. Further studies which will compare the level of cell free fetal DNA between both early and late preeclampsia subgroups that have different etiopathogenesis will be helpful to explain pathogenesis of enigmatic disorder. Since cell free fetal DNA firstly detected in maternal circulation in 1997 [5], in such a short period, laboratories in some European Countries have already started to use cell free fetal DNA routinely as part of their prenatal testing for determining fetal RhD status of the RhD-negative pregnant patients [23]. When this rapid progress is considered, using cell free fetal DNA in the routine clinical practice of obstetrical complications like preeclampsia should not be a surprise.

References


Address reprint requests to:
M. M. SEVAL, M.D.
Ankara University School of Medicine
Department of Obstetrics & Gynecology
Tip Fakultesi Street, 001
Mamak, Ankara (Turkey)
e-mail: seval@ankara.edu.tr
Middle cerebral artery Doppler in prediction degree of fetal anemia and the best timing for the second intrauterine intravascular transfusion in red cell alloimmune disease

I. Babović1,2, S. Plešinac1,2, Z. Radojičić4, O. Antonović1,2, R. Sparić2, D. Plevaš1,2, N. Radunović1,2,3

1 School of Medicine, University of Belgrade, Belgrade
2 Department of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade
3 Serbian Academy of Sciences and Arts, Belgrade
4 Faculty of Organizational Sciences, University of Belgrade, Institute for Statistics, Belgrade (Serbia)

Summary

Aim: To determine the role of fetal multiples of the median of middle cerebral artery peak systolic velocity (MoM MCA-PSV), predicts the rate of decline in fetal hematocrit (Hct) for determination of the best timing for the second intrauterine intravascular transfusion (IUIVT) in fetuses with Rh alloimmunisation. Materials and Method: Retrospective study of 59-monofoetal alloimmunized pregnancies from 2005 to 2012 that underwent first and second IUIVT were assessed in Department of Gynecology and Obstetrics, Belgrade, Serbia. Result: There was an inverse statistically significant correlation between measurements MCA MoM-1 and fetal Hct-1 before the first IUIVT $r = -0.622; \ p = 0.001$ and MCA-MoM-3 and Hct-3 before the second IUIVT $r = -0.381; \ p = 0.001$, also as the significant correlation between the interval between both procedures (expressed in day) and measurement MCA-MoM-3, before the second IUIVT $r = -0.284; \ p = 0.029$. Conclusion: The measurements MoM-MCA before every IUIVT can be useful for prediction of the best timing for the next IUIVT.

Key words: Doppler; Hemolytic disease; Hydrops fetalis; Intrauterine intravascular transfusion; RhD antibodies.

Introduction

The introduction in the late 60s and 70s of prophylactic anti-D immunoglobulin (IG) for RhD negative women has changed the landscape of hemolytic disease of fetus and newborn (HDFN) and counts as one of the great of success stories in modern medicine. However, is a still remains a relevant pregnancy complication in some countries, mainly because of failure in prophylaxis [1].

Hemolytic anemia due to Rh D antibodies can be of different intensity. Therefore, diagnosing fetal anemia in a non-invasive and accurate way is fundamental [2-4]. Doppler assessment of the fetal middle cerebral artery (MCA) peak systolic velocity (PSV) has emerged as the best tool for predicting fetal anemia in at-risk pregnancies. It is based on the principle that the anemic fetus preserves oxygen delivery to the brain by increasing cerebral flow of low viscosity blood [5].

Standardized fetal hematocrit (z-Ht) was defined as the number of the standard deviations (SDs) from the normal mean for gestational age. Severe fetal anemia was defined as $z$Hct $\leq -5$ SDs. Mary et al. have proposed the cut off value for multiples of the median of middle cerebral artery peak systolic velocity (MoM MCA-PSV) of 1.29 for mild and one of 1.50 MoM for moderate, as finally MoM-MCA of 1.55 for severe anemia. These cutoff values results in 100% sensitivity, based on their retrospective analysis of 111 fetuses. The sensitivity of increased MCA-PSV above 1.5 MoMs for the prediction of moderate or severe anemia was 100 percent (95% CI 86-100), either in the presence or absence of hydrops [6].

The first fetal blood sampling was indicated in pregnancies with an antibody titer 1:64 Coombs (critical titer in the present laboratory), when $\Delta$ OD 450 reached Liley zone 3 or the upper zone 2, or MCA-PSV values of >1.50 multiples of the median (MoM). IUT is an effective treatment for severe fetal anemia. Perinatal loss occurs in about 1.6% of the procedures. Additional complications include thrombosis v. umbilicalis (includes emergency cesarean section), infection, and rupture of the membranes. [7, 8] Fetal medicine teams aim at optimizing the number of intrauterine intravascular transfusions (IUIVTs) and avoiding unnecessary procedures.

After the first IUIVT, most centers time subsequent procedures based on the expected rate of decline in fetal hemoglobin (Hb) or hematocrit (Hct) levels or MCA PSV measurements. Indeed, Scheier et al. showed that MCA PSV is useful in the prediction of fetal anemia in the second transfusion but less accurate for the third transfusion [3]. Possibly, this is due to hemodynamic changes induced
by the presence of transfused adult cells in the fetal circulation. Recent studies show an estimated fetal Hct drop of 0.7% to 1% per day. This parameter depends on the presence of fetal hydrops, because of the association between fetal hydrops and higher fetal Hct decline [9].

Since 1987, Department for Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade has been the national referral center for the management and intrauterine treatment of fetal anemia. The first IUIVT was performed on November 1987. In over 22 years, 498 IUIVTs were performed in 149 fetuses. The incidence of HBFN due to red cell Rh alloimmunization in Serbia is 1.5-2%.

The aim of the present study was to determine standardized MCA peak velocity MoM-MCA as a predictor fetal Hct decrease between first and second IUIVT, which indicated the best time for the second IUIVT for fetal anemia due to red-cell alloimmunization.

Materials and Methods

Fifty-nine monofetal Rh D alloimmunized pregnancies were retrospectively studied at the Department of Gynecology and Obstetrics, Clinical Center of Serbia, from January 2005 to January 2012. A computer database search was performed to identify all pregnancies with maternal Rh D alloimmunization that underwent first and second IUIVTs during the study period.

The ultrasound and Doppler examination was performed using an ultrasound scanner with 3.75 MHz curvilinear probe. Axial section of brain, including thalamus, cavita septi pellucidi was obtained and the Circle of Willis was identified. All Doppler measurements were performed with the angle between the ultrasound beam and the direction of the blood flow as close to 0° as possible and never exceeding 30°. If the angle was > 0°, an angle correction was applied. MCA-PSV measurements were performed before IUIVT (24 hours) and the day after (12-24 hours). The highest point of the flow velocity waveform (peak systolic velocity) was measured.

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The rate of fetal Hct fall after the first IUIVT was calculated by dividing the difference between the post-transfusion (post-2 Hct) and the pre-transfusion Hct (pre-Hct-3) at the second IUIVT and interval in days between both transfusions.

Hct decline (% / day) = post-transfusion (post-Hct-2) – pre-transfusion (pre-Hct-3) / time interval between IUIVT-1 and IUIVT-2

from the study the authors excluded: pregnancies submitted to only one IUIVT and cases in which post-transfusion or pre-transfusion blood samples were not obtained, multifetal pregnancies, intrauterine fetal demise and suspected fetal congenital malformations and fetal anemia due to other antibodies (C, Kell).

Results

During the study period, 59 singleton pregnancies underwent first and second IUIVTs to treat fetal anemia because of Rh alloimmune disease. Thirty-one women (52.54%) had previous history: 19 (32.2%) moderate titer (1:64) and 12 (4.83%) were with high (1:128) and of anti-D antibodies. Hydrops was present at the first IUIVT in 28/59 (47.45%) fetuses.

First IUIVT was performed at a mean gestation 26.46 (SD 3.9), and mean volume of blood transfused was 38.3 ml (SD16.8). The mean pre-transfusion Hct-1 was 24% (SD 9%), the mean post-transfusion Hct-2 was 41% (SD 8%). The mean pre-transfusion Hct-3 before second IUIVT was 27% (SD 7%) Mean interval to the second procedure was 12.19 (SD 6.04) days and average Hct decline rate 0.7% to 1% per day. This parameter depends on the presence of fetal hydrops.

The PSV-MCA decreased immediately after transfusion in 58 cases but no changed in one case. This study also showed that MCA MoM-1 before the first IUIVT exhibits inverse statistically significant correlate with pre-transfusion Hct-1 r = - 0.622; p = 0.001 and MCA-MoM-2 with post-transfusion Hct-2 r = 0.512; p = 0.001. The study also exhibited a significant correlation MCA-MoM-3 with.
pre-transfusion Hct-3 before the second IUIVT. \( r = -0.381; p = 0.001 \). The sensitivity of pre-transfusion MCA-MoM-1 for severe anemia before the first IUIVT was 100% (95% CI for difference 78.12 - 100%) and the sensitivity of pre-transfusion MCA-MoM-3 before the second IUIVT was 88.89% (95% CI for difference 63.93 - 98.05%). The specificity MCA-MoM-3 before the second IUIVT was 51.22% (95% CI for difference 35.37 - 66.85), slightly higher than the specificity MCA-MoM-1 before the first IUIVT was 24.39% (95% CI for difference 12.91 - 40.64).

Pearson’s test demonstrated that mean decline rate in fetal Hct levels between first and second IUIVT (expressed in percentage/day) insignificant correlate with post-transfusion Hct level (Hct-2) \( r = -0.047; p = 0.755 \) and pre-transfusion Hct-3 \( r = -0.012; p = 0.939 \). There were also insignificant correlations between the mean decline rate in fetal Hct levels and measurements MCA-MoM-2 \( r = 0.109; p = 0.469 \) after the first IUIVT as so as between the mean decline rate in fetal Hct levels and MCA-MoM-3 before second IUIVT \( r = -0.038; p = 0.804 \).

The study documented significant negative correlation between the interval between both procedures (expressed in day) \( T \) and measurement MCA MoM-3 before the second IUIVT \( r = -0.284; p = 0.029 \). *T-interval between both procedures (expressed in day). **PSV-MCA MoM-3 before the second IUIVT/ (multiples of the median of middle cerebral artery peak systolic velocity).

**Discussion**

IUIVTs for severe fetal anemia are performed between 19 to 34 weeks of gestation in the present Department (Table 1). Before 18 weeks, fetal transfusions are rarely successful due to limited visualization and small size of the relevant anatomic structures [11]. As Plećaš et al. have reported [10] after 34 weeks, the procedure is generally considered riskier than late preterm delivery and neonatal treatment of severe anemia.

As Egberts et al. reported [12], significant correlation of mean decline rate and time interval between both procedures mean the study was not determinated. In fact, it has been shown that transfused adult red cells destruction in nonlinear fashion, possibly reflecting mechanical effects of hemoconcentration, and biochemical effects of a fetal circulation on transfused adult red cells and membranes. Besides this, adult red cells are supposed to have a reduced lifespan in more severely anemic fetuses. Sumacher et al. [13], and Egberts et al. [14] have hypothesized that more fetal red cells disappear during the first days after the transfusions.

The most centers that treat alloimmunized pregnancy consider an average decline rate of 0.3 to 0.4 g/dl Hb per day, to calculate the expected fetal hemoglobin concentration at the time of the second transfusion [9]. In the present study, the authors notice that the average decline rate of 1.13 %/Hct per day. Scheier et al. [3] have reported that MCA PSV exhibits a significant correlation with fetal hemoglobin concentration before the first and before the second IUIVT. However, the present study confirmed statistical signifi-
cant correlations between MCA MoM-1 and fetal Hct-1 before the first IUIVT, as so as between MCA MoM-3 and fetal Hct-3 before the second IUIVT. It can be useful to time subsequent transfusions. As Nishie et al. [15] have reported studies on the evaluation of fetal myocardial performance may help establish the best moment for fetal treatment before myocardial function is affected. Radunović et al. [16] previously reported that volume of blood to be transfused could increase fetal Hct and viscosity. The fetal cardiac failure would be eventually developed [17]. Nishie et al. [15] showed that MCA Doppler prediction performance is slightly lower in subsequent transfusions compared with first time transfusions. In the present study, the authors found a sensitivity of pre-transfusion MCA-MoM-1 for severe anemia before the first IUIVT was 100% and the sensitivity of pre-transfusion MCA-MoM-3 before the second IUIVT was 88.89% in the prediction of severe fetal anemia (cut-off value for severity anemia MCA MoM ≥ 1.5). The present study documented that MCA Doppler can be useful to predict severity of fetal anemia before the first as before the second IUIVT. The sensitivities are always lower in the prospective than in the retrospective series.

The study did not demonstrate statistical significance between MCA-MoM-3 before the second IUIVT and the mean decline rate in fetal Hct levels between first and second IUIVT (expressed in percentage/day). Hct value is described as relative number or percentage, as well time interval in day. The present authors documented that all of three variables are correlated (Hct-2), (Hct-3), and the third MCA MoM insignificant correlate to interval between both procedures (expressed in day), as well as the fetal hematocrit mean decline rate insignificant correlate to interval between transfusions. During IUIVT we documented changes only in fetal hematocrit, although after IUIVT a relatively large fetal blood volume exists in the vascular space, the fetal blood volume does not change in 24 hours. It is known though, that through loss of plasma from fetal circulation, after packed red cell transfusion, the fetal blood volume increases by only half of the transfused volume As Loboto et al. reported [9] in cases of severe fetal anemia, the amount of blood that can be transfused is limited by fetal tolerance to volume overload and fetal hemoglobin concentration after treatment may still be below the optimal level The mean decline rate in fetal Hct levels between first and second IUIVT and the volume of blood to be transfused insignificant correlate as well as the volume of blood to be transfused and interval between both procedures (T).

Although an estimated fetal Hct decline of approximately 1% / day, as in the present study, this parameter is quite variable and independent of the volume of blood to be transfused. The present authors supported the results of study Mary et al. [6] that the optimal interval between Doppler examination for MCA PSV has not been determined, but appears to be one or two weeks. As Steel et al. [18] reported, the wide range in pre-transfusion MCA MoM for fetuses with identical Hct, other factors such as, blood viscosity, cardiac output, and peripheral resistance must play an important role in determination MCA peak. Scheier et al. [3] confirmed that more precise predictive models have been investigated. Unexpectedly, the present study showed the significant negative correlation between MCA-MoM-3 before the second IUIVT and the time interval between both procedures (T) (Figure 1). The present authors think that may not be a rule, but it can be useful for future investigations. The present study involving 47% hydropic fetuses with low pre-transfusion Hct and high post-transfusion Hct values, should be candidates for shorter interval between two measurements MCA-MoM than the timing approximately 12 days between the first and second IUIVT, as we documented in the study.

In the present Department, in cases of severe fetal anemia, ultrasound-directed fetal blood sampling (ie, cordocentesis) allows direct access to the fetal circulation to obtain important laboratory values such as Hct, fetal blood type, reticulocyte count, and platelet count. Doppler velocimetry were performed initially before the first and before each IUIVT can be usefully for determine the best timing for the next procedure.

Conclusion

An increasing fetal Hct after IUIVT has effect on maximum MCA PSV. The present study documented high sensitivity MCA-MoM before the first and second IUIVT in predicting severity of the fetal anemia. The wide range in pre-transfusion MCA-MoM, for fetuses with identical Hct, exist, besides other factors must determine MCA peak or MoM-MCA. The measurements MCA MoM before every IUIVT can be useful for determining severity of fetal anemia as well in predicting the best time interval for the next IUIVT.

Acknowledgement

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References

Middle cerebral artery Doppler in prediction degree of fetal anemia and the best timing for the second intrauterine intravascular etc.


Address reprint requests to:
I. BABOVIC, M.D.
63/27 Luke Vojvodica Street
Belgrade (Serbia)
e-mail: ivana.r.babovic@gmail.com
Application of acoustic radiation force impulse imaging (ARFI) in quantitative evaluation of neonatal brain development

Y. Su1, J. Ma2, L. Du1, J. Xia1, Y. Wu1, X. Jia1, Y. Cai1, Y. Li1, J. Zhao1, Q. Liu1
1 Department of Ultrasound, Shanghai First People’s Hospital, Affiliated Shanghai JiaoTong University, Shanghai
2 Department of Cardiovascular Ultrasound, Shanghai East Hospital Affiliated to Tongji University, Shanghai (China)

Summary
Objective: This study aims to quantitatively evaluate the effect of acoustic radiation force impulse imaging (ARFI) in neonatal brain development. Materials and Methods: The authors observed 41 neonatal brain different tissues by using traditional two-dimensional gray scale ultrasound and color Doppler flow imaging and frequency spectrum ultrasound. After that they used ARFI to quantitative evaluate white and gray matter of neonatal different tissues in brain with different gestational ages. They also used new technical index: virtual touch tissue quantification (VTQ) to evaluate elastic changes of brain tissues. Results: Different tissues in brain had different elastic numerical values. Neonatal with different gestational ages had different elastic numerical values. The more gestational ages were, the more the elastic numerical values. Elastic numerical values between preterm and full-term infants were different. Elastic numerical values of full-term infants were higher than preterm infants. Conclusion: ARFI provides a new quantitative index to evaluate neonatal brain development. It increases objectivity and reliability of clinical analysis. Ultrasound was a noninvasive examination method, safe, simple, and convenient, and it has more usefulness of ARFI in quantitative evaluation of neonatal brain development.

Key words: Neonatal; Brain development; Acoustic radiation force impulse imaging.

Introduction
Neonatal brain tissue and the function is in the process of development, and as a result of the existence of a variety of diseases, brain development can be blocked or brain damage, cause the functional and structural abnormalities, clinical manifestation of cerebral palsy, epilepsy and other neurological diseases [1-3]. Neonatal head ultrasound in neonatal cerebral disease diagnosis is of great significance, through analyzing the characteristics of the cerebral ultrasound, observation of brain volume size, the width of the gyri and craniocerebral overall the echo intensity, can evaluate neonatal brain development mature conditions, and change with gestational age can present different characteristics [4-7]. However, how to quantify the change to guide the evaluation of the degree of brain development is still a clinical problem remaining to be solved [8-10]. Acoustic radiation force impulse imaging (ARFI) is a good method for us in evaluating the maturity of infants’ brain. Now ARFI has been widely used in liver, kidney, thyroid, breast, pancreas and so on [11-16]. However, ARFI has been little used in brain. This article analyzed quantitatively neonatal cerebral white and gray matter with different gestational ages by Virtual touch tissue quantification (VTQ) of ARFI, and discussed the value of VTQ to evaluate neonatal brain development.

Materials and Methods
Patients
Forty-one consecutive newborns were selected from the department of gynecology and obstetrics of First Hospital Affiliated to Shanghai Jiaotong University from March 2012 to October 2012; male 22 cases, female 19 cases, gestational ages < 37 weeks 16 cases, ≥ 37 weeks 25 cases. All neonates were appropriate for gestational ages (AGA). The exclusion criterion were jaundice of the newborn, aspiration pneumonia, neonatal brain injury and pregnancy complicated with diabetes mellitus, pregnancy induced hypertension, anemia and heart and lung diseases, et al. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Shanghai First People’s Hospital, School of Medicine, Shanghai Jiaotong University. Written informed consent was also obtained from all participants before ARFI examination.

Acquisition of the ARFI
Real-time ARFI was performed by using diagnostic ultrasound system equipped with 4C1 (3.5 MHz) probe. All the examinations were performed in succession by two independent sonographers. Both of them had more than ten years’ experiences in ultrasonic scanning. They were blinded to the colposcopic findings and physical exam results when performing.

Conventional ultrasound
Neonates that were born in one to three days were placed in a supine position in a quiet state. All participants were examined by brain US using the anterior fontanelle as the acoustic window. Conventional sonography was used to observe skull continuity, midline brain was centered, echo of brain parenchyma, lateral
ventricle, cerebral transverse diameter, lateral ventricle width, and superior frontal gyrus width were measured. Color Doppler was used to access the blood supply of the brains. Including middle cerebral artery, anterior cerebral artery, posterior cerebral artery and vertebral basilar artery.

ARFI
Switching to the ARFI elastic model, VTQ was used to measure the elasticity of neonatal cerebral white and gray matter, which included parietal white matter, thalamic and basal ganglia, cerebellum, a hippocampus, medulla oblongata, and cerebral falx.

Virtual touch tissue quantification
In the VTQ model, ROI (5×10 mm) was placed inside the brain and the depth of the regions of interest (ROI) was less than 80 mm. All the structures of newborns brain were observed. For more accurate and objectively derived elastic parameters, operators were asked to handle with care and probe on the cranial surface. It was continuously measured three times randomly, and the average value was calculated as the VTQ value (meter per second). Care was taken to avoid vascular structures when measured.

Statistical analysis
All statistical analysis used SPSS version 17.0 software. All measured data are presented as the mean ± standard deviation. Groups of premature infants and full-term infants were compared using the Student U test and Analysis of variance. A p < 0.05 was considered statistically significant.

Results

Echo intensity quantitative analysis
The authors used VTQ to quantitative analysis echo intensity in different parts of brain tissues. It showed that the value of VTQ of parietal white matter was 1.34 ± 0.33 m/s, thalamic and basal ganglia were 1.53 ± 0.35 m/s, cerebellum was 1.62 ± 0.31 m/s, and cerebral falx was 2.23 ± 0.48 m/s (Figure 1A-C).

Table 1. — Comparison of VTQ between preterm and full term infants.

<table>
<thead>
<tr>
<th>Position</th>
<th>VTQ (m/s)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal white matter</td>
<td>1.12 ± 0.43</td>
<td>1.34 ± 0.33</td>
</tr>
<tr>
<td>Thalamic</td>
<td>1.21 ± 0.51</td>
<td>1.53 ± 0.35</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.33 ± 0.41</td>
<td>1.62 ± 0.31</td>
</tr>
<tr>
<td>Cerebral falx</td>
<td>1.95 ± 0.52</td>
<td>2.23 ± 0.48</td>
</tr>
</tbody>
</table>

Figure 1. — The value of VTQ of different parts of brain tissues in full-term infants. A) The value of VTQ of parietal white matter in full-term infants was 1.26 m/s; B) the value of VTQ of thalamic and basal ganglia in full-term infants was 1.46 m/s; C) the value of VTQ of cerebellum in full-term infants was 1.65 m/s.

Figure 2. — The value of VTQ of cerebellum in preterm infants was 0.84 m/s.

Brain echo intensity
The authors measured the value of VTQ of echo intensity in different parts of brain tissues in different gestational ages of 41 infants; it showed that the value of VTQ of parietal white matter, thalamic and basal ganglia, cerebellum and cerebral falx gradually increased with increased gestational ages. The value of VTQ of preterm infants were obviously lower than full term infants (p < 0.05) (Table 1, Figure 2).

Discussion
ARFI is a new elastic imaging technology, it uses acoustic radiation force to fire pulse, and it can cause instantaneous (<1 s) and tiny micron displacement in ROI of the body. At the same time, it produces shear wave at transverse vibration. The computer can test and calculate the speed of shear wave, which is called shear wave velocity and shear wave velocity is on behalf of the elasticity of tissue. The higher the
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shear wave velocity is, the larger the coefficient of elasticity is. This is VTQ of ARFI. It does not rely on the pressure of the probe, so it has more objectively [17-21]. This technology can be utilized to quantitative evaluate the echo intensity of neonatal brain.

From the present study, brain tissue of different parts of echo intensity quantitative analysis in full-term infants showed that the value of VTQ of parietal white matter was 1.34 ± 0.33 m/s, thalamic and basal ganglia was 1.53 ± 0.35 m/s, cerebellum was 1.62 ± 0.31 m/s, and cerebral falx was 2.23 ± 0.48 m/s. The proportion of increase was accordance with brain development. It can be more intuitive to understand the situation of echo intensity in different brain tissues.

The brain development originates in ectoderm. Myelination of brain takes place at about 20 weeks of gestational ages. From the bottom to top, from central to peripheral, from dorsal to ventral, from sensory fibers into motor fibers. That is to say, the development of myelination of brain is from brain stem to thalamic and basal ganglia to each lobe of brain. It suggests that development of thalamus and cerebral falx were more early and mature [22, 23].

The present results show that different brain echo intensity occur at different gestational ages. The value of VTQ in preterm infants were obviously lower than that of in full term infants. Brain development in preterm infants are more immature than full term infants [24, 25].

Neonatal head ultrasound is with the aid of before and after anterior fontanelle and lateral anterior fontanelle as the acoustic window, for cerebral coronal and sagittal scans. Its advantages include bed check, no radiation damage and low cost, do not need to be calm, can be repeated many times, and is the first choice for the premature infant brain injury. Its disadvantages are the need of the operator to have experience and has certain limitations of craniocerebral peripheral lesions. Therefore, it remains to be combined with other imaging examination methods such as MRI [26, 27].

In conclusion, the value of VTQ is a very useful quantitative index in evaluation of the neonate brain development. ARFI is a safe, noninvasive, simple, and convenient technology; it can play a greater role not only in evaluation of the neonate brain development, but in diagnosing diseases in neonate brain, including preterm, and full term infants, and will be the topic of the present authors’ future study. While AFRI is still in the preliminary clinical application stage, more cases must be observed to accumulate experience.

Acknowledgements

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References

[1] Salmaso N., Tomasi S., Vaccarino FM.: “Neurogenesis and matura-
roimaging in predicting neurodevelopmental outcomes of preterm
D.E., Henderson J.L. et al.: “Prenatal antecedents of newborn neu-
rological maturation”. Child Dev., 2010, 81, 115.
rodevelopmental outcome of premature infants”. Am. J. Perinatol.,
2010, 27, 803.
trasound findings in neonates treated with intrauterine transfusion for
P.W., Shen S. et al.: “Neonatal head ultrasound abnormalities in
preterm infants and adolescent psychiatric disorders”. Arch. Gen.
sonographic diagnosis and treatment”. Paediatr. Drugs., 2012, 14,
143.
[10] Nelly F.P., Goya E., Tomas J., Gratacos E., Hernandez-Andrade E.:
“Quantitative Tissue Echogenicity of the Neonatal Brain Assessed
of cervical cancer detection with acoustic radiation force impulse
acoustic radiation force impulse imaging for the clinicopatholog-
“The application of Virtual Touch tissue quantification (VTQ) in
diagnosis of thyroid lesions: a preliminary study”. Eur. J. Radiol.,
2013, 82, 797.
G.V. et al.: “Evaluation of ultrasound based acoustic radiation force
impulse (ARFI) and eSie touch sonoelastography for diagnosis of
sults of acoustic radiation force impulse (ARFI) ultrasound imaging
[16] Huang M.T., Ping Z., Ying Q., Li-Rong C., Ping Z., Rui-Zhen L.:
“Usefulness of acoustic radiation force impulse imaging in the dif-
ferential diagnosis of benign and malignant liver lesions”. Acad. Ra-
diol., 2011, 18, 810.
radiation force-induced displacements”. IEEE Trans. Ultrason. Fer-
radiation force impulse imaging with real-time measurement of pla-
dental elasticity: acoustic radiation force impulse imaging”. Placenta,
2013, 34, 1009.


Address reprint requests to:
L. DU, M.D.
Department of Ultrasound
Affiliated Shanghai JiaoTong University
No. 1878 Sichuan North Road
Shanghai 200080 (China)
e-mail: yjlfcn@126.com
Interleukin-6 and C-reactive protein levels in the amniotic fluid as indicators of preterm delivery in Turkish women

M. Öz, B. Polat, E. Ö zgü, K.D. Seçkin, C. Taşın, N. Danişman
Zekai Tahir Burak Women’s Health Hospital, Ankara (Turkey)

Summary
Objective: The aim of this study was to determine the value of amniotic fluid interleukin-6 (IL-6) and C-reactive protein (CRP) levels in the prediction of preterm delivery in singleton pregnancies without any known risk factors for preterm delivery in Turkish women. Materials and Methods: Patients in the present perinatology department who underwent mid-trimester genetic amniocentesis due to evidence of increased risk of aneuploidy in their prenatal serum screening tests were included in the study. A sample of amniotic fluid from each patient was assessed for IL-6 and CRP. Concentrations of IL-6 and CRP in the amniotic fluid of preterm delivery and term delivery groups were compared. Results: Of 151 singleton pregnancies, 142 participants were included in the study. The participants were assigned to either the preterm or term delivery group based on pregnancy outcome. IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group, and there was a statistically significant negative correlation between IL-6 concentrations in the amniotic fluid and gestational age at delivery (correlation coefficient (CC): -18.5%, \( p < 0.05 \)). A negative correlation was also detected between CRP levels in the amniotic fluid and gestational age at delivery, but the correlation was not statistically significant (\( p = 0.068 \)). Conclusion: Measuring IL-6 in the amniotic fluid can identify women at risk of preterm delivery. Because it is not acceptable to perform amniocentesis for this screening, it is more convenient for patients in whom genetic amniocentesis is performed.

Key words: Preterm delivery; Interleukine-6; C-reactive protein; Midtrimester amniocentesis.

Introduction

Preterm birth, defined as delivery that occurs prior to 37 weeks of gestation, accounts for approximately 10% of all pregnancies [1]. Prematurity and prematurity-related complications are the major cause of infant mortality and morbidity [2, 3]. The survival rate of a premature infant depends primarily on birth weight and gestational age. The survival rate is greater than 90% after the 30th gestational week, but less than 10% before the 24th week [4]. Preterm parturition is a syndrome rather than a diagnosis, as it encompasses several different conditions. Preterm deliveries prior to 32 weeks of gestation frequently involve infections, infant morbidity, and higher long-term sequel risk, and they tend to recur in subsequent pregnancies.

Bacterial infection is one of the most important mechanisms related to preterm delivery [5,6]. It has been shown that infections are responsible for at least 35–40% of all preterm deliveries. Bacterial chorioamnionitis is the main cause of infection-related preterm labor. Amniotic fluid is supposed to be sterile, and bacteria are detected in less than 1% of women at term who are not in active labor. The presence of bacteria in the amniotic cavity is a pathologic condition known as microbial invasion of amniotic cavity (MIAC). Most MIAC cases are subclinical and cannot be diagnosed unless a microbial study of the amniotic fluid is performed. MIAC alone is not sufficient reason to promote preterm labor, as bacterial presence in the chorioamniotic membranes does not always induce a maternal and/or fetal inflammatory response [7].

An elevated level of interleukin-6 (IL-6) in the amniotic fluid is an indicator of an inflammatory process in the amniotic cavity and, in most cases, is related to bacterial infection [8-11]. IL-6 is released from either T-lymphocytes or macrophages, and it can act as both proinflammatory and anti-inflammatory cytokines. IL-6 is one of the most important cytokines that play a major role in acute phase response and fever pathogenesis. C-reactive protein (CRP) is a prototype for acute phase reactants. It is named C-reactive protein because it binds to the “capsule” antigen of pneumococci [12]. In fact, CRP binds to the phosphocholine on the surface of most bacteria, fungi, and parasites and causes complement activation to eliminate the circulating antigens [13]. High-sensitive CRP (hs-CRP) is a more accurate method of quantifying CRP that is sensitive to concentrations lower than 0.4 mg/L. Based on this information, the purpose in this study was to predict preterm delivery rates in the antenatal population without any known risk factors for preterm delivery by measuring IL-6 and CRP levels in the amniotic fluid.
Materials and Methods

For this prospective study, amniotic fluid samples were collected from patients who underwent mid-trimester genetic amniocentesis in the perinatology department of the present tertiary care hospital between January and March 2011. Clinical data were approved by the Institutional Review Board of our hospital. All of the patients provided written informed consent in accordance with the Helsinki criteria.

All amniocentesis procedures were performed under transabdominal ultrasound guidance, and in all cases, detailed ultrasonography was performed to assess the fetal anatomy and determine the location of the placenta. The amniocentesis procedures were performed via a 21-gauge amniocentesis needle. The first one to two cc of amniotic fluid were discarded to avoid possible blood contamination; the following 18–20 cc were used for the genetic study; and the last two cc were used to determine IL-6 and CRP levels. The amniotic fluid samples were stored at -80°C until analysis.

The inclusion criteria were as follows: singleton pregnancy; age 18–45 years; mid-trimester genetic amniocentesis. Exclusion criteria included: known systemic disease or infection; use of antibiotics for any reason in the previous month; major fetal malformation detected in the ultrasound examination prior to amniocentesis; abnormal fetal karyotype; pregnancy loss during the first month after the amniocentesis (possibly procedure related); iatrogenic preterm delivery due to maternal or fetal indications such as preeclampsia, intrauterine growth restriction, gestational diabetes, or amniotic fluid disorders;

Pregnancy outcomes were obtained by accessing labor and delivery records or by contacting the patient if the delivery was not in the present hospital. IL-6 quantitative measurements were performed with an immunoassay system, and hs-CRP quantitative measurements were performed with a chemistry analyzer using a Tina-quant cardiac C-Reactive protein high-sensitivity kit.

Statistical analysis

Results are expressed as mean and standard deviation according to the distribution of data. Kolmogorov Smirnov’s test was used to evaluate the normality of the distribution of the continuous data. The Mann–Whitney U test, Student’s t test, and Spearman’s rho correlation test were used according to the distribution of the variables (Mann-Whitney U test for continuous variables without normal distribution, and Student’s t test for normal distributed variables) for the comparison and correlation of proportions. The diagnostic value of IL-6 and CRP levels for the prediction of preterm delivery was evaluated using receiver operating characteristic (ROC) curves. Sensitivity, specificity, and negative and positive predictive values were calculated for optimal cutoffs. The data were analyzed using SPSS 17 software.

Results

Amniocentesis was performed on 151 pregnant women with singleton gestations who met the inclusion criteria; the amniotic fluid samples were studied immediately after the procedure. Amniocentesis was performed when there were proper clinical indications, such as suspected fetal anomalies, family history of chromosomal abnormalities, advanced maternal age, and abnormal first or second trimester screening test. Nine patients were excluded from the study: two patients for intrauterine exitus, one patient for immune hydrops fetalis, three patients for undergoing a cesarean section due to a diagnosis of severe preeclampsia prior to 37 weeks of gestation, and three pregnancies were terminated due to diagnoses of Down syndrome according to amniocentesis. Thus, 142 patients’ results were analyzed in this study.

The demographic and clinical characteristics of the subjects are shown in Table 1. The participants were 18–42 years of age (mean 31±6.25); 61.9% were over 30 years old. The authors found no correlation between maternal age and gestational age at delivery. Total gestational duration was 245–297 days according to last menstrual period or first trimester ultrasound imaging (mean gestational age 272±10 days). Fifteen patients (10.56%) who delivered prior to 259 days formed the preterm delivery group; 127 patients (89.44%) delivered at term. The preterm delivery rate in the present study was similar to that reported in the literature [14].

IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group than in the term delivery group. No correlation was found between CRP levels in the amniotic fluid and preterm delivery (Table 2).

There was a statistically significant negative correlation between IL-6 concentration in the amniotic fluid and gestational age at delivery (correlation coefficient (CC): -18.5%, p < 0.05). A negative correlation was detected between CRP levels in the amniotic fluid and gestational age at delivery, but the correlation was not statistically significant (p = 0.068).

### Table 1. — Demographic and clinical features of the subjects.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Preterm delivery</th>
<th>Term delivery</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (wks)</td>
<td>32.6 ± 6.1</td>
<td>31.1 ± 6.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.4 ± 1.2</td>
<td>2.1 ± 0.9</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*p Mann-Whitney U Test.

### Table 2. — IL-6 and CRP levels in the amniotic fluid in the preterm and term delivery groups.

<table>
<thead>
<tr>
<th></th>
<th>Preterm delivery</th>
<th>Term delivery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>0.15 ± 0.03</td>
<td>0.10 ± 0.009</td>
<td>0.527</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>473 ± 346</td>
<td>313 ± 208</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*p < 0.05, Mann–Whitney U Test.

### Table 3. — Coordinates for sensitivity, specificity, PPV, and NPV to predict preterm delivery using IL-6 levels in the amniotic fluid.

<table>
<thead>
<tr>
<th>IL-6 threshold level (pg/ml)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>NPV %</th>
<th>PPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>228.5</td>
<td>93.3</td>
<td>52.8</td>
<td>98.5</td>
<td>18.9</td>
</tr>
<tr>
<td>360.5</td>
<td>80</td>
<td>72.4</td>
<td>96.8</td>
<td>25.5</td>
</tr>
</tbody>
</table>

*Mann-Whitney U Test.*
Interleukin-6 and C-reactive protein levels in the amniotic fluid as indicators of preterm delivery in Turkish women

If a 360.5 pg/ml threshold was determined for IL-6 level in the amniotic fluid, preterm delivery would then be predicted with 80% sensitivity and 72.4% specificity with 95% confidence interval (CI). Positive predictive value (PPV) and negative predictive value (NPV) would then be 96.8% and 25.5%, respectively. Preterm delivery would be predicted with 93% sensitivity and 52.8 specificity with 95% CI if the threshold level was determined at 228 pg/ml for IL-6 level in the amniotic fluid. PPV and NPV would then be 18.9% and 98.5%, respectively (Figure 1 and Table 3).

Discussion

Prematurity and prematurity-related neonatal complications are known to be the most important reasons for neonatal mortality and morbidity in the past decade. Various markers and methods that can be measured during the antenatal period have been put forward to prevent prematurity and prematurity-related neonatal complications. Inflammatory cytokines, especially IL-6, have become more of an issue recently, and recent studies have suggested that IL-6 is a promising marker for predicting preterm delivery [15, 16]. In these studies, not only were IL-6 concentrations in the amniotic fluid significantly higher in the preterm delivery group than in the term delivery group, but they also showed a negative correlation with gestational age. Cobo et al. stated the superiority of amniotic fluid IL-6 levels to predict microbial invasion of amniotic fluid as an inflammatory biomarker [17].

According to the present literature search, 32 studies in the literature associated IL-6 levels in the amniotic fluid with preterm delivery; however, only six of the studies investigated second-trimester singleton pregnancies [16, 18-21]. One of the most powerful and well-designed studies was conducted by Wenstrom et al. in 1998 [19]. In that study, the researchers determined a cutoff level of 250 pg/ml second trimester amniotic fluid IL-6 to predict preterm delivery. In the present study, if the cutoff level was set at 228 pg/ml, the sensitivity of IL-6 for predicting preterm delivery was calculated as 93.3% (14 out of 15 preterm pregnancies). These data are also similar to the study published by Thomakos et al. in 2010 [16]. Cobo et al. reported 46% sensitivity and 93.8% specificity to predict preterm delivery, as well as 52.6% NPV and 92% PPV, when the cutoff level for amniotic fluid IL-6 level was determined to be 134 pg/ml [22]. Kim et al. showed significantly higher levels of amniotic fluid IL-6 levels in midtrimester amniocentesis for preterm delivery group and determined the cut-off value of 134.35 pg/ml with a sensitivity of 77.8% and a specificity of 61.1% for preterm delivery, slightly lower than the present cut-off value [21]. This may be due to higher overall amniotic fluid IL-6 levels in Turkish women. However, cytokine response to the inflammatory process may be different for each individual; some individuals may not show a cytokine response to inflammatory processes as others do.

There are fewer published studies regarding the relationship between CRP levels in the amniotic fluid and preterm delivery. The present research of the literature identified only two published studies on mid-trimester CRP levels in the amniotic fluid and preterm delivery [23, 24], only one of which was designed as a prospective study [23]. Özer et al. reported 92.9% sensitivity and 78.7% specificity with 99% NPV and 32.5% PPV for predicting preterm delivery if a 0.65-mg/L cutoff value was set for CRP concentration in the amniotic fluid. In addition, Ghezzi et al. reported that CRP concentrations in the amniotic fluid were found to be higher in the preterm delivery group [24]. However, several studies in the literature found no significant correlation between amniotic fluid CRP concentrations and preterm delivery, as in the present study [25]. These findings suggest that the inflammatory process of the fetomaternal unit is more important than the maternal systemic inflammatory response in the etiology of preterm delivery. Özer et al. suggested that the high-risk population for preterm delivery could be predicted by determining mid-trimester CRP levels in the amniotic fluid, and that proper precautions could take months before delivery. Other reports in the literature concluded that CRP is a commonly used laboratory parameter predicting inflammatory response, but measurement of CRP is very non-specific and therefore unreliable in predicting preterm labour [21, 23, 26].

Conclusion

The main purpose of the present study was to predict preterm delivery with a reasonable sensitivity and specificity.
in the antenatal population without any risk factors for preterm delivery. For this purpose, the authors examined IL-6 and CRP levels in amniotic fluid. Determining IL-6 and CRP levels in amniotic fluid for genetic investigation is low in cost, and it is an easy procedure to perform; it increases the cost of the second trimester genetic amniocentesis procedure by only 2–3%. While some studies in the literature have shown that IL-6 and CRP levels in amniotic fluid are useful for predicting preterm delivery, other studies have indicated that these markers are useless for this purpose. In the present study, IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group. CRP levels in the amniotic fluid were also higher in the preterm delivery group, but the difference was not statistically significant. In addition, in the preterm delivery group, amniotic fluid IL-6 levels increased as gestational age at delivery decreased. According to the results of the present study, IL-6 measurement in the amniotic fluid seems to be an appropriate screening test to exclude preterm delivery rather than a predictor, because it has a higher sensitivity and NPV; amniotic fluid IL-6 study may be reasonable for patients in whom genetic amniocentesis is performed. In conclusion, further prospective studies are needed to detect a more solid relation between IL-6 levels in amniotic fluid and preterm delivery.

References

Antenatal counseling against passive smoking may improve birth weight for gestational age

O.B. Akkar1, C. Yildiz1, S. Karakus1, I. Akkar2, A. Cetin1, A. Yanik1, A.G.I. Yenicesu1, A. Boztosun1

1 Departments of Obstetrics and Gynecology, Cumhuriyet University Faculty of Medicine, Sivas
2 Department of Pediatrics, Sivas State Hospital, Sivas (Turkey)

Summary

Aim: The authors determined the impact of antenatal counseling against exposure to environmental cigarette smoke on the prevention of reduced neonatal birth weight according to gestational age. Materials and Methods: A cross-sectional study was conducted in pregnant women, with 77 passive smokers and 88 non-smokers. During motivational interviews, passive smoking status was monitored and additional follow-up visits were arranged to increase the knowledge regarding perinatal risks of passive smoking, including intrauterine growth restriction and low birth weight. The authors aimed to increase the woman’s motivation to avoid second-hand tobacco smoke exposure. Results: The demographic and clinical findings of the study groups were found considerably similar, in this context, and the authors found positive and strong correlations between the gestational age and neonatal birth weight (r = 0.80 and r = 0.76, respectively; p < 0.05). Conclusions: During antenatal care of women, regular counseling against second-hand smoke exposure may prevent negative effect of passive smoking on neonatal birth weight according to gestational age. This promising finding needs to be supported by further studies with larger sample size considering covariates relevant to passive smoking.

Key words: Passive smoking, pregnancy; Antenatal care; Neonatal birth weight.

Introduction

Smoking during pregnancy is one of the leading preventable causes of adverse maternal and perinatal outcomes. Maternal cigarette smoking during pregnancy is associated with many perinatal complications such as congenital anomalies, placental abruption, placenta previa, preterm birth, and even infant mortality [1-4]. Smoking during pregnancy is accepted as an important risk factor for low birth weight (LBW) [5]. Exposure to environmental tobacco smoke also causes similar adverse maternal and perinatal consequences [6].

In a recent review evaluated the association of passive smoking and neonatal birth weight, the authors summarized the findings of pertinent literature and concluded that maternal exposure to environmental smoke decreases the neonatal birth weight in addition to its several other adverse effects [2]. In a study investigating the independent effects of maternal exposure to second-hand smoke and maternal body mass index on the anthropometric measurements of term infants and on the prevalence of macrosomia and low birth weight, the authors found an increased risk of low birth weight and decreased risk of macrosomia in women who were exposed to second-hand smoke in the same body mass index category [7]. There are several previous studies that provided comparable findings consistent with those findings [8-12].

Similar to other reproductive health problems, there is paucity of information regarding the magnitude of passive smoking during pregnancy on the prevention of reduced neonatal birth weight according to gestational age and long-term impacts, and about interventions to reduce its negative influences on general health of pediatric population. According to the present authors’ knowledge, there is no study investigating the effect of antenatal counseling related to prevention of environmental tobacco smoke exposure to improve birth weight for gestational age. In this context, they attempted behavioral counseling against exposure to environmental cigarette smoke, including increasing awareness of the dangers of exposure to cigarette smoke, motivating the mother to avoid from the environment cigarette smoke and to ensure that she recognized and understood the reasons to change her living conditions. The aim of this study was to evaluate the effect of antenatal counseling against exposure to environmental cigarette smoke on the prevention of reduced neonatal birth weight according to gestational age.

Materials and Methods

A cross-sectional study was conducted in pregnant women, with 77 passive smokers and 88 non-smokers. Pregnant women admitted to the present outpatient service, who satisfied the inclusion and exclusion criteria, were consecutively included in the study. After the approval of Human Ethics Committee and obtaining...
written informed consents from patients, data was collected during a period of 12 months, from August 2012 to August 2013 with personal interviews with the mothers. A person was accepted to be a passive smoker if a family member or colleague had regularly smoked cigarettes in their presence for more than one year [13].

The method of smoking cessation education recommended by American College of Obstetricians and Gynecologists [14] was used to develop an education program against passive smoking for the patients. In brief, during motivational interviews, passive smoking status was monitored and additional follow-up visits were arranged to increase the knowledge regarding the perinatal risks of passive smoking, including intrauterine growth restriction and low birth weight in about 20% of cases, and its long-term effects during postnatal life. The authors focused on the pregnant woman’s motivation to avoid second-hand smoke exposure.

The inclusion criteria were women aged 18–40 years, with 30–41 weeks of gestation, and with a BMI of 19–25. The exclusion criteria were the pregnancies with obstetrical conditions including genetic abnormalities, congenital anomalies, multiple gestation, infection, preeclampsia, placental abruption, fetal growth restriction, low pre-pregnancy maternal weight, or poor weight gain during pregnancy, pregnancy after use of assisted reproductive technologies, toxic exposure to warfarin, anticonvulsants, antineoplastic agents, folic acid antagonists, antihypertensive drugs, uterine malformations, and chronic maternal diseases including diabetes, hypertension, renal insufficiency, collagen vascular dis-

| Table 1. — Selected demographic and clinical data of the study population. |
|------------------------------------------|-----------------|-----------------|
| Demographic data                        | Non-smoking     | Passive smoking |
|                                        | (n=88)          | (n=77)          |
| Age, years                              | 27.2±5.6        | 27.1±5.0        |
| Maternal weight, kg                     | 62.7±13.0       | 63.9±11.8       |
|                                       | 74.8±13.7       | 78.4±11.3       |
| Occupation                              | Employed        | Unemployed      |
|                                        | 13 (15%)        | 75 (85%)        |
|                                        | 1 (1%)          | 76 (99%)        |
| Education                               | High school     | University      |
|                                        | 74 (84%)        | 14 (16%)        |
|                                        | 76 (99%)        | 1 (1%)          |
| Socioeconomic status                    | Low             | Average         |
|                                        | 15 (17%)        | 73 (83%)        |
|                                        | 14 (18%)        | 63 (82%)        |
| Obstetrical history                     | Gravidity       | Parity          |
|                                        | 2 (1-10)        | 2 (1-6)         |
|                                        | 3 (1-5)         | 2 (1-5)         |
|                                        | 0 (0-5)         | 0 (0-2)         |
| Stillbirth                              | Yes             | No              |
|                                        | 8 (9%)          | 80 (91%)        |
|                                        | 13 (17%)        | 64 (83%)        |
| Live child                              | 2 (1-5)         | 2 (1-5)         |
| Diabetes mellitus                       | Yes             | No              |
|                                        | 7 (8%)          | 81 (92%)        |
|                                        | 3 (4%)          | 65 (96%)        |
| Hypertension                            | Yes             | No              |
|                                        | 14 (16%)        | 74 (84%)        |
|                                        | 10 (13%)        | 67 (87%)        |
| Consanguinity                           | Yes             | No              |
|                                        | 4 (5%)          | 84 (95%)        |
|                                        | 12 (15%)        | 65 (85%)        |
| Fetal malformation                      | Yes             | No              |
|                                        | 0              | 88 (100%)        |
|                                        | 3 (4%)          | 74 (96%)        |
| Demographic data                        | Non-smoking     | Passive smoking |
|                                        | (n=88)          | (n=77)          |
| Age, years                              | 37.8±2.7        | 37.5±2.5        |
| Maternal weight, kg                     | 6 (7%)          | 8 (10%)         |
|                                       | 82 (93%)        | 69 (90%)        |
| History of                              | Gestational diabetes mellitus | No             |
|                                        | Yes             | No              |
|                                        | 13 (15%)        | 75 (85%)        |
|                                        | 8 (10%)         | 69 (90%)        |
| Premature rupture of membrane           | Yes             | No              |
|                                        | 2 (2%)          | 86 (98%)        |
|                                        | 6 (8%)          | 71 (92%)        |
| Complications                           | Threatened abortion | No              |
|                                        | Yes             | No              |
|                                        | 8 (9%)          | 80 (91%)        |
|                                        | 2 (3%)          | 75 (97%)        |
| Antenatal bleeding                      | Yes             | No              |
|                                        | 4 (5%)          | 84 (95%)        |
|                                        | 2 (3%)          | 75 (97%)        |
| Fetal distress                          | Yes             | No              |
|                                        | 5 (6%)          | 83 (94%)        |
|                                        | 6 (8%)          | 71 (92%)        |
| Neonatal variables                      | Newborn weight, g | 3069±758       | 3110±788        |
| Apgar scores, minutes                   | 1               | 8.2±1.8        | 8.1±1.3         |
|                                      | 5               | 9.3±1.8        | 9.4±1.0         |
| Need for respiratory support at birth   | Yes             | No              |
|                                        | 2 (2%)          | 82 (98%)        |
|                                        | 2 (3%)          | 75 (97%)        |
| Need for neonatal intensive care unit   | Yes             | No              |
|                                        | 9 (10%)         | 79 (90%)        |
|                                        | 8 (10%)         | 69 (90%)        |
| Respiratory distress syndrome           | Yes             | No              |
|                                        | 2 (2%)          | 86 (98%)        |
|                                        | 4 (5%)          | 73 (95%)        |
| Congenital malformations                | Yes             | No              |
|                                        | 2 (2%)          | 86 (98%)        |
|                                        | 1 (1%)          | 76 (99%)        |

\*\*p < 0.05 vs. no smoking group. Data are expressed as mean ± SD, median (min-max), or percentage as appropriate.
Antenatal counseling against passive smoking may improve birth weight for gestational age

ease, antiphospholipid syndrome, pulmonary disease, cyanotic heart disease, or severe anemia.

Collected data included information about demographic, obstetrical history, last pregnancy, complications, and neonatal variables of patients. Demographic data included age, maternal weight before pregnancy and during delivery, occupation, education, and socioeconomic status. The socioeconomic status was classified according to the minimum wage in the present country. Obstetrical history data included gravidity, parity, miscarriage, stillbirth, live child, diabetes mellitus, hypertension, consanguinity, and fetal malformation. Last pregnancy data included gestational age, number of antenatal visits, and history of gestational diabetes mellitus, preeclampsia, and premature rupture of membrane. Complication data included threatened abortion, antenatal bleeding, and fetal distress. Neonatal variables were newborn weight, Apgar scores at one and five minutes, and the rates of need for respiratory support at birth, need for neonatal intensive care unit, respiratory distress syndrome, and congenital malformations.

Statistical analysis

Data are presented as mean ± SD, median (min-max), or percentage as appropriate. For the analysis of parametric data, t-test was used. For the analysis of non-parametric data, Mann-Whitney U test was used. For the analysis of categorical data, chi-square test was used. Association of gestational age and neonatal weight was examined with Pearson correlation test. A p value of less than 0.05 was accepted as significant.

Results

Table 1 presents the selected demographic and clinical data including obstetrical history, last pregnancy, complications, and neonatal variables of the study population.

After the analyses of obstetrical history, the study groups were comparable with regards to gravidity, miscarriage, history of stillbirth, and number of live children (p > 0.05). The parity in the passive smoking group was lower than that of the non-smoking group (p < 0.05). While the rates of maternal diabetes mellitus, hypertension, and history of fetal malformation were comparable in the study groups (p > 0.05), the rate of consanguinity in the passive smoking group was higher than that of the non-smoking group (p < 0.05).

After the analyses of clinical data during last pregnancy, the study groups were comparable with regards to the gestational age, number of the antenatal visits, and history of gestational diabetes mellitus, preeclampsia, and premature rupture of membranes (p > 0.05).

After the analyses of complications, the study groups were comparable with regards to the threatened abortion, antenatal bleeding, and fetal distress (p > 0.05).

After the analyses of neonatal variables, the study groups were comparable with regards to the newborn weight, Apgar scores at one and five minutes, and the rates of need for respiratory support at birth, need for neonatal intensive care unit, respiratory distress syndrome, and congenital malformations (p > 0.05).

After the correlation analyses of gestational age and neonatal birth weight in the passive smoking and nonsmoking groups, the authors found positive and strong correlations between the gestational age and neonatal birth weight (r = 0.80 and r =0.76, respectively; p < 0.05) (Figures 1 and 2).

Discussion

In the current study, the authors aimed to determine the effect of antenatal counseling against exposure to environmental cigarette smoke on the prevention of reduced neonatal...
tal birth weight according to gestational age in 77 passive smoking women. The higher rates of unemployment and high school education were in accordance with the status of exposure to environmental cigarette smoking. The lower number of parity and higher rate of consanguinity may be related to the exposure to environmental cigarette smoke; however, it is not appropriate to draw a conclusion because of the small sample size of this study. The fact that the values of other demographic, obstetrical history, last pregnancy, complication, and neonatal data are comparable supports the similarity of study groups to enhance the importance of the association of gestational age and neonatal birth weight. The authors found that the correlation coefficients of the association of gestational age and neonatal birth weight in the non-smoking and passive smoking groups were considerably similar. In addition, overall, this is important for revealing the success of intervention to decrease the negative impact of passive smoking on neonatal birth weight according to gestational age. According to the present authors’ knowledge, in this country, this is the first study demonstrating the importance of antenatal counseling against passive smoking to improve fetal and neonatal health.

Quitting smoking and prevention of passive smoking are among the main interventional measures to improve perinatal health of mother and fetus. Counseling about smoking habits and environmental smoke exposure must be a requirement of routine antenatal care [15]. Clinicians need to offer effective interventions against smoking and environmental smoke exposure during the first antenatal visit and the following visits during pregnancy [16]. The obstetrician needs to keep this in mind because smoking during pregnancy is not socially accepted as an appropriate behavior, and in general, pregnant women did not mention their smoking status or level of environmental smoke exposure [17].

Overall, smoking during pregnancy can cause several health problems in women and in their fetuses and neonates. It increases the rate of many pregnancy complications, including preterm birth, low birth weight, and sudden infant death syndrome [3, 14, 18]. Ko et al. [5] investigated the association of low birth weight with the amount of parental smoking during the different pregnancy periods. They found that maternal smoking caused meaningful decrease in birth weight. Compared with the non-smoking groups, all the smoking mothers had higher incidences of low birth weight, especially when the mothers smoked more than 20 cigarettes per day. The association of paternal smoking with LBW, SGA, and preterm birth infants was insignificant. They concluded that pregnant women should be advised to stop or decrease smoking to reduce neonate morbidity.

One of the important abnormalities related to passive smoking during pregnancy is low birth weight seen in about 20% of cases [10, 19]. During second-hand smoke exposure, several chemicals including nicotine, carcinogens, and toxic substances found in tobacco smoke are inhaled [20]. They affect many aspects of fetal development, from conception to birth. It is important to inform pregnant women about the absence of any safe level of exposure to second-hand smoke [21]. There are studies evaluated the relationship between prenatal passive smoking and neonatal intensive care unit admission; they demonstrated that the rate of neonatal intensive care unit admission increased meaningfully. The passive-smoking pregnant women had two to four times more risk of perinatal complications compared to non-smoking mothers [17]. In a recent study by Wahabi et al. [11], the authors assessed the impact of second-hand smoke on the neonatal birth weight of term infants. They found that the prevalence of exposure of their study population to second-hand smoke is high at 31% and this has an important contribution to the reduced neonatal birth weight.

There are some inherent limitations of this study. The authors preferred to exclude several obstetric conditions with a potential to reduce neonatal birth weight. Because of this, during the study period, they enrolled 88 non-smoker and 77 passive smoker women into the study. No interaction of selected demographic and clinical parameters with the association of gestational age and neonatal birth weight may be related to the small sample size of the study groups. In this study, the exposure to second-hand smoke was based on information given by pregnant women without the use of biomarker to verify cigarette smoke exposure. In addition, the exposure to second-hand smoke was not measured according to the number of hours the mother was exposed. Another limitation of this study was that there was no study group exposed to second-hand smoke but received standard antenatal care in the setting of the present obstetric unit.

In conclusion, during antenatal care of women, regular counseling against passive smoking may prevent negative effects of passive smoking on neonatal birth weight according to gestational age. This promising information is important for women, their families, and healthcare professionals, and reinforces the continued need for programs to increase awareness on prevention of passive smoking to improve perinatal health.

References

Antenatal counseling against passive smoking may improve birth weight for gestational age


Address reprint requests to:
O.B. AKKAR, M.D.
Department of Obstetrics and Gynecology
Cumhuriyet University Faculty of Medicine
Yenisehir Street, 5446
58140 Sivas, Turkey
e-mail: dr.ozlemakkar@yahoo.com
A novel case of an adenomyosis-related uterine rupture in pregnancy

U. Indraccolo, A. Iannicco, G. Micucci

Complex Operative Unit of Obstetrics and Gynecology, Hospital of Civitanova Marche, Area Vasta 3 – Marche, Civitanova Marche (Italy)

Summary
To date, few cases of uterine rupture related to adenomyosis have been reported. The current case report briefly describes a novel case of an adenomyosis-related uterine rupture, while focusing on few symptoms that this kind of uterine rupture may have. Due to increasing rate of adenomyosis in Western countries, practicing obstetricians should carefully take into account silent uterine rupture related to adenomyosis.

Key words: Adenomyosis; Uterine rupture; Pregnancy.

Introduction
Spontaneous uterine rupture of the unscarred pregnant uterus is an uncommon accident. Miller et al. [1] have reported that uterine rupture in unscarred uterus in labor is linked with prostaglandin use, oxytocin infusion, malpresentation, and multiparity. Before the onset of labor, however, Sun et al. [2] have reported that sometimes the uterine rupture of unscarred uterus may not relate with any risk factors. More recently, Nikolaou et al. [3] have suggested why adenomyosis may be another risk factor for uterine rupture of unscarred uterus after reviewing 12 cases. Therefore, readers may feel interesting to assess the clinical course of the aforementioned uterine rupture in a pregnant woman with adenomyosis.

Case Report
The patient was 37-years-old, with two previous uneventful pregnancies and vaginal deliveries. The year before her actual pregnancy, she underwent laparoscopy with adhesiolysis for chronic pelvic pain. During ultrasonographic scans and vaginal examinations in the actual pregnancy, the gynecologist found a huge nodule of the uterine posterior wall, diagnosing an adenomyosis. The pregnancy was uneventful until 36 weeks, when the patient complained of nausea and abdominal pain. A cardiocotography (CTG) at this time was normal, but ultrasonography shows a large image resembling a hypoechoic cyst between the uterine fundus and the liver. It was suspected to be the cause of the symptoms. Moreover, the fetus was in breech presentation. In the succeeding hours, abdominal pain increased and the patient underwent another CTG. Regular uterine contractile activity was found, but the patient was not laboring, based on cervical exploration. Repetitive variable decelerations depicted an abnormal CTG pattern, leading the onward obstetrician to perform a cesarean section. A healthy fetus was extracted in breech (Apgar 9-10). At the delivery of the placenta, the obstetrician found a large pouch of amnion behind the placenta, which was removed by pulling the placenta. The uterus was externalized from the abdomen. At this time, a three-cm tear in length on the uterine fundus, close to the right angle, was surprisingly found. The tear was not bleeding significantly and it was easily sutured (Figure 1). Moreover, no cysts were found at exploration of the abdomen and of the adnexa, but the obstetrician confirmed the diagnosis of wide zones of adenomyosis on the entire uterus, with signs of decidualized endometriosis in the pelvis. The post-cesarean course was uneventful.

Figure 1. — Uterine tear during suture.
After epicrisis, the patient was informed that she likely suffered from a primitive rupture of the uterus occurred not-in-labor and without the common complications as bleeding and fetal distress. The suspected cyst was a pouch of amnion herniating across the uterine tear. Patient was counseled that the uterine rupture could reoccur in another pregnancy and that it was likely to be caused by adenomyosis.

Discussion

The present authors were unable to strongly relate the uterine rupture to the behavior of the adenomyosis in a pregnancy. A severe burning damage of the uterus during the previous laparoscopy could have occurred. However, they believed that laparoscopy with adhesiolysis should not involve the uterine wall. Moreover, the multiparity per se should not justify the uterine rupture not-in-labor. Therefore, they mostly agree with what was already reported by Nikolaou et al. [3]: that perhaps transmural adenomyosis leads to splay of uterine smooth fibers and uterine rupture not-in-labor. Such interpretation could explain the evolution of the present case, with herniation of the amnion across the uterine tear in absence of significant bleeding and severe pain.

In conclusion, the transmural adenomyosis during pregnancy can be a subtle risk factor for uterine rupture, because it could favor the splaying of the smooth muscle without symptoms. Since adenomyosis rate is increasing in women, practicing obstetricians should carefully consider the possibility of a subtle uterine rupture due to adenomyosis during pregnancy.

References


Address reprint requests to:
U. INDRACCOLO, M.D., Ph.D.
Via Montagnano, 16
62032 Camerino (MC) (Italy)
e-mail: ugo.indraccolo@libero.it
Mature cystic teratoma of both the fallopian tube and contralateral ovary: a case report

J.M. Rhymes¹, T. Gorman², R.A. Sasso¹

¹ Ross University School of Medicine, Department of Clinical Medicine, North Brunswick, NJ
² Department of Pathology, Concord Hospital, Concord, NH (USA)

Summary

Intratubal teratoma is a very rare condition. The authors believe to present the first case of a completely intratubal mature cystic teratoma with a contralateral intraovarian teratoma. Preoperative ultrasound examination allowed the intraoperative diagnosis of this rare condition, hence allowing appropriate surgical management. Materials and Methods: A 19-year-old woman presented with a history of pelvic pain and severe dysmenorrhea. Ultrasound examination initially suggested bilateral ovarian dermoids. Upon laparoscopy, the distal left fallopian tube was obstructed and contained an inflammatory mass adhered to the rectosigmoid. The left ovary was entirely normal. A contralateral intraovarian dermoid was also identified. Conclusion: Although rare, when an intratubal mass is identified, consideration of intratubal dermoid should be given. Preoperative ultrasound can be of critical importance to the intraoperative diagnosis.

Key words: Fallopian tube tumor; Dermoid cyst; Pelvic pain; Salpingectomy; Laparoscopy.

Introduction

Mature cystic teratomas are composed of differentiated tissue originating from all three germ cell layers: ectoderm, mesoderm, and endoderm [1]. Mature cystic teratomas of the ovary are fairly common. However, teratomas occurring in the fallopian tube are rare occurrences with only about 60 cases in the literature to date [2]. Here, the authors describe a mature cystic teratoma in both the fallopian tube and the contralateral ovary.

Materials and Methods

Patient presentation

A 19-year-old white female presented with a two-year history of progressive pelvic pain and severe dysmenorrhea. The patient had an otherwise unremarkable past medical history and no previous surgeries. Ultrasound examination was initially felt to be consistent with bilateral ovarian teratomas.

Surgical procedures

Laparoscopy was performed demonstrating a right ovary containing a deep 2.5 cm intraovarian teratoma attached to the ovarian hilus (Figure 1). The right fallopian tube was entirely normal. The distal left fallopian tube was obstructed and firm, with dense adhesions to the rectosigmoid colon, at the level of the pelvic inlet, and surrounding inflammation (Figure 2). The left ovary was entirely normal and uninvolved with the pathology. Based on the preoperative ultrasound finding of bilateral pelvic dermoids, the intraoperative diagnosis of a left intratubal teratoma was now made.

Results

A left salpingectomy was then performed with left ovarian preservation, as well as a right ovarian cystectomy with removal of its contained teratoma. The left tube was removed with its contained cystic teratoma intact, hence avoiding spillage of its content into the peritoneal cavity. Two separate lesions consistent with superficial endometriosis were also detected and excised. Pathologic examination of the left fallopian tube revealed a mass completely encased within the tube containing components...
of all three germ layers, confirming a complete left intratubal teratoma (Figure 3). Patient had an uneventful postoperative recovery.

Discussion

Mature cystic teratomas are composed of differentiated tissue originating from all three germ cell layers: ectoderm, mesoderm, and endoderm [2]. They are the most common ovarian tumors in women in their second and third decades and can be associated with ovarian torsion and rupture of the cystic component [3]. In contrast, neoplasms of the fallopian tube are the rarest tumors of the female reproductive system [4]. Intrafallopian tube teratomas have been associated with reduced parity, menstrual irregularity, leukorrhea, postmenopausal bleeding, and abdominal pain [5].

The authors believe that this is the first report of a mature cystic teratoma of the fallopian tube occurring concomitantly with a mature cystic teratoma of the contralateral ovary. Also, preoperative pelvic ultrasound allowed the intraoperative diagnosis of intratubal dermoid without opening the fallopian tube and risking spillage of its content into the peritoneal cavity, which can lead to chemical peritonitis.

Conclusion

When an intratubal mass is encountered during pelvic surgery, although rare, the possibility of intratubal dermoid should be considered. In this instance, with the use of preoperative ultrasonographic evaluation of the pelvis, the intraoperative diagnosis of an intratubal dermoid with a contralateral intraovarian dermoid was possible. This allowed for the most appropriate surgical intervention with the smallest risk of pelvic contamination.

References


Address reprint requests to:
J. RHYMES, M.D.
Department of Clinical Medicine
Ross University School of Medicine
630 US Highway #1 Suite 500
North Brunswick, New Jersey 08902 (USA)
e-mail: jadarhymes1@rossu.edu
Acardiac acephalus fetus – report of distinctive anatomical anomalies with regards to pathophysiology of TRAP sequence

P. Lewitowicz1, A. Wincewicz2, D. Koziel3, J. Matykiewicz3, A. Horecka-Lewitowicz4, M. Koda5,6, L. Kanczuga-Koda6, S. Gluszek3, S. Sulkowski4

1 Department of Pathology, Faculty of Health Sciences, Jan Kochanowski Memorial University, Kielce
2 Department of Anatomy, Faculty of Health Sciences, Jan Kochanowski Memorial University, Kielce
3 Department of Surgery and Surgical Nursing, Faculty of Health Sciences, Jan Kochanowski Memorial University, Kielce
4 Department of Public Health, Faculty of Health Sciences, Jan Kochanowski Memorial University, Kielce
5 Department of General Pathomorphology, Medical University of Bialystok, Bialystok
6 Department of Pathology, Bialystok Oncology Centre, Bialystok (Poland)

Summary

Acardiac fetuses are consequences of twin reversed arterial perfusion (TRAP). Here the authors present a case of 40-year-old gravida IX who gave birth to a healthy, 2,900 g female child by a cesarean section. Additionally amorphic 1,020 g maldeveloped fetus was removed. There was a diamnion monochorionic type of twin placenta with incorrect single umbilical arteries (SUA) both in umbilical cord of healthy fetus and in atrophic second umbilical cord. A malformed fetus developed a rather well formed lower leg with four digital foot and oval shape amorphous body mass with omphalocele and evagination of the intestines. X-ray picture showed well visible metatarsal and femur bone and anatomically undefined bones cluster in the central part. A cavity of fetal body contained intestines - the only one well-formed organ, nests of heterotopic pilosebaceous residues, remnants of adrenal glands, well-formed ganglia, and nests of neural tissue covered by neuroepithelium.

Key words: Acardius acephalus; Organ underdevelopment; Disruption; Twin reversed arterial perfusion; Hypoxia.

Introduction

Acardiac acephalus twin is a rare fetal malformation that occurs in one to 1.5% monochorionic multiple pregnancies [1-3]. Generally a disturbance of placental circulation could be associated with conjoined twins or more severe malformations as acardiac fetus. In 1850, Meckel first identified the pathogenetic mechanism of reverse fetal perfusion, and next in 1859 Claudius articulated the thesis that after normal initial development, the heart degenerates as result aortal thrombosis [4]. According to updated definition, twin reversed arterial perfusion (TRAP) or twin-twin transfusion syndrome is caused by incorrect intraplacental arterial-arterial and venous-venous anastomoses that convey reversed blood flow in umbilical vessels and aorta in twin fetus that is therefore destined to serious malformation [4,5]. Precisely, twin reversed arterial perfusion (TRAP) is constituted by three determinants: (1) there is no heart or cardiac development is severely impaired; (2) artery-artery shunt conveys arterial blood flow from a normal so-called “pump twin” to the acardiac partner; (3) vein-vein shunt is a way of outflow of venous blood from the acardius back to the normal twin [6]. Dysmorphogenesis has still not be unequivocally defined to be a case or result of reversal of blood flow [6]. In consequence of such circulatory defect, prolonged hypoxia is responsible for a underdevelopment and dysmorphogenesis that are expressed by atrophy and hypoplasia of one of twins that is termed a recipient. Organ agenesis could also be another eventual explanatory mechanism in pathogenesis of this malformation due to the same but more aggravated causative factors as hypoperfusion and hypoxia due to ischemia [2,3]. Thus, growth of upper parts of the body is abolished severely, while development of inferior organs is less impaired in the manner of disruption [5]. In this condition the severity of organ’s ananomaly depends mainly on the rate of incorrect blood flow, hypoxia level, and the gestational period. A few types of acardiac fetuses can be distinguished on the basis of the stage of undergrowth. They include acardiac acephalus, acardius anceps, acardius ancormus, and acardius amorphous fetus. If severe vascular disturbances commenced within first trimester, it can be a cause of early death of the twin whose fetal relics are vividly termed a fetal papyraceus.

Case Report

A 40-year-old gravida IX without history of multiple pregnancy was admitted to hospital for delivery. Her pregnancy was not under gynecological control until hospitalization. Hence, a suspicion of twin pregnancy with ultrasonography described a pathological amnion tissue mass was diagnosed just before the delivery.
Figure 1. — Macroscopic presentation of the acardiac fetus. A, B: The dorsal and ventral view of acardius acephalus fetus with evident intestinal eventration. C: Four-digit foot. D: X-ray picture of maldeveloped fetus with centrally locked bones cluster.

Figure 2. — Microscopic presentation of acardiac fetus. A, B: Rudimentary neural tissue with prominent ganglia cells surrounded by primitive glial tissue and covered by neuroepithelium - H&E stain x20 and x10 magnification. C: Papillary projections of mesothelial surface covered by two-layer cuboidal epithelium - H&E stain x10 magnification. D: Well-formed and nearly matured adrenal gland - H&E stain x10 magnification.
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at 39 gestational week. A cesarean section revealed a healthy, with absence heart failure signs, 2,900 g female new born child (NBC) and amorphic 1,020 g maldeveloped fetus with discernibly formed only one lower extremity and another rudimentary lower limb in a form of very small process of skin and subcutaneous tissue.

In the histopathological placental report the authors recorded diamnion monochorion type of twin placenta with incorrect single umbilical arteries (SUA) both in healthy fetus and in atrophic second umbilical cord. Microscopic evaluation of placenta denotes indirect signs of anastomosing circulation such as focal villous edema and partial high density of villous vasculature.

Macroscopic evaluation of malformed fetus showed relatively well formed lower leg with four digital foot and oval shape amorphous body mass measuring 16 x 11 x 7 cm with omphalocele and evagination of the intestines (Figure 1A-C). X-ray picture revealed well visible metatarsal and femur bone and anatomically undefined bones cluster in the central part which could be some compounds of axial skeleton and the pelvic girdle (Figure 1D). Most of the body’s relics consisted of skin that covered spherical tissue mass with centrally located skeletal cluster.

Anatomical preparation showed many different anomalies. A small cavity was encountered after dissection of skin and edematous soft tissue cover. In this space there were nine-cm long loops of intestines. One of the ends of intestine was conjoined with funicular tissue, which led around the femur into centre of body mass that contained a cavity space. This umbilical cord contained umbilical artery and well-formed urachus with purulent exudates. In maze of disorganized skeleton there were a few long bones which were surrounded by adipose tissue, skeletal muscle fibers, nerves, lacunar lymphatic channels, small lymph nodes, and vessels. There were also rudimentary central nervous system represented by well-formed ganglia, nests of neural tissue with many neurocytes, and covered by prominent neuroepithelium (Figure 2 A, B). Histological examination of the cavity space showed its multilocular appearance with persisting papillary projections of two-layer-thick cuboidal epithelium (Figure 2C) and focal hyperpigmentation due melanin content.

Moreover adrenal glands tissue was found to be three mm in length (Figure 2D). The evaluation of intestines, which remained the only one well-formed organ, demonstrated a multifocal lumen atresia, blind terminal ends, but relatively well-formed and persisting appendix. The macroscopic analysis of circulatory system allowed only distinction of umbilical artery that grew into bone cluster. It is worth mentioning that there was a complete lack of fetal large arteries and veins. The authors observed only plenty of other vessels mainly small veins and small muscular arteries. (Figure 3A). Thus, they supposed that arterial supply of bones is continued by anastomosing vascularity type only from the end of umbilical artery.

The intestine directly communicated with tunnel covered by mesonephroidal-like epithelium, which showed a focal goblet cell differentiation. (Figure 3B, D) It was also interesting to note that anatomically ileum –like intestine possessed partially colon texture in a caecum region. Histologically, the authors observed intestine of colon type texture with small nests of heterotopic pilosebaceous units and urothelial epithelium, which interfered with colon epithelium of intestinal mucosa (Figure 3C) and formed primitive cloacal-like space.

Discussion

The present case report is only one of a few descriptions of greatly variable mythic forms of fetal deformation. Atrophy, agenesis, aplasia or dysgenesis are terms which do not com-
pletely explain the pathogenesis of gradually advanced malformation of fetal tissue. The severe anatomical malformations could indicate not only mechanism of atrophy and hypoplasia but rather complex causes with severe disturbances during organogenesis. Hypoxia/ischemia is the mechanism, which is crucial in pathogenesis of this entity according to the majority of the investigators [7]. However, hypoxia is not an mechanism that completely explains fetal malformations with evident hypoplasia of organs and minor tissue structures. The presented observations of intestine wall heterotopy would rather indicate an incorrect tissue differentiation or -what is more likely- disturbances of cell migration during organogenesis. In the present case study the authors did not find a liver at all. In this context it is worth to mention that Giménez-Scherer and Davies rightly noticed that the liver is located first in the circulatory way from the placenta to the fetus so this organ was expected to be relatively well-developed in TRAP syndrome [8]. Taking this into account, hypoxia-ischemia mechanism does not explain absence of liver in the present case of acardiac fetus. The present authors postulated that hypoxia-exposed fetus is more flexible to modeling pressure of the donor twin, whose growth can be expanding enough to compress the twin recipient in uterine cavity. Nonetheless, there are decreased blood oxygen saturation levels in the body of cardiac twin and this could be a triggering factor for hypoxia mediated angiogenesis, which employs such factors as hypoxia inducible factor and vascular endothelial growth factor. Such a process augments vascular bed capacity and simultaneously decreases vascular resistance of the acardiac body that may result in intrauterine fetal death [9]. Conclusions of De Groot et al. [7] are supported by the present finding of prominent vascular density in this observation.

The present findings of only rudimentary nervous system are not astonishing in front of the fact that neural tissue particularly brain tissue is the most sensitive to hypoxia-ischemic injury in TRAP sequence. Sergi and Schmitt reported microcephaly and a residual brain anlage, which was consistent with impairment of brain growth at the prosencephalic stage -holoprosencephaly that was accompanied with formation of cystic cavity (hydranencephaly) due to hypoxic injury to the holospheric brain mantle [9]. Their findings were in agreement with the opinion that oxygen deficiency causes disruptions of head-brain and heart development during early embryogenesis on animal experimental model [9]. In other report of severely underdeveloped acardius that constituted ovoid mass with skin, rudimentary legs, and vertebral skeleton, there was a disorganization in structure of brain tissue, which suggested that a hypoxic injury occurred after neurolization to impair the prosencephalic stage of brain development [10]. Namely, cerebellar cortex was the only well-formed element in the present inspection. Moreover, an encountered slit-like space was consistent with the third ventricle that was limited with ependymal covering and choroid plexus [10]. As an evidence to hypoxia induced augmented neovascularization, an area of increased vascular density was also encountered to draw a resemblance to cavernous hemangioma [10]. In comparison to the present findings of more modest neural residues, the authors conclude that this case of acardiac fetus presents with much less developed nervous system than plenty of reports in literature on TRAP syndrome.

There are different subtypes of acardiac fetuses depending on the level of underdevelopment: acardius acephalus, acardius amorphus, and acardius anceps [1]. The present case was classified as acardius acephalus because the malformed twin was devoid of a head, a thorax, and upper limbs but in opposition to acardius amorphous there were relatively well-developed adrenal glands and large intestines in the body of the fetus [11]. Acardius acephalus is also termed with an older name chorangiopagus parasiticus (CAPP) and is found in 1% of monozygous twins. The description of CAPP fetus is the most suitable to the present case as it was an edematous fetus with discernible lower limbs, incomplete pelvis and lower spine elements, incomplete abdominal viscera like intestines, but without any thoracic organs [12]. A case reported by Kakkar et al. contained edematous cystic area in upper part of the fetus, similarly to the present case which contained a cystic cavity [12]. The need of ultrasound surveillance and control should be emphasized here because this malformation can be identified by ultrasound as early as 12 weeks gestation [12].

The present case of acardiac fetus seems to resemble slightly the fetus reported by Sharbaf et al. [13]. They described a normal fetus and acardiac twin with a underdeveloped lower extremity with a sac containing some intestinal loops [13]. However, the present case better fits this classification on the ground of combined Doppler waveform ultrasound and pathological findings presented by Shih et al. [11, 14]. Namely, artery-artery pump-in pattern is one distinguished types and is characterized by two rudimentary lower limbs (in the present case the second one extremity was actually an cutaneous process-like relic), agenesis of upper extremities, underdeveloped visceral organs, and brain and presence of omphalocele [14]. The present case was a subject of differential diagnosis between artery-artery pump-in pattern and collision summation artery–artery pattern [14]. The latter one is defined by presence of cardiac motion in malformed twin because of preserving a cardiac substitute in a form of a rudimentary, contractile heart and artery-artery shunts [14]. Moreover collision summation artery–artery pattern is characterized by an underdeveloped leg, omphalocele, agenesis of liver, spleen, kidneys, lungs, and brain [14]. Both patterns result in live birth of co-twin.

Acardia can occur in an unusual setting with cyclopia and aprotencephaly in a twin fetus because of TRAP [5]. A variety of abnormalities could indeed be very broad to note also that an acardiac, anencephalic twin harbored a serious cardiac type defect, which was a transposition of great arteries [15]. The cardiac development could be impaired at a very varied stage, ranging from a complete lack of the heart through hypoplastic cor triloculare to a malposition of a relatively well-developed heart [16].
Fetal karyotype can be very often normal regardless of subtype of acardiac twin [1]. Nonetheless acardiac fetuses may carry chromosomal abnormalities as trisomy 2 (karyotype 47,XX,+2), which was reported by Blaicher et al. with extensive review on cytogenetic defects in acardia [17]. The other chromosomal aberrations like XXY karyotype in Klinefelter syndrome were also recorded at increased rate in monozygotic pregnancies with acardiac twin and twin reversed arterial perfusion sequence [18]. Acardiac twin was also reported in other genetic disorders like Duchenne muscular dystrophy (DMD) [19]. In every case such an acardiac twin recipient causes additional load for heart output of “pump” twin and could result in cardiac insufficiency of the donor twin [19]. Actually, TRAP sequence can be the life-threatening condition also for the mother as in the case of hydropic acardiac fetus in triplet pregnancy [20]. Namely, it is a risk factor for occurrence of mirror syndrome that is defined as simultaneous placental, maternal and maternal edema with maternal preeclampsia [20].

TRAP sequence could be managed in early fetal life. As acardiac twinning is a complication of at least 1% of monozygotic twins with incidence of one in 35,000 births, a proper protection of pump twin is recommended [21, 22]. This covers prenatal diagnostics with ultrasound Doppler imaging and genetic testing. Nowadays modes of therapy include numerous procedures from hysterotomy with delivery of the acardiac twin to techniques of thermocoagulation and radiofrequency, fetoscope-guided or ultrasound guided occlusive ligation of umbilical cord of acardius, and many others, if only benefit of pump twin is expected to be achieved by the prenatal intervention [21]. Among these methods, the least invasive treatment seems to be intrafetal laser ablation of acardiac fetus that is performed by laser coagulation of pelvic vessels and umbilical cord [22]. This is method of choice particularly at early stage of monochorionic diamniotic twin pregnancy [22].

The present acardiac fetus was in advanced pregnancy which in spite of the lack of gynecological care, resulted in birth of healthy donor twin. Nonetheless, due to possible circulatory complications in acardiac twin pregnancy, this case calls for careful and efficient ultrasonographic screening for early detection of TRAP sequence.

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References


Address reprint requests to:
A. WINCEWICZ, M.D., Ph.D. FEBP
Department of Anatomy Faculty of Health Sciences
Jan Kochanowski Memorial University
Kielce IX Wieków Kielce 19, 25-317
Kielce Holy Cross District (Poland)
e-mail: rualpolin@yahoo.com
andwine@gmail.com
Early operative treatment of anti-N-methyl D-aspartate (anti-NMDA) receptor encephalitis in a patient with ovarian teratoma

S. Yanai, Y. Hashiguchi, M. Kasai, T. Fukuda, T. Ichimura, T. Yasui, T. Sumi

Department of Obstetrics and Gynecology, Osaka City University, Graduate School of Medicine, Osaka (Japan)

Summary

Background: Anti-N-methyl D-aspartate (anti-NMDA) receptor encephalitis is often accompanied by ovarian teratoma. Early tumor resection is reported to be effective as a treatment. Case: A 21-year-old woman presented with anti-NMDA receptor encephalitis which was accompanied by ovarian teratoma. The present case was a very rare case of an early stage of anti-NMDA receptor encephalitis receiving operative treatment before confirming the presence of anti-NMDA receptor antibody. The diagnosis was established postoperatively by identifying anti-NMDA receptor antibody. Conclusion: In case of suspecting anti-NMDA receptor encephalitis in a patient with ovarian teratoma, early operative treatment should be considered even before confirming the presence of anti-NMDA receptor antibody.

Key words: Anti-NMDA receptor encephalitis; Ovarian teratoma.

Introduction

Anti-N-methyl D-aspartate (anti-NMDA) receptor encephalitis often occurs in young females [1, 2]. Patients follow a very similar course, gradually improving after progressing through common cold symptoms, a period of psychiatric symptoms, an immobile period, and a hyperactive period [1-3]. This disorder results in a characteristic syndrome that presents with prominent psychiatric symptoms or, less frequently, memory deficits, followed by a rapid decline of the level of consciousness, central hyperventilation, seizures, involuntary movements, and dysautonomia [2, 4]. Definitive diagnosis is possible when anti-NMDA receptor antibody is detected in the cerebrospinal fluid and blood serum [3]. As a therapy, the immunotherapy including corticosteroids, intravenous immunoglobulin or plasma exchange are often effective [2-4].

Anti-NMDA receptor encephalitis is often accompanied by ovarian teratoma [1-6]. In patients with ovarian teratoma, early tumor resection is reported to be effective [1-6]. In most previously reported cases, the ovarian teratoma was removed a few months after neurological symptom presentation [1-6]. Moreover, operation was performed after confirming the presence of anti-NMDA receptor antibody in most cases [1-6]. There is no report that operation was performed before identifying anti-NMDA receptor antibody for patients with an early stage of disease.

The authors present a rare case of an early stage of anti-NMDA receptor encephalitis patient with ovarian teratoma, who received early operative treatment before confirming of the presence of anti-NMDA receptor antibody.

Case Report

A 21-year-old woman without a past medical history of interest developed epilepsy 25 days before admission to the present hospital. She visited a local doctor and was initially diagnosed with epilepsy. She was treated with antiepileptic drug such as diazepam, phenytoin, and acyclovir was started for suspicion of herpes simplex virus (HSV) encephalitis. Although the convulsions were under control by treatment and the disturbance of consciousness was improved, the involuntary movement of fingers of left hand was not responding to the treatment. Antiepileptic drug such as carbamazepine, phenobarbital, levetiracetam, and topiramate was added to treatment. She was admitted to the Department of Geriatric Medicine of the present hospital because of persistent symptoms.

The disturbance of consciousness and the involuntary movement of fingers of left hand was observed. No other psychiatric symptoms were observed. Magnetic resonance imaging (MRI) examination of the brain showed no remarkable findings. Single photon emission computed tomography (SPECT) study showed slight hypoperfusion in the left temporal region and the left occipital region. Electroencephalogram (EEG) showed no remarkable findings. On hospital day 8, contrast-enhanced computed tomography (CT) examination of the abdomen and chest demonstrated a right cystic adnexal mass with an internal focus of fat and high-attenuation material, suggesting an ovarian teratoma (Figure 1). Anti-NMDA receptor encephalitis accompanied by teratoma was initially considered the most likely diagnosis. Therefore, anti-NMDA receptor antibody was examined in the blood serum and steroid pulse therapy was initiated. On hospital day 10, she visited the present gynec-
cology outpatient clinic and also underwent MRI examination of the pelvis, which demonstrated a right cystic adnexal mass with an internal focus of fat and calcification, suggesting an ovarian teratoma (Figure 1). Laboratory investigations showed no remarkable findings without slightly elevated liver enzymes. Serum tumor markers were within the normal ranges (CA125: 18 U/ml; CEA: 0.8 ng/ml; CA19-9: 11 U/ml; SCC: 0.6 ng/ml). The cytological examination of cervix showed no abnormality. The authors decided to perform operative treatment of right ovarian teratoma as a treatment of anti-NMDA receptor encephalitis before confirming the presence of anti-NMDA receptor antibody.

On hospital day 11, she underwent a laparoscopy-assisted cystectomy of right ovary. Macroscopically, there was right ovarian tumor (size: 5 x 3 cm) which contained fat tissue and hair. The left ovary and uterus were unremarkable (Figure 2). There were no other remarkable findings in the peritoneal cavity.

Finally, the pathological examination showed a mature cystic teratoma, including adipose tissue, epithelial tissue and nervous tissue (Figure 3). The authors confirmed the diagnosis of mature cystic teratoma of right ovary. On hospital day 35, they confirmed the presence of anti-NMDA receptor antibody and the diagnosis was established postoperatively.

On hospital day 40, the disturbance of consciousness was almost completely cured and the involuntary movement of fingers of left hand improved completely without progression of the disease.

Discussion

Anti-NMDA receptor encephalitis is an autoimmune and paraneoplastic encephalitis first reported in 2007 by Dalmau et al. [1]. They reported clinical data gathered from around the world in 2008 [2]. This type of encephalitis often occurs in young women (approximately 80% of the patients are females and the median age is 23 years) [2]. Patients follow a very similar course, gradually improving after progressing through common cold symptoms such as fever, headache or malaise, a period of psychiatric symptoms, an immobile period, and a hyperactive period [1-3]. This dis-
order results in a characteristic syndrome that presents with prominent psychiatric symptoms or, less frequently, memory deficits, followed by a rapid decline of the level of consciousness, central hypoventilation, seizures, involuntary movements, and dysautonomia [2,4]. Definitive diagnosis is possible when anti-NMDA receptor antibody is detected in the cerebrospinal fluid and blood serum [3].

As a therapy, immunotherapy including corticosteroids, intravenous immunoglobulin or plasma exchange are often effective [2-4]. In non-responders, second-line immunotherapy including rituximab or cyclophosphamide or combined therapy is required [5, 6]. Despite the severe symptoms and prolonged clinical course, 75% of patients achieve complete recovery [2]. The mortality rate is reported to be 7% [2]. On the other hand, anti-NMDA receptor encephalitis is often accompanied by teratomas in various regions such an ovary [1-6]. Dalmau et al. reported that 31% of female patients had a tumor and all tumors were ovarian teratomas [2]. In patients with ovarian teratoma, early tumor resection is reported to be effective [1-6]. In most previously reported cases, the ovarian teratoma was removed in a few months (median: nine weeks) after neurological symptom presentation, sometimes when symptoms had already partially responded to immunotherapy [1-6]. Moreover, surgery was performed in most cases after confirming the presence of anti-NMDA receptor antibody. Although operation was performed before confirming the presence of anti-NMDA receptor antibody in a few cases, these patients were in advanced stage of disease and were transferred to the intensive care unit (ICU). There was no report that operation was performed before identifying anti-NMDA receptor antibody for patients with an early stage of disease. In this report, the authors present a rare case of an early stage of anti-NMDA receptor encephalitis patient with ovarian teratoma, who received early operative treatment before confirming the presence of anti-NMDA receptor antibody.

In the present case, a 21-year-old woman presented with neurological symptom onset 25 days before admission to the present hospital. On hospital day 8, ovarian teratoma was detected by CT and anti-NMDA receptor encephalitis was initially considered the most likely diagnosis. On the same day, anti-NMDA receptor antibody was examined in the blood serum and steroid pulse therapy was initiated and operative therapy was performed three days later. The present authors confirmed the presence of anti-NMDA receptor antibody and the diagnosis was established postoperatively. Iizuka T et al. reported that early tumor resection seemed to be the most important factor enabling prompt and full recovery from anti-NMDA receptor encephalitis [7]. They also reported that early treatment could shorten the duration of ventilatory support and dyskinesia compared with that of patients without tumor resection [7]. The present case was a case of an early stage of anti-NMDA receptor encephalitis. Considering possibility of worsening of the disease status, the authors performed operative treatment before confirming the presence of anti-NMDA receptor antibody. By early operative treatment, the present patient seemed to recover early and achieve almost complete recovery without progression of disease.

If young female patient with ovarian teratoma presents encephalitis or psychiatric symptoms, anti-NMDA receptor encephalitis should be considered as a diagnosis. Moreover, early operative treatment should be considered even before confirming the presence of anti-NMDA receptor antibody.

References


Address reprint requests to:
Y. HASHIGUCHI, M.D.
Department of Obstetrics and Gynecology
Osaka City University, Graduate School of Medicine
1-4-3 Asahimachi, Abeno-ku
Osaka 545-8585 (Japan)
e-mail: hashiguchi@med.osaka-cu.ac.jp
Introduction

Congenital syphilis is preventable and curable if maternal infection is detected early, and pregnant women in Korea are screened routinely for this disease. Nevertheless, the incidence of congenital syphilis is not decreasing. Prenatal diagnosis of congenital syphilis is difficult and treatment is usually based on maternal syphilis serology. Prenatal ultrasonographic examination may sometimes reveal abnormal features suggesting congenital infection. The authors report a case of congenital syphilis that was diagnosed in both fetus and asymptomatic mother following detection on prenatal ultrasonography of transient fetal pleural effusion. The case is noteworthy for its sonographic presentation as fetal pleural effusion rapidly resolved spontaneously.

Case Report

A 21-year-old woman, gravida 2, para 1, was referred to the present clinic following detection of a fetal pleural effusion at 27+5 weeks’ gestation. The patient’s antenatal progress had been unremarkable to that point. Ultrasonographic examination revealed a unilateral fetal pleural effusion in the right thorax (Figure 2A). The effusion was small and did not compress adjacent organs and no fluid collection was detected in other organs. No other fetal abnormalities were neither observed in this examination nor in fetal echocardiography. The patient was informed of the sonographic findings and underwent appropriate serological, blood type, and antibody screening tests. A genetic amniocentesis was also performed. A subsequent ultrasound scan at 28+5 weeks’ gestation revealed that the fetal pleural effusion had spontaneously resolved (Figure 2B). However, the patient’s blood tests for rapid plasma regain (RPR) test were positive at 1:48 dilution. The Treponema pallidum hemagglutination assay (TPHA) was reactive at 1:2,000 dilution and the fluorescent treponemal antibody–absorbed immunoglobulin M (FTA-ABS IgM) and immunoglobulin G (FTA-ABS IgG) assays were reactive. Early latent syphilis was diagnosed because the patient showed no clinical signs or symptoms of syphilis, and fetal syphilis was strongly suspected. At 28+6 weeks’ gestation the patient received a single injection of 2.4 million units of penicillin G benzathine. One week later (29+6 weeks), she was admitted for spontaneous preterm labor with vaginal bleeding. A 1,370-g male neonate was delivered by repeat cesarean section due to placental abruption. He had an Apgar index of 5 at one minute and 7 at five minutes. His serological test for syphilis showed a reactive RPR (>1:48), positive TPHA (>1:2,000), reactive FTA-ABS IgG, and nonreactive FTA-ABS IgM. Laboratory studies of the cerebrospinal fluid (CSF) showed the following values: WBCs, 9/uL; protein, 142.0 mg/dL; glucose, 58 mg/dL, with a plasma glucose level of 123 mg/dL. The venereal disease re-
search laboratory (VDRL) test of CSF was weakly reactive. The newborn was treated with aqueous penicillin G for 21 days and discharged at 64 days of age. As of the last follow-up visit at 14 months of age, the child had suffered frequent infections and showed neurodevelopmental disability.

Discussion

With increasing use and distribution of penicillin since 1960s the prevalence of syphilis decreased in many regions of the world. Congenital syphilis is preventable and even treatable through treatment of the mother with intramuscular penicillin when maternal syphilis is diagnosed. During the last decade, however, congenital syphilis has re-emerged [3-5]. This follows a rising incidence of maternal primary and secondary syphilis, which has been attributed to inadequate prenatal care [4], illicit drug use [6], the sex trade and social upheaval in developing countries [6, 7], and regional changes in demographic and socioeconomic patterns [8]. In South Korea, the increase in congenital syphilis reflects an increase in the primary and secondary syphilis rates, which is presumably associated with liberalized sexual attitudes. Diagnosis of congenital syphilis during pregnancy usually relies on maternal serological testing. However, abnormal signs in a fetus may potentially be detected earlier and more dependably, because syphilis in the mother may be...
asymptomatic and difficult to diagnose if not detected on routine screening at the first prenatal visit. The sonographic findings of fetal ascites, hepatosplenomegaly, placental thickening, and hydrops fetalis are associated with fetal syphilis [2]. In the present case, prenatal ultrasonography led the authors to diagnose fetal syphilis infection by revealing transient fetal pleural effusion, which is not often associated with congenital syphilis. Fetal pleural effusion is a rare condition, with an estimated incidence of 1/10,000 pregnancies [9]. The clinical course varies according to the underlying pathophysiology from spontaneous resolution to progressive accumulation of fluid with a high risk of stillbirth and poor perinatal outcome [10]. Fetal pleural effusion may resolve spontaneously in about 22% of cases [11], but this benign course does not guarantee that the fetus will be normal and healthy. Once fetal pleural effusion is detected, the clinician should rule out associated fetal conditions by detailed ultrasonic analysis of fetal anatomy, fetal karyotyping, and maternal serological testing for congenital infection, regardless of its clinical course. In the present patient, the authors diagnosed a congenital syphilis infection through fetal and maternal evaluation, although the mild pleural effusion was completely resolved at the next follow-up visit.

Pregnant women who are treated with penicillin should be monitored for serologic response to treatment. Treatment failed in the present patient because preterm labor began secondary to placental abruption at only one week after treatment. Neonatal examination confirmed the congenital syphilis, and neurologic impairment proceeded in spite of postnatal treatment.

In summary, the present authors report a case of congenital syphilis diagnosed following detection of a transient unilateral pleural effusion on routine prenatal ultrasonography. As the incidence of congenital syphilis has increased in Korea, the authors recommend the serological testing of all pregnant women for this disease at the first prenatal visit. In addition, all physicians should be aware that this sexually transmitted disease is re-emerging and may be revealed as a fetal abnormality in an asymptomatic mother.

References


Address reprint requests to:
H.J. SEOL, M.D.
Department of Obstetrics and Gynecology
Kyung Hee University Hospital at Gangdong
892, Dongnam-ro, Gangdong-gu
Seoul 134-727 (Korea)
e-mail: seolhj@khu.ac.kr
Introduction

Hypoparathyroidism is rarely seen during pregnancy and the most common cause is after injury that happens accidentally to the parathyroid glands during head and neck surgery [1, 2]. Usually, it lasts for only a short period of time in about 12% of patients undergoing surgery, but 3% of patients present permanent hypocalcemia [3, 4].

The parathyroid glands produce too little parathyroid hormone (PTH) and this causes blood calcium levels to fall and blood phosphorus levels to rise. The symptoms of hypoparathyroidism vary depending on the cause, the speed at which the condition develops, and the effectiveness of treatment [5]. Women who develop hypoparathyroidism quickly can have a tingling sensation in the hands or feet or around the mouth, such as paraesthesia, unusual muscle movements, muscle cramps, feeling tired, irritable, anxious or depressed [6].

Obviously, our target should be to maintain normocalcemia before, during, and after pregnancy, in order to avoid any adverse effects on the outcome. At every trimester of pregnancy and at regular intervals after delivery, serum calcium levels should be determined. The obstetrician and pediatrician have to collaborate and be aware of the situation [5].

Unfortunately there is no proven therapeutic management for treatment of hypoparathyroidism during pregnancy. This is particularly due to unknown usefulness of vitamin D or its analogues, as in various animal experiments have been reported teratogenic side-effects. Nevertheless, vitamin D or its analogues are necessary to control tetany predisposing to preterm labour and abortion [7, 8].

In the present article, the authors present the case of a 36-year-old female with persisted hypoparathyroidism after total thyroidectomy for cold nodules. They describe in detail the treatment strategies undertaken in this patient and their impact on the outcome of pregnancy and lactation.

Case Report

A 36-year-old gravida 2 para 1, was admitted in the antenatal clinic of Aretaieion University Hospital in Athens, Greece, at her 39 weeks of pregnancy with known hypoparathyroidism for the last ten years.

During her first pregnancy, she had an increased calcium requirement during the first two trimesters and was found to be hypocalcemic peripartum. The infant was born in July 2010 with cesarian section (c-section) and reports that she suffered a significant fall in calcium levels with developing symptoms of titanic spasm that required urgent management.

In her second pregnancy, she changed hospital and clinician and the medication included calcitriol (0.25 μg) two tabs, one gm of calcium and 200μg L-thyroxine for her hypothyroidism daily. Serum calcium level was normal (1.9 mmol/L) and was monitored monthly. The dose of calcitriol was increased to one μg/day from 34 weeks of pregnancy. She did not mention tetanic convulsions or other symptoms during the period of her pregnancy. Elective c-section was performed at 39 weeks and one day of pregnancy. Before the surgery, serum calcium level was measured, and was found decreased, actually below 1.7 mmol/L. So, it was decided to be treated intraoperatively and until the patient was able to eat postoperatively with calcium gluconate iv diluted in a saline solution of 500 ml, in order to establish again normal levels. A female baby of 3.380 gr was delivered. Serum calcium level of baby was 2.3 mmol/L after delivery. Mother and baby were healthy at the time of discharge from the hospital. The mother was advised to continue with above dose of calcitriol and calcium and follow up was continued with her endocrinologist.
Discussion

In normal pregnancy, it is noticed that maternal serum concentration of 1,25(OH)2D3 rises early in the first trimester of pregnancy, and continues to increase during the third trimester [9-12]. After delivery and especially on the third day, it decreases to a non-pregnant level [13, 14]. The reason why synthesis of 1,25(OH)2D3 is increased in the mother especially in the third trimester, can be explained by the high fetal uptake of calcium into the skeleton in this period of time [15].

The lack of PTH in case of hypoparathyroidism during pregnancy reduces metabolism of endogenous vitamin D to 1,25(OH)2D3. Substitution therapy in this case requires a compound, which bioavailability is immediate and predictable. Calcitriol, in contrast to prodrugs such as cholecalciferol and tachysterol, has a much shorter, dose-independent half-life. The risk of teratogenicity in humans and animals seems to be small as long as the concentration of serum calcium and the 1,25(OH)2D3 remain in lower levels [7, 15].

In the present case the maximum dose of calcitriol was 1.00 μg/day and was given after 34 weeks of pregnancy. No teratogenicity or toxicity signs were found in the outcome and serum calcium level was normal after birth.

Pitkin recommends a daily dosage of calcitriol ranging between 0.5 and 3.0 μg/day with advancing gestation in order to maintain a normal maternal serum calcium level [16].

Conclusions

In case of secondary hypoparathyroidism during pregnancy, the treatment of choice consists on supplementation of oral calcium with suggested dose of calcitriol. The normal range which serum calcium concentration should be kept is between 2.00 and 2.20 mmol/L., and generally requires a calcitriol dose between 0.25 and 3.00 μg/day. A combination of 0.25 μg/day calcitriol and one g/day of calcium supplementation should be the correct initial dose, but has to be adjusted according to the physiological requirements during pregnancy. The dosage has to be increased after the second trimester with further elevation in the third trimester. Serum calcium levels should not fall below 1.70 mmol/L. Intraoperatively and until oral intake is possible, the patients should be treated with intravenous administration of calcium gluconate with parallel frequent measurement of serum calcium levels in order to main calcium levels within the desired levels.

References


Address reprint requests to:
N. CHADOS, M.D.
2nd Department of Obstetrics and Gynecology
Medical School of Athens,
Aretaieion University Hospital
Vas. Sofias, 76, 11528 Athens (Greece)
e-mail: nchados@yahoo.com
MRI in the assessment of prolapsed pedunculated submucous leiomyomas: two case reports

V. Fiaschetti¹, M. Fornari¹, V. Cama¹, M. Rascioni¹, V. Liberto¹, G. Sorrenti², G. Simonetti¹

¹Department of Diagnostic Imaging, Interventional Radiology and Radiation Therapy, University of Tor Vergata, Rome
²Department of Obstetrics and Gynecology, University of Tor Vergata, Rome (Italy)

Summary
Uterine leiomyomas are the most common benign gynecological tumors affecting 20-30% of women in reproductive age. Despite their benignity, in some cases several symptoms may require surgical intervention. Submucosal leiomyomas are less frequent (5-10%), but are usually asymptomatic. Approximately 2.5% of the myomas are pedunculated and can protrude in the cervical canal. Symptomatic leiomyomas can be treated either by hysterectomy or myomectomy, and these procedures can be performed with several techniques. Whenever possible, hysteroscopic myomectomy is better because it has many advantages, as it also preserves future fertility. Two interesting cases of prolapsed pedunculated submucous leiomyomas are reported in order to prove that magnetic resonance imaging (MRI) is essential to choose the most appropriate treatment and to perform an adequate presurgical planning, which must be based on an overall assessment of the leiomyoma’s characteristics (number, location, size and presence or absence of a stalk) and the patient’s characteristics.

Key words: Prolapsed pedunculated submucous leiomyomas; Magnetic resonance imaging; Stalk; Hysteroscopic myomectomy.

Introduction
Uterine leiomyomas (myomas or fibroids) are the most common benign gynecological tumors affecting 20-30% of women in reproductive age [1, 2]. They are composed of whorls of smooth muscle cells with varying amounts of fibrous connective tissue. Usually they involve the myometrium of the uterine corpus, but they can also occur in the cervix (8%) [2].

Only 20-50% of uterine leiomyomas are symptomatic and the most common symptoms include menorrhagia, sometimes with associated anaemia, pressure symptoms (including urinary frequency, urgency, pressure on the bowel and on the back, and lower abdominal pain), dysmenorrhea, and infertility; but the precise relationships between their sizes and locations and their clinical implications are still not clear. Despite the benignity of uterine leiomyomas, in some cases several symptoms may require surgical intervention [2-4].

Leiomyomas may be classified into: submucosal, intramural or subserosal. The intramural leiomyomas are the most common and often asymptomatic (occasionally causing menorrhagia and infertility) and are located within the myometrium. The Subserosal leiomyomas are located beneath the serosa and are usually asymptomatic; but the pedunculated ones, however could undergo stalk torsion with acute pain [2]. Submucosal leiomyomas project into the endometrial canal. These are the less frequent (only 5-10%) [2,5], but usually symptomatic (dysmenorrhea, menorrhagia, and infertility) [2]. Approximately 2.5% of the myomas are pedunculated and [6] can protrude in the cervical canal (gradually dilating the cervix), or in the vagina [2]. When this occurs, the myoma becomes usually necrotic and sometimes infected, because an adequate blood supply is difficult through a long pedicle [7]. Moreover, although the pathogenesis of leiomyomas is still not clear, it is evident that estrogen and progesterone promote their growth [1, 2]. If they outgrow their blood supply or if there is stalk torsion, a degeneration could occur [2, 8]. The following two interesting cases of prolapsed pedunculated submucous leiomyomas are reported in order to prove that magnetic resonance imaging (MRI) is essential to choose the most appropriate treatment and to perform an adequate presurgical planning, which must be based on both the leiomyoma type and the patient’s characteristics [8].

Case Report
Case 1
In November 2013, a 44-year-old woman came to the present institute to perform a transvaginal ultrasound (TVUS). She reported regular menses every 28-30 days with smelling, vaginal bleeding, between menses, for several months. She also reported the presence of mucus during menses. She complained of mild chronic pelvic pains increasing during menstruation. The obstetric history of the patient revealed four miscarriages, between the second and the fifth month of pregnancy. Later, in 2007, she had a cesarean section at 36 weeks, with prophylactic deltacortene. In her general medical history she reported multiple sclerosis.
patient reported a previous gynecological examination, in which no significant findings were evidenced, except for the presence of a bulky uterus and vaginal bleeding after the introduction of the speculum. On TVUS an inhomogeneous and ill-defined, mainly hypo-echoic, gross area was found in the body of uterus, close to the endometrial line, displacing it. Since this area was suggestive of adenomyosis, although dubious, the authors decided to perform a pelvic MRI. It was performed using a 1.5T scanner with a surface coil (pelvic phased-array coil). A peristaltic inhibitor (ten mg of hyoscine-N-butylbromide, buscopan) diluted in ten ml of saline solution, was intravenously administered to the patient, to reduce gastrointestinal tract peristalsis. The imaging protocol consisted of T1-weighted (w) Turbo Spin-Echo (TSE) on axial (perpendicular to the major axis of the uterus) (TR/TE 1035/15 ms; section thickness 3.5 mm; intersection gap 0 mm) and sagittal plane (TR/TE 606/14 ms; section thickness 3.5 mm; intersection gap 0.35 mm) and T1w spectral presaturation inversion recovery (SPIR) on axial (TR/TE 1280/15 ms; section thickness 3.5 mm; intersection gap 0 mm) and sagittal plane (TR/TE 606/14; section thickness four mm; intersection gap one mm), T2w sequences on axial (TR/TE 4406/90 ms; section thickness 3.5 mm; intersection gap 0.35 mm) and sagittal (TR/TE 3814/90 ms; section thickness 3.5 mm; intersection gap 0 mm) and coronal plane (TR/TE 3790/90 ms; section thickness 3.5 mm; intersection gap 0.35 mm); T1 high-resolution isotropic volume excitation (THRIVE) dynamic three-dimensional sequence turbo gradient echo with fat suppression (TR/TE 4/1 section thickness four mm; intersection gap one mm), after gadolinium bolus injection (gadopentetic acid and dimeglumine salt) of 0.1 mM/kg at two ml/second, followed by saline solution (20 ml), and axial T1w TSE after contrast media (TR/TE 575/10 ms; section thickness 3.5 mm; intersection gap 0 mm). On MRI an oval well-defined and pedunculated formation was found in the uterine cavity (Figures 1A-D). It was linked with the uterus by a soft-tissue stalk, approximately 16 mm wide and located in correspondence with the right postero-lateral wall of the body-fundus of the uterus (Figure 1B). It extended caudally for about 6.3 cm up to the uterine cervix (internal cervical os). This formation had heterogeneous signal intensity, mostly low signal intensity on T1w images (Figure 1C), high signal intensity on T2w images (Figures 1A-B), and heterogeneous contrast enhanced (Figure 1D). The images were suggestive of prolapsed pedunculated submucous leiomyomas with degeneration. Another very small pedunculated submucous leiomyoma (approximately six mm), at the postero-lateral left wall of the fundus, was found. MRI also showed a uterus increased in size and noticeably fibromatous. Then the patient was hospitalized in the Department of Gynecology and Obstetrics of the present Institute and laboratory exams revealed an

Figure 1. — Axial (A) and sagittal (B) T2-weighted images, and axial T1-weighted images before (C) and after (D) the administration of the contrast medium, in a 44-year-old patient, showing an enlarged and fibromatous uterus. On axial T2-weighted image (A), there is an oval well-defined formation located in the uterine cavity. This formation has heterogeneous (mostly high) signal intensity on T2w images. On T1w images, this formation has heterogeneous (mostly low) signal intensity, before the administration of the contrast medium (C), and heterogeneous contrast enhanced after the administration of the contrast medium (D). Sagittal T2-weighted image (B) clearly shows that this formation is linked with right postero-lateral wall of the body-fundus of the uterus by a soft-tissue stalk (approximately 16 mm wide), that is not visible on axial T2 image. This image is suggestive of prolapsed pedunculated submucous leiomyomas with degeneration, that extends caudally for about 6.3 cm up to the uterine cervix (internal cervical os).
iron deficiency anemia (hemoglobin 9.8 g/dl). Given the patient’s age and the diffuse uterine fibromatosis, it was decided to perform a hysteroscopic hysterectomy under general anesthesia. Histological examination confirmed the diagnosis of fibromatous uterus with a large prolapsed pedunculated submucous leiomyomas, with areas of ischaemic infarction. The patient made an uneventful recovery and was able to go home on the second day after surgery.

Case 2

In December 2013, a 46-year-old woman, with the suspicion of a uterine cervical mass, was referred for a pelvic MRI from the Department of Gynecology and Obstetrics of the present Institute. She reported irregular menses with vaginal bleeding between menses for several months and mild anemia. She did not report pelvic pain. The obstetric history of the patient revealed two normal pregnancies with vaginal delivery. In 2005, the patient was subjected to conization for grade III cervical intraepithelial neoplasia (CIN3). She reported normal follow-up smear results, although the last smear had been done two years earlier. She had no significant problems in her general medical history, but she reported family history of cervical cancer. A gynecological examination revealed the presence of vaginal bleeding after the introduction of a speculum and a palpable mass in the cervix. On TVUS, a homogeneous and well-defined area, mainly hypoechoic, was found in the cervix. Because of her personal and family history, it was decided to perform a pelvic MRI in order to quickly clarify the diagnosis. An imaging protocol similar to that of the previous patient was done. On MRI a round well-defined formation with the largest component located in the cervix was found (Figures 2A-D). This formation was markedly hypointense with an hyperintense rim on T2w sequences (Figures 2A-B) and had a contrast enhanced, similar to that of the myometrium, but slightly heterogeneous, with a highly enhanced rim on T1w sequences after the administration of the contrast medium (Figure 2D). Through careful examination, a thin soft-tissue stalk in correspondence of the anterior wall of the uterine isthmus-cervix, was found (Figure 1B). The images, suggestive of prolapsed pedunculated submucous leiomyomas without degeneration, ruled out the diagnosis of malignancy. At the postero-lateral left wall of the fundus, another very small intramural leiomyomas (approximately seven mm), markedly hypointense on T2w sequences, was found. Given the likely benign nature, the patient preferred to avoid the hysterectomy. Therefore it was decided to perform an hysteroscopic myomectomy. Histological examination confirmed the diagnosis of leiomyoma without degeneration. The patient made an uneventful recovery and was able to go home the following day.
Discussion

MRI is the most accurate imaging technique for the detection and localization of leiomyomas. It is more sensitive and has a wider field of view than the ultrasound (US) [2]. In both cases the MRI has proven to be better than TVUS, especially in the first case where the TVUS was not diagnostic. In both cases the MRI clearly showed the uterine submucosal leiomyoma prolapsing into the uterine cavity. Especially, the T2w sequences have allowed the present authors to carefully circumscribe and define the leiomyomas and to detect their stalk. On MRI, small and non-degenerated leiomyomas (composed of whorls of uniform muscle cells with various amounts of collagen) are well-circumscribed masses with homogeneous low or intermediate signal intensity on both T1w and T2w images. In the second case, the round well-defined formation, markedly hypointense on T2w sequences, was found to be a non-degenerated leiomyoma on the histological examination. Cellular leiomyomas (with dense muscle cells and little or no collagen) have relatively higher signal intensity on T2w images and enhancement after contrast media. The larger and degenerated leiomyomas had heterogeneous signal intensity, mostly low signal intensity on T1w images and high signal intensity on T2w images, corresponding to degeneration and infarction observed at pathologic examination [2, 8]. However, it was difficult to distinguish the type of degeneration (hyaline, myxomatous, fatty, mucinous, or malignant) on MRI [8]. In the first case, the oval well-defined formation, with heterogeneous signal intensity and heterogeneous contrast enhanced, was found to be a degenerated leiomyoma on the histological examination.

MRI is essential for an adequate pre-surgical assessment, because the choice of a surgical intervention is based on both leiomyoma and patient’s characteristics [8]. Symptomatic leiomyomas, indeed, can be treated either by hysterectomy or myomectomy, or by uterine artery embolization (UAE) in selected cases. These procedures can be performed with different techniques, which have been significantly developed over the years. In the past, abdominal hysterectomy and laparotomic excisions were the two standard treatments for symptomatic submucous leiomyoma. The first successful procedure with fibroid size representing the main limiting factor was performed by Atlee in 1845 [7]. Whereas the first reported hysteroscopy myomectomy was performed in 1976, when Neuwirth and Amin resected a fibroid using an urologic resectoscope [5]. Since then, instrumentation evolution enabled the development of several techniques. Therefore the procedure and the technique must be chosen on the basis of an overall assessment of the leiomyoma characteristics (number, location, size, and presence or absence of a stalk) the patient’s characteristics (age, desire to preserve fertility, and clinical condition), the surgeon’s experience, and the equipment available [5, 8]. In order to preserve future fertility in young women or teenagers, the preferred surgical procedure for a submucosal myoma is a myomectomy [1]. Whenever possible, hysteroscopic myomectomy is better because it has many advantages, including the lack of an abdominal incision, short recovery time, and less blood loss [1]. Moreover, in the suspicion of a superadded infection of leiomyoma, the intervention through the vaginal route (compared to major abdominal surgical procedure), reduces the risk of infection spread, while minimizing the peritoneal exposure [9]. The possible complications, however, must not be forgotten, including bleeding, fluid overload (related to the complexity and duration of the procedure), incomplete removal of leiomyoma in one surgical time, and intrauterine adhesions (IUA) [1].

In order to perform a safe and complete hysteroscopic myomectomy, it is essential to establish the intramural extension that could considerably vary, influencing, therefore, the chance of achieving complete resection. The European Society for Gynaecological Endoscopy (ESGE) adopted a classification system, developed by Wamsteker et al. in 1993 [10], and is used worldwide. According to this classification, submucosal myomas are classified in the following types: G0, that is intracavitary with no intramural components, and appears joined with the cavity wall only through a thin pedicle (pedunculated submucous leiomyomas); G1, that is mostly intracavitary (>50%) with some intramural components and G2 with a larger intramural component (>50%) and lower intracavitary components (<50%). Lasmar et al. [11] recently proposed a new preoperative classification which considers also other parameters including the size of the nodule, the extension of its base with respect to the wall of the uterus, and the topography of the uterine cavity. A pedunculated submucous leiomyomas (G0) can be easily and safely treated with hysteroscopic myomectomy in a single procedure with fibroid size representing the main limiting factor [5, 7, 12]. In this case several effective techniques have been proposed (including resectoscopic slicing, ablation by neodymium-yttrium-aluminum-garnet laser, morcellation, and even outpatient procedure) [5, 13].

However, the hysteroscopic myomectomy for fibroids with intramural extension (G1, G2) may be sometimes a very technically complex procedure and the intramural extension influences the chance of achieving complete resection in one surgical time. This must only be done by expert surgeons and its real feasibility must be thoroughly evaluated preoperatively in order to minimize the incidence of complications, that are higher than in other hysteroscopic procedures. Several techniques have been developed to completely remove such fibroids but there is still no single technique proven to be unequivocally superior for treating these fibroids (G1- G2). Most techniques aim at transforming an intramural fibroid into a totally intracavitary lesion, to avoid a deep cut into the surrounding myometrium, thus reducing the risk of operative (i.e. bleeding, perforation) and long-term complications (i.e. IUA and uterine rupture). Unlike fibroids G0, advanced equipment must be used for a safe hysteroscopic
myomectomy. Whereas G1 fibroids may be often completely removed with one-step hysteroscopic myomectomy, G2 fibroids may require a two-step technique which is very effective and safe. Despite a second surgical step does not seem to be necessary in case of an incomplete removal, patients should always be advised of this possibility, because repeated hysteroscopies can cause greater distress in patients [5, 14].

Providing excellent delineation of the uterine zonal anatomy, a MRI enables accurate classification of leiomyomas and allows to determine their components (submucosal, intramural or subserosal) [1, 2]. However, attention must be paid because some prolapsed pedunculated submucous leiomyomas may have a considerable intramural component [7]. In both cases, the MRI enabled us to rule out the presence of a significant intramural component.

Hysterectomy is required in patients with multiple myomas [7], also because the myomectomy effectiveness is limited by the myoma recurrence rate, that is higher in women with multiple myomas, compared to those with a single myoma [1]. In the second case, a hysteroscopic myomectomy was decided instead of a hysterectomy to respect the patient desire, since only a further very small fibroid was found on MRI. Conversely, in the first case, given the diffuse uterine fibromatosis and the patient’s age, it was decided to perform a hysteroscopic hysterectomy.

It is very important to obtain a correct assessment of the leiomyoma size. In 30% of the prolapsed tumors the mean diameter varies from three to five cm [15]. A tumor size over five cm in diameter is more often associated with lower hemoglobin level and usually it requires hysterectomy, such as in the first case. [16] In case of huge prolapsed leiomyoma it is imperative to have a thorough preoperative evaluation [12]. An underestimation of the leiomyoma size must be avoided with the imaging [15]. According to the present authors’ experience, MRI is better than ultrasound for this purpose, especially in case of large leiomyomas (such as in the first case) because MRI has a wider field of view than US. In both cases the T2w sequences enabled the present authors to carefully circumscribe and determine the leiomyoma sizes.

Moreover also in the case where pedunculated submucosal leiomyoma is diagnosable from gynecologic examination because the mass has prolapsed into the vaginal canal, on gynecologic examination, it is not often possible to precisely identify the origin of the mass and the relationship of the mass with uterus and cervix. Therefore MRI can be really helpful to accurately delineate the stalk and uterine attachment of the prolapsed leiomyoma [8, 17]. In both cases it was possible to determine the stalk on T2 sequences, but in the second case a careful assessment of the images was necessary. This can help in planning a correct surgical approach [8]. Indeed the first-choice treatment for prolapsed pedunculated submucous leiomyomas is the hysteroscopic resection, but it is important to locate the stalk in order to avoid excessive bleeding by its ligation (avoiding hysterectomy) [15]. In particular, in the second case, the detection of a stalk and its exact localization prompted the present authors to perform a stalk ligation at the beginning of the procedure in order to avoid a heavy bleeding. Kim et al. suggested the “broccoli sign” as the radiologic findings of the prolapsed submucosal leiomyoma [17]. Indeed the “broccoli-like” appearance is due to a lobulated bulky mass (corresponding to the broccoli floret) connected to the endometrial cavity by a long stalk (corresponding to the broccoli stem) [17, 18]. This sign is readily identifiable on sagittal MRI or on reformatted sagittal CT scan [17]. CT is less accurate than MRI in the detection of the broccoli sign [18]. The broccoli sign is useful in differentiating an uterine tumor prolapsed inferiorly through the cervical canal for a primary cervical malignancy, because the largest component of the prolapsed uterine tumors is often in the cervix and it may be mistaken for a cervical cancer, both clinically and radiologically [18]. Therefore, the true origin of the mass must be identified by detecting the connecting stalk with a careful assessment of the images, in order to exclude the cervical origin. Cervical cancer usually involves the uterine body by simply direct mass-like growth and superficial endocavitary growth seems to be rare and without pedicle [18]. In the second case, the MRI findings and stalk detection allowed to rule out a malignancy, encouraging a less invasive surgery, as preferred by the patient. However the broccoli sign is not specific for prolapsed submucosal leiomyoma, and it may also be due to a malignant prolapsed uterine tumor (i.e. endometrial carcinoma, adenosarcoma, and carcinosarcoma) or it may be secondary to a complication of UAE [18]. The transcervical passage of leiomyoma after UAE can occur in 2.2% to 7.7%, over three years following the procedure [4]. Nonetheless, malignant prolapsed uterine tumors often appear to have a relatively good prognosis. Despite a bulky and aggressive imaging appearance, their growth pattern tend to be endoluminal (following the “path of least resistance” and prolapsing through the cervix), rather than systemic. However tumors do retain an invasive capacity and sometimes a uterine tumor prolapsed through the cervical canal may invade the cervix. Usually, a gross cervical invasion is evident on MRI [18].

A non-puerperal uterine inversion must also be excluded. Non-puerperal uterine inversion is a very rare event, usually secondary to a submucosal leiomyoma (71.6%), which can be classified as incomplete, complete, or prolapsed, depending on the degree of the extension of the uterine corpus beyond the cervix. It can be difficult, both clinically and sonographically, to determine the degree of the inversion and the point of the leiomyoma attachment causing the inversion. MRI may help in making a correct preoperative diagnosis and a proper plan of the surgery, which are both important to avoid damage to the uterus and surrounding organs (bladder, ureters), especially in the cases where the procedure by the vaginal route is chosen [9, 19].

The MRI allows the differential diagnosis with other possible causes of pain or pressure complaints, such as adeno-
myoma (which can be treated only with hysteroscopy, while for the leiomyoma, a myomectomy can be performed), endometriosis, adnexal masses, pelvic inflammatory disease, pelvic varices, or bowel- or spine-associated causes [2, 3]. In the first case at TVUS, the degenerated prolapsed pedunculated submucous leiomyomas was mistaken for adenomyosis, whereas the MRI allowed a correct diagnosis. Although irregular margin of a uterine leiomyoma on MRI, may suggest sarcomatous transformation, the diagnosis of leiomyosarcoma is often done on the histological examination of a resected mass (presumed benign) [2]. Finally, by pelvic MRI we can assess and monitor the results of a previous myomectomy to establish the need for further treatment [15].

Conclusion

MRI is an accurate imaging technique for the detection and localization of leiomyomas and is better than TVUS, because it is more sensitive and has a wider field of view. Especially the T2w sequences allow to carefully circumscribe and define the leiomyomas, to determine their components (submucosal, intramural or subserosal), to obtain a correct assessment of the their size, to detect their stalk and uterine attachment, to precisely identify the origin of the mass, and the relationship of the mass with uterus and cervix. Whenever possible, especially in young women, myomectomy is preferable (with respect to hysterectomy) for a submucosal prolapsed myoma because it is less invasive and enables future fertility preservation. Several techniques have been developed to completely remove such fibroids but in each case the real feasibility must be thoroughly evaluated preoperatively in order to minimize the incidence of complications. Therefore MRI is essential for an adequate pre-surgical assessment, allowing a thorough preoperative evaluation.

References


Pulmonary embolism during delivery – treatment and outcome

M. Zamurović, D. Damnjanović
University Clinic for Obstetrics and Gynecology “Narodni front”, Belgrade (Serbia)

Summary
Pulmonary embolism during delivery is not a frequent occurrence. It is often impossible to ascertain whether it is a case of embolism by amniotic fluid or thromboembolism. Diagnostics of pulmonary embolism in labor is based solely on clinical symptoms. Immediate interdisciplinary treatment with cardiopulmonary resuscitation, hemodynamic stabilization, and correction of haemostasis disorders play a decisive role in prognosis. This paper presents diagnostics, treatment, and consequences of pulmonary embolism in expulsion phase during delivery in epidural anesthesia of a multiparous patient aged 37.

Key words: Pulmonary embolism; Delivery; Amniotic fluid embolism.

Introduction
Amniotic fluid embolism is a life-threatening obstetric complication in pregnancy and delivery. According to data from references, it occurs in two to eight of every 100,000 deliveries [1]. The fact that mortality is 11% to 44%, places it among the leading causes of maternal death.

Etiopathogenesis of amniotic fluid is still not thoroughly clarified. Amniotic fluid can reach mother’s circulation by endocervical veins, lesions of the uterus or the site of placental attachment. According to references, amniotic fluid embolism occurs most commonly during cesarean section (76% of all cases described in literature), followed by vaginal deliveries of patients with polyhydramnion and multiple pregnancies (55% of all cases described in literature). In rare cases, it also occurs during pregnancy following intrauterine surgery or blunt abdominal trauma.

As far as pulmonary thromboembolism during labor is concerned, according to data from references, venous thromboembolism is the major cause of maternal morbidity and mortality during pregnancy or early after delivery and it remains a diagnostic and therapeutic challenge. The Confidential Enquiry into Maternal Deaths (2006-2008) showed that venous thromboembolism is nowadays the third leading cause of direct maternal mortality, beside sepsis and hypertension [2]. The prevalence of venous thromboembolism has been estimated to be one per 1,000-2,000 pregnancies.

There is not much data to be found in references concerning pulmonary embolism during delivery itself. Mortal outcome can be avoided by quick clinical diagnostics and urgent treatment either in case of thromboembolism or amniotic fluid embolism [3].

This paper presents diagnostics, treatment, and consequences of pulmonary embolism occurring in the expulsion phase of delivery in epidural anesthesia in the case of a multiparous patient aged 37.

Case Report
Patient whose case is described in this paper was admitted to the Clinic for delivery which started by spontaneous uterine contractions at 37 weeks of gestation. The pregnancy was complicated by gestational diabetes, diagnosed at 21 weeks of pregnancy. During pregnancy, a hygienic dietary regime was followed. Even though glycemia was maintained within normal physiological range, fetal biometric parameters during the last trimester indicated a macrosomic fetal growth and polyhydramnion. Aside from gestational diabetes mellitus, the patient did not suffer from any other illnesses. During labour, epidural anesthesia was performed with 0.125% bupivacaine in bolus, followed by continuous anesthesia by pump. Uterine contractions were made painless. Induction of delivery was stimulated with oxytocin. Amniotomy was performed, showing clear and abundant amniotic fluid. Fetal heartbeat and uterine contractions were monitored throughout delivery, which had a normal course. The dosage of epidural anesthetic was reduced when cervical dilation reached eight cm, so that the patient gradually started to feel uterine contractions, followed by labor pains with two expulsion uterine contractions, baby’s head reached the pelvic floor. At this moment the patient started to complain of nausea and urge to vomit. Epidural anesthesia was discontinued. During the next contraction patient complained about chest pains, and consequently started to cough. Coughing then became more severe. Bloody secretion discharged from the mouth. Uterine contractions and pains continued while patient first became cyanotic, then lost consciousness, pulse in large blood vessels became impalpable, and arterial pressure immeasurable. Patient went into cardiac arrest. The baby was extracted by vacuum extractor. At the same time, the patient was intubated and cardiopulmonary resuscitation was started. A live female baby was born, weighing 3,350 grams and 51 cm in length, having the Apgar score of 5/6. Baby was immediately put in care of...
a pediatrician. The placenta was extracted manually, followed by manual revision of the uterine cavity, and perineal laceration was sutured. Cardiopulmonary resuscitation was performed simultaneously with obstetric interventions in the fourth stage of labor. Due to suspected pulmonary embolism, indicated by the clinical picture, bolus dose of 5,000 i.v. of heparin was administered intravenously. Patient was ventilated through the tube by Ambu balloon accompanied by external heart massage and therapy by medicaments. Since heartbeat was not reestablished by urgent cardiopulmonary reanimation, heart defibrilation was performed. Heart function was reestablished only after the third defibrilation cycle and stabilized after two to three minutes. After heart function was restored, patient started to bleed vaginally, probably due to previous intravenous administration of heparin. Thus manual revision of the uterine cavity had to be repeated and intravenous therapy by uterotonics was administered. Protamine sulphate was prescribed to contrast the hemiparetic action and uterine bleeding was stopped.

Mechanical ventilation type IPPV was continued in the ICU, monitored by an anesthesiologist and accompanied by therapy. A consultation with a neurologist was performed immediately. It was concluded that patient’s condition was caused by hypoxic-ischemic encephalopathy which occurred as a consequence of cardiac arrest caused by acute pulmonary embolism. Differential diagnostic dilemma remained whether this was a case of pulmonary embolism by amniotic fluid or thromboembolism. General state of the patient required acute recouperm of blood, blood derivatives, plasma expanders, fluids, and other medicaments under intensive monitoring. MR scan, performed several days later, when patient’s condition became more stable, verified subacute ischemic lesions in temporofrontoparietal region and to a lesser extent in the occipital lobe, accompanied by lacunar infarctions in corpus callosum. Since gynecological findings after childbirth were normal, treatment was continued under supervision of a neurologist. Patient recovered, but consequences remained. Hypoxia related sequelae, such as hemiparesis of the right arm and global aphasia were present. Patient was sent to rehab including speech therapy to which she responded well; only a mild, almost imperceptible, hemiparesis of the right arm still persists, while auditory comprehension and verbal capabilities have been significantly improved.

Discussion

Amniotic fluid embolism is a disorder with a high mortality rate, because it often causes sudden respiratory failure, circulatory collapse and disseminated intravascular coagulation (DIC) [4]. Fortunately, both pulmonary thromboembolism and amniotic fluid embolism rarely occur.

According to available data from references, amniotic fluid embolism occurs more frequently during labor than during pregnancy, while pulmonary thromboembolism is less frequent during delivery but occurs during pregnancy. Both described conditions are very complex and difficult to diagnose and choose appropriate treatment. They are life threatening both for the mother and for the child. During delivery, it is very difficult to diagnose the pathogenetic mechanism of pulmonary embolism. Pulmonary embolism, amniotic fluid embolism, cardiomyopathy, arrhythmias, sepsis, and non-cardiogenic pulmonary oedema were considered as differential diagnoses [5]. Rapid diagnosis and immediate interdisciplinary treatment are essential for good outcome.

As it was ascertained that the most significant risk factors for pulmonary embolism during delivery are mother’s age over 35, placenta previa and multiple pregnancies, and keeping in mind that the number of expectant mothers with above described risk factors has been on the rise recently, an increase in the number of embolic complications during pregnancy and delivery itself is expected in the future. In order to prevent sudden maternal death, the following is necessary: urgent treatment with cardiopulmonary resuscitation, hemodynamic stabilisation, correction of haemostasis disorders, as well as prompt extraction of the baby and timely application of intensive therapy.

References

Cesarean scar pregnancy (CSP) is a very rare type of ectopic pregnancy, with an estimated incidence ranging from 1:1800 to 1:2226 pregnancies [1–2]. The true incidence of CSP is not known because of the very limited literature on this subject [3], and the exact etiology and mechanism of action of CSP are poorly understood. However, it is thought that a CSP occurs if a blastocyst implants on fibrous scar tissue within a wedge-shaped myometrial defect in the anterior lower uterine segment at the site of a previous cesarean scar [4]. It is often misdiagnosed as an aborting pregnancy. CSP may result in life-threatening complications such as uterine rupture, massive hemorrhage, disseminated intravascular coagulation, and even maternal death [5]. Transvaginal ultrasonography can be used to diagnose CSP in its early stages [6] but management guidelines for this disease are lacking. Thus, each patient with CSP should be evaluated on an individual basis [5].

A pseudoaneurysm is an extraluminal collection of blood with turbulent flow that communicates with the flowing arterial blood through a defect in the arterial wall. Pseudoaneurysms typically form because of local trauma with vascular injury. If a punctured or lacerated artery does not seal completely, blood may escape and diffuse through adjacent tissues, thereby collecting in a perivascular location. If this blood maintains a communication with the patent vessel, a pseudoaneurysm may form [6, 7]. A “covert” pseudoaneurysm can easily result in massive hemorrhage, potentially resulting in the necrosis of hemorrhagic tissue and life-threatening hemorrhagic shock. Uterine artery pseudoaneurysm (UAP) is a rare consequence of cesarean section [8, 9]. UAP can be diagnosed with angiography and color Doppler ultrasound [10, 11], which reveal a blood-filled cystic structure with a “swirling” arterial flow pattern [6, 12].

Here, the authors review a rare case of CSP associated with UAP. They also discuss the presentation, diagnosis, management strategies, and outcome for the patient.

**Case Report**

A 36-year-old female (G2P1) with a previous early miscarriage was admitted to the present hospital because of irregular vaginal bleeding. She had undergone a low-segment cesarean delivery 16 years previously, and had regular menstrual cycles since. Uterine curettage had been conducted 46 days previously due to an aborted pregnancy that was diagnosed by ultrasound at another hospital. Massive uterine bleeding had been noted during that procedure, which was controlled with medication.

Clinical examination revealed a normal pulse rate and normal blood pressure (110/65 mmHg). Gynecological examination demonstrated a smooth, normal-sized cervix but some dark-red blood in the vagina. The uterus was in a forward position and the uterus size was typical of a pregnancy in the third month. The uterus showed a medium mass, suitable activity, and pain upon gentle palpation. Both adnexa were normal.
Ultrasound images revealed a complex area of mixed echogenicity measuring 7.5 × 6.5 × 6.1 cm in the anterior wall of the middle–lower uterine cavity, with no clear border with the muscle layer and ≈ four mm away from the nearest placenta percreta. Color flow Doppler confirmed a large serpiginous vessel ≤ nine mm in diameter (Figure 1). The peak systolic velocity was 15.24 cm/s, and the resistance index was 0.58. According to the ultrasonographic images, a diagnosis of trophoblastic disease was considered.

After hospital admission, laboratory data revealed levels of hemoglobin (Hb) to be 85.0 g/L, beta-human chorionic gonadotropin (β-hCG) to be 1,127 IU/L, and progesterone to be 7.66 mmol/mL. A diagnosis of CSP was established according to the aforementioned findings and absence of cardiac, renal, hepatic, and blood system diseases. After appropriate counseling, the patient willing to receive uterine artery embolization (UAE) and intra-arterial methotrexate (MTX) infusion was transferred to Interventional Radiology for embolization. The next day, the patient was treated by UAE, during UAE, right UAPs were observed by angiography (Figure 2). The patient subsequently accepted embolization with an intra-arterial infusion of MTX 75 mg and polyvinyl alcohol (PVA) granules. UAP and extravasation of contrast agent disappeared after transmicrocatheter PVA embolization for the parent artery (Figure 3). The patient showed a serum level of β-hCG of 403.70 IU/L one day after UAE, which was reduced to 22.08 IU/L five days after UAE. A subsequent reduction in vaginal bleeding was observed. Repeat ultrasound imaging of the pelvis seven days after UAE showed a hematoma measuring 7.0 × 6.0 × 5.0 cm (Figure 4) without blood-flow signals within the mass. Uterine curettage was undertaken under ultrasound guidance. Blood loss during curettage was ≈ 50 mL. Histopathological examination revealed that most of the removed tissue consisted of blood clots, with inclusions of degenerative and necrotic tissue and highly degenerative chorionic villi.
Eight days after uterine curettage, the patient displayed a serum level of β-hCG of 10.41 U/L. The bleeding had stopped, and she was discharged from hospital. Weekly outpatient assessment of β-hCG levels and monthly transvaginal sonographic examinations to evaluate the size of retained products of conception were recommended [10]. Weekly outpatient clinical assessment of β-hCG until it is undetectable and monthly transvaginal sonographic examinations to evaluate the size of retained products of conception were recommended [13]. Two weeks after leaving the hospital, serum levels of β-hCG fell to normal levels. Transvaginal sonographic imaging of the pelvis one month after discharging from hospital showed a hematoma measuring 2.5 × 1.6 × 2.0 cm (Figure 5). Three months after hospitalization, ultrasound images appeared to be normal. The patient returned a normal menstrual cycle 2.5 months after hospitalization.

Discussion

In cases of CSP, the gestational sac is embedded in the myometrium, and the fibrous tissue of the cesarean scar (a long-term complication of abdominal delivery) separates from the endometrial cavity. According to recent reports, CSP may be more common than was previously thought, with an incidence of 6.1% in women with ectopic pregnancy and at least one previous cesarean delivery [2]. The lower uterine segment lacks muscle fibers, and the cesarean scar has poor levels of contractility. As a result, bleeding in a CSP can be difficult to control, and can lead to potentially life-threatening massive uterine hemorrhage or rupture.

Transvaginal color Doppler sonography is important in the early diagnosis of a CSP [1]. The criteria for CSP diagnosis was proposed by Jurkovic et al. [1] and Vial et al. [14] involved the following criteria: (i) an empty uterine cavity; (ii) an anteriorly located gestational sac at the level of the internal os, covering the visible presumed site of the previous lower uterine segment of the cesarean scar; (iii) evidence of functional trophoblastic/placental circulation on Doppler imaging; and (iv) a trophoblast between the bladder and the anterior uterine wall as a sign of deep implantation.

CSPs and UAPs can cause massive life-threatening uterine hemorrhage but are rarely seen together. To avoid massive hemorrhaging of the uterus, blind uterine curettage is not recommended once a CSP or a UAP is suspected. At the initial diagnosis by ultrasound, the clinician at the patient’s previous hospital may have mistaken the CSP for an abortion due to the lower gestational sac. Blind curettage in this case could result in massive uterine hemorrhage, and iatrogenic mechanical injury could lead to the formation of a UAP. At the present hospital the patient was misdiagnosed with gestational trophoblastic disease (GTD) by ultrasonography. GTD is a type of hypersecretory hCG tumor that is secondary to vesicular moles in 60% of cases. The misdiagnosis of GTD may have been due to several reasons: a prolonged disease course, excessive bleeding, large lesion size, and an unclear boundary between the lesion and scar. Moreover, the rich internal blood supply of the lesion meant that the UAP may have been mistaken for the new vessels of GTD. The characteristic ultrasonic imaging findings for GTD are that the interior and borders of the lesion have a rich blood supply, with an abundance of high-speed, low-resistance arteries and arteriovenous fistulae [15].

Conclusion

CSPs and UAPs can cause massive, life-threatening hemorrhage of the uterus, but simultaneous occurrence of both diseases is rare. UAE is the most effective hemostatic method for the treatment of CSPs and UAPs. This method can spare the uterus and reduce the chance of death [4, 15, 16]. If a subject presents with vaginal bleeding after a uterine-cavity procedure, clinicians should be vigilant with regards to CSP and UAP. To provide timely and effective treatment, a definitive diagnosis should be made quickly via ultrasound and/or angiography.

References


Address reprint requests to:
Q. XUE, M.D.
Department of Ultrasound Diagnosis
The Affiliated Jiangyin Hospital of
Southeast University Medical College,
163 Shoushan Rd, Jiangyin,
Jiangsu 214400 (China)
e-mail: xueqin010305@hotmail.com
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