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M.G. Porpora, S. Resta, E. Fugetta, P. Storelli, F. Megiorni, L. Manganaro, E. De Felip
Chronic pelvic pain in Spanish women: prevalence and associated risk factors. A cross-sectional study

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Summary

Objective: To determine the prevalence of chronic pelvic pain (CPP) symptoms and associated risk factors in Spanish women. Design: A cross-sectional study. Setting: in Malaga and its province in Spain. Population: women aged 18-65 years using non-probability sampling by quotas (n = 940), stratified by age and county. Materials and Methods: All the participants completed the CPPQ-Mohedo, a self-administered validated questionnaire able to discriminate between patients with and without CPP. Results: Taking a CPPQ-Mohedo score of ≥ 6 as an indication of CPP symptoms, the prevalence of CPP symptoms was 26.8% in the general population in women aged 18-65 years. After adjustment for age, those women who exercised had a lower CPPQ-Mohedo score than those who did not exercise (mean difference -3.02 ± 4.27). Higher (worse) scores were associated with the following factors: lifting and/or moving heavy loads during activities of daily living (3.57 ± 4.51), laxatives and/or a high-fiber diet (4.71 ± 5.07), history of recurrent urogenital infection (vulvovaginitis, cystitis; 4.40 ± 5.22), pelvic trauma (4.77 ± 4.55), irritable bowel (5.10 ± 5.50), anal fissure (7.46 ± 6.50) or uterine prolapse (13.66 ± 2.36). Conclusions: The prevalence of CPP symptoms in Spanish women is high and is associated with risk factors that should be addressed by multidisciplinary preventive, diagnostic, and therapeutic strategies. More prevalence studies are needed to determine the true situation concerning chronic pelvic pain in Spain.

Key words: Chronic pelvic pain; Prevalence; Risk factors; Urogenital infection; Uterine prolapse.

Introduction

Chronic pelvic pain (CPP) is generally defined as pain in the lower abdomen, pelvis or intrapelvic structures, lasting at least three to six months, which presents either continuously or intermittently, and in women is not associated exclusively with the menstrual cycle or pregnancy [1-2]. Although CPP affects 10-16% of men, with a greater frequency in the age range of 36-50 years [3], epidemiologically it has a higher incidence in women [4]. In the UK, an annual prevalence in primary care of 38/1,000 was found in women aged 15-73 years, a rate comparable to that of asthma (37/1,000) and back pain (41/1,000). The monthly incidence in primary care was 1.6/1,000 [5].

The impact of CPP on quality of life has been studied by several authors. Estimates are that 15% of women with CPP report time lost from paid work and 45% report reduced work productivity; 26% reported that they had stayed in bed more than half the day (bed day) on one or more days during the previous month because of pelvic pain (the mean number of bed days was 2.6 ± 2.4) [6]. Approximately half of those women with pelvic pain (52.7%) reported that their pain affected their activities (inability to carry out activities without taking analgesics or resting, and limitations in mobility, particularly moving and walking), and felt that their social, family, and sexual activities were affected [7].

Studies generally agree about the high prevalence of the syndrome [4, 6, 8, 9], affecting two to 16% of the population worldwide [10], with a lifetime incidence of 33% [11]. No agreement exists, though, on the terminology to denominate the syndrome or about the associated symptoms. Thus, while some studies define CPP as lower abdominal or pelvic pain lasting at least six months, which can be either continuous or intermittent, but is not associated with the menstrual cycle or sexual activity [1-2]; other definitions include wider regions affected by the pain (abdominal wall, umbilical area, and lumbosacral region) and also contemplate the resulting limitations in activities of daily living [12-13]. Other definitions, though, exclude pain related with intestinal or urogenital infection [6,9] or consider CPP to be present if the pain has lasted for more than three months [14]. Proof of all this confusion is the use and standardization of the questionnaire NIH-CPSI [15] not only for the disease it was designed for (chronic prostatitis), but also to provide prevalence data for several other disorders causing CPP. The suitability and relevance of this questionnaire, though, have not yet been assessed [16]. Accordingly, the prevalence data, and consequently the resulting conclusions, should be interpreted with caution.

The definition of CPP in the present study, as well as including lower abdominal or pelvic pain lasting at least six months, which can be either continuous or intermittent, but is not related with the menstrual cycle or sexual intercourse, also includes a score ≥ 6 on the CPPQ-M (Cuestionario Dolor Pélvico Crónico – Mohedo [Chronic Pelvic Pain Questionnaire – Mohedo]) [16]. The discriminative capacity of this instrument has already been validated among persons with and without CPP [16]. The purpose of this study,
therefore, was to determine the prevalence of CPP symptoms in women aged 18-65 years in Malaga and its province using the CPPQ-M questionnaire and, depending on the results, detect the main risk factors associated with a higher score.

Materials and Methods

Study type
The authors undertook a cross-sectional population-based study in 2011 in Malaga and its province.

Participant selection
The sample size needed was calculated to be 850 persons to estimate the confidence intervals for percentages with error margins less than three percent in the situation of greatest uncertainty. The sample size was proportional to the population pyramid of Malaga in age, and proportional to the nine counties in the province of Malaga. The participants were selected by non-probabilistic sampling and by quotas assigned to interviewees. The sample for this study included supposedly healthy non-institutionalized adults, aged between 18 and 65 years (n = 940 women).

Inclusion and exclusion criteria
The inclusion criteria required the participant to be resident in Malaga or its province, aged 18-65 years, and with a minimum cognitive level sufficient to read and understand the questionnaire and then complete it.

To study the risk factors associated with higher CPPQ-M scores, and taking into account the diagnosis and/or etiological factors considered to cause CPP having Vercellini evidence levels A and B [17], the authors analyzed the most prevalent risk factors for which they had a sufficient number of cases (Table 1). Thus, persons were excluded from the analysis if, at the time of the study, they had had any one of these factors for less than six months, as this was an exclusion factor. A total of 141 women were excluded.

Data collection
The data were collected by 154 students from Malaga university. According to their place of residence and family and/or social setting, each student was given a quota (varying in age group) of questionnaires to be completed. These quotas were assigned to represent the population pyramid of Malaga, in age and sex, and proportional to the nine counties in the province of Malaga. The students underwent a prior training period to learn about the justification and aims of the study, the inclusion criteria, and the methodology in order to guarantee the veracity of the data and their confidentiality. They also had to be available at all times to answer any doubts the participants might have about the questionnaire. The tool used was the self-administered CPPQ-M questionnaire.

This questionnaire (http://www.salud.uma.es/cppq-mohedo/) groups the items in two dimensions (pain and quality of life) and provides a weighted score corresponding to the sum of the affirmative answers. The maximum score is 27 points [16].

Additional information was also collected regarding personal and demographic data, education level, occupation, pregnancies and births, contraception, physical activity, and postural, sexual, and dietary habits. The participants also provided relevant information about their past and present medical conditions, including any diagnoses and the corresponding treatment. This information was used for the risk-factor association study.

<table>
<thead>
<tr>
<th>System</th>
<th>Evidence A</th>
<th>Evidence B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynaecological</td>
<td>Endometriosis</td>
<td>Adhesions</td>
</tr>
<tr>
<td></td>
<td>Gynaecological</td>
<td>Benign cysts</td>
</tr>
<tr>
<td></td>
<td>malignancy</td>
<td></td>
</tr>
<tr>
<td>Ovarian remnant</td>
<td>Syndrome</td>
<td>Myomas</td>
</tr>
<tr>
<td>Pelvic inflammatory</td>
<td>Disease</td>
<td>Peritoneal disease</td>
</tr>
<tr>
<td></td>
<td>postsurgical cysts</td>
<td></td>
</tr>
<tr>
<td>Urological</td>
<td>Interstitial cystitis</td>
<td>painful bladder syndrome</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Colon carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td>Diverticular disease</td>
</tr>
<tr>
<td></td>
<td>Inflammatory bowel disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irritable bowel disease</td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Back or coccygeal region pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Postural alterations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuralgia (iliohypogastric, ilioinguinal, genitourinary)</td>
<td>or pudendal nerve)</td>
</tr>
</tbody>
</table>

Evidence A: Good consistent evidence between the cause and the chronic pelvic pain. Evidence B: Limited inconsistent evidence between the cause and the chronic pelvic pain

The level of education and the occupation were classified according to the scale proposed by the Spanish Society of Epidemiology working group on the measurement of social class in health sciences [18]. To analyze the influence of these factors on the prevalence of CPP symptoms, these factors were grouped into the following categories: low (illiterate or uncompleted primary education); medium (primary education completed, elemental or basic general education, or professional training); and high (higher education, university entrance exam, university graduates, and doctors of any discipline). The socio-economic status was classified as low, medium or high. The living environment was considered to be that of the usual place of residence, grouped by county: Antequera, Axarquia, Serranía de Ronda, Guadalteba, Valle del Guadalhorce, Costa del Sol, Malaga, Comarca Nororiental, and Sierra de las Nieves.

Physical activity was defined as specific physical activity for longer than 30 minutes at least three times a week, and a fiber-rich diet as the consumption of 20-35 grams of vegetable fiber per day (at least two pieces of fruit and three servings of vegetables and cereals).

The results obtained were transferred to a specially designed database. This enabled us to perform later verification by phone of all the data in those persons who reported symptoms of CPP and who had provided contact information.

Ethical considerations
The voluntary completion of the questionnaire implied acceptance to participate in the study, ensuring at all times the confidentiality of the data.

Statistical analysis
The results are shown as estimated mean differences between individuals with and without risk factors, 95% confidence interval and statistical significance for the null hypothesis that this difference is zero, adjusted by sex and age. The statistical analysis was performed with SPSS and verified with the R package, with the “boot” bootstrap libraries [19].
The main epidemiological characteristics of the study population are summarized in Table 2. The prevalence of CPP symptoms in women aged 18-65 years in Malaga and its province was 26.8%. Table 3 shows the prevalence by age group. The mean score on the questionnaire was 3.51 ± 4.7. No associations were found between the CPPQ scores and age.

The present study detected a group of 158 women (23.7%) who, despite having CPP symptoms for over six months, still had no obvious diagnosis (women who had no current medical diagnosis, but had CPP symptoms and, therefore, discriminatory scores on the CPPQ-M).

After correcting the epidemiological data for age, no significant differences were found between the women who had and did not have a risk factor, as well as the differences after adjusting for age are presented in Table 4. Those women who undertook physical activity had a lower CPPQ-M score than those who did not (mean difference: -3.02±4.27). Risk factors associated with a higher score were lifting and/or moving heavy loads during everyday activities (3.57 ± 4.51), consuming laxatives or a fiber-rich diet, recurrent urogenital infectious disease, pelvic trauma, irritable bowel, anal fissure, and uterine prolapse.

Table 2. — Main epidemiological characteristics of the population sample.

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>18-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-65</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>60.2 (± 8.8)</td>
<td>63.2 (± 8.8)</td>
<td>66.5 (± 12.5)</td>
<td>67.3 (±11.2)</td>
<td>69.0 (±13.4)</td>
<td>64.6 (±11.7)</td>
</tr>
<tr>
<td>Height</td>
<td>162.7 (± 15.9)</td>
<td>163.6 (± 6.8)</td>
<td>162.4 (± 5.7)</td>
<td>160.0 (±6.7)</td>
<td>155.3 (±22.9)</td>
<td>161.5 (±12.3)</td>
</tr>
<tr>
<td>BMI*</td>
<td>23.1 (± 3.2)</td>
<td>24.3 (± 4.0)</td>
<td>25.8 (± 4.5)</td>
<td>26.8 (±4.4)</td>
<td>27.4 (±4.4)</td>
<td>25.0 (±4.3)</td>
</tr>
</tbody>
</table>

Table 3. — Prevalence by age group

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age Group (Years)</th>
<th>Frequency and Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>18-29</td>
<td>65 (25.8%)</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>65 (25.8%)</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>48 (19.0%)</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>49 (19.4%)</td>
</tr>
<tr>
<td></td>
<td>60+</td>
<td>25 (9.9%)</td>
</tr>
<tr>
<td>Men</td>
<td>18-29</td>
<td>65 (25.8%)</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>65 (25.8%)</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>48 (19.0%)</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>49 (19.4%)</td>
</tr>
<tr>
<td></td>
<td>60+</td>
<td>25 (9.9%)</td>
</tr>
</tbody>
</table>

Table 4. — Factors associated with higher CPPQ-Mohedo scores

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Mean± SE</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>-3.02± 4.27</td>
<td>-0.29 — -1.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Moving and/or lifting heavy loads</td>
<td>3.57± 4.51</td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>Recurrent urogenital infectious</td>
<td>4.40± 5.22</td>
<td>3.02 — 0.02</td>
<td>0.047</td>
</tr>
<tr>
<td>Pelvic trauma</td>
<td>4.77± 4.55</td>
<td>3.59 — 0.18</td>
<td>0.030</td>
</tr>
<tr>
<td>Irritable bowel</td>
<td>5.10± 5.50</td>
<td>4.24 — 0.18</td>
<td>0.032</td>
</tr>
<tr>
<td>Anal fissure</td>
<td>7.46± 6.50</td>
<td>6.69 — 2.52</td>
<td>0.000</td>
</tr>
<tr>
<td>Uterine prolapse</td>
<td>13.66± 13.66</td>
<td>15.07 — 6.47</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Mean of the effect between persons who had the risk factor and those who did not. **ADL: Activities of daily living.
and/or a fiber-rich diet (4.71 ± 5.07), and a history of recurrent urogenital infection (e.g., vulvovaginitis, cystitis, (4.40 ± 5.22), pelvic trauma (4.77 ± 4.55), irritable bowel (5.10 ± 5.50), anal fissure (7.46 ± 6.50) or uterus prolapse (13.66 ± 2.36). The CPPQ-M scores for other conditions reported (inflammatory pelvic disease, endometriosis, hemorrhoids, constipation, bladder prolapse, benign cysts, myomas, or prior surgery) presented no significant differences.

Discussion

To the authors’ knowledge, this is the first time that a high prevalence of symptoms of this disease has been reported in Spain; 26.8% for Spanish women. This places Spain among the countries with the highest prevalence according to a recent review showing that the worldwide prevalence of CPP ranges from two to 24% [20].

CPP affects 14.7% of American women aged 18-50 years [6], 21.5% of Australian women [21], 24%-25.5% of women in New Zealand [4,7,9], and 15.1% of women of reproductive age in Brazil [22]. The high figure found in this study may be due to the wide age range contemplated.

The finding that 23.7% of the participants had discriminatory scores on the CPPQ-M although they had no actual diagnosis, may be due to the diagnostic difficulty of the syndrome, not seeking medical help, or the association in women with pain, which may lead to an underestimation of the diagnosis by the primary care physician [22-23].

Although an association has recently been found between CPP and current sexual intercourse and abdominal surgery (cesarian) [22], the epidemiological characteristics of the present sample agree with those of other studies concerning the lack of association between CPP and level of studies or education, socioeconomic status, parity, use of oral contraceptives, a low body mass index, living in the mountains, no sexual intercourse, and cesarian delivery or episiotomy [6, 24-26].

The current results coincide with those of Bartoletti et al. (although this study only assessed CPP in men) in finding significant differences (p < 0.001 in his study and p = 0.000 in the present) between CPP and mild alterations in eating habits, e.g., need for a fiber-rich diet and/or laxatives [8]. Since the study design did not contemplate verification of symptoms of constipation using the Rome III criteria, the present can only note that just 12.4% of those who had these eating habits reported suffering from constipation; and consequently reflect on the possible role of a fiber-rich diet in the development of CPP symptoms.

The present results also coincide with those of Vercellini et al., detecting a lower risk for CPP in those women who undertook physical exercise [27]. The present authors are unaware of the reason for this, although the presence or otherwise of pain may affect the practice of physical exercise.

Although the authors recorded information about postural habits (hours spent standing, seated, and driving), lifting heavy loads during activities of daily living, the type of delivery (natural vaginal, episiotomy, instrumental), and the type of contraception (barrier, IUD, surgery), they have found no relevant data with which to make comparisons and draw conclusions.

Although Vercellini et al. consider recurrent urologic infections with evidence grade C (causal relationship to CPP based on expert opinion) [17], the significance found in the current study and that in the study of Oliveira et al. [22] suggests the need to examine the etiologic role of these infectious processes, in an attempt to determine whether they occur before the CPP and thus contribute to its development or whether they are in fact the consequence of CPP.

Concerning other risk factors associated with CPP, the current results are in agreement with those of others who also found a link with intestinal, urinary, gynecological, and myofascial disorders [28-29], but with the associated diagnostic difficulty.

Specifically, concerning intestinal disorders, the present results also coincide with cross-sectional studies showing that approximately one-third of women with chronic pelvic pain have irritable bowel syndrome (IBS) [23,30]. In the present study, 42.1% (n = 16) of those who had a high CPPQ score reported having suffered from IBS in the past. As treatment of this disorder involves dietary manipulation and antispasmodic agents with fiber supplementation to relieve symptoms, this may partly explain the significance found in the present study with a fiber-rich diet.

Every year 235,000 new cases of anal fissure are reported in the US and about 40% of them persist for months and even years [31]. Lateral internal sphincterotomy remains the gold standard for definitive management of anal fissure [32]. In the present study, only seven percent of women had had surgery; it is therefore possible that the others had had an increased anal tone and chronic tension of the pelvic floor muscles for many years, something often associated with anal fissure [31]. In these patients, often with tenderness on palpation of the pubo-rectalis muscle, a paradoxical contraction of the pelvic floor muscles on attempted defecation has often been documented and its therapeutic reversal would correlate with clinical benefit [33-34].

Concerning gynecological disorders, endometriosis is the most common gynecological disorder causing CPP [35]. Thus, endometriosis plays a significant role due to its frequency and its effects on the quality of women’s lives. The lack of significance in the present study was probably due to the low number of women with endometriosis (n = 5), which explains why this did not reach statistical significance despite the high score on the questionnaire.

The exact prevalence of uterine prolapse is unknown. Forty percent of participants in the women’s health initiative trial in the United States had some degree of prolapse [36]. Delancey’s three levels of support are now accepted worldwide [37]. Uterine prolapse causes myofascial alterations; specifically, the cardinal-uterosacral ligament com-
plex breaks or is attenuated. Many symptoms have been attributed to prolapse, including perineal pain. One study showed that the number and intensity of symptoms increased with an increasing stage of prolapse [38]. In the present study, the women had high CPPQ scores but they received no gynecological examination to assess the level of prolapse; accordingly, the present authors are unable to determine any correlation with this. The association between prolapse and connective tissue metabolism is well established [39-40]. However, the causality of this association is unclear [41]. The results of these latter studies suggest that pelvic organ prolapse is an acquired disorder of the extracellular matrix and that therapies targeting matrix proteases may be successful for preventing or ameliorating pelvic organ prolapse in women [40].

If we consider that the myofascial component is an important protagonist in this dysfunction, mention should be made of the interesting studies undertaken relating to myofascial therapy. Manual myofascial therapy involves an elongation of tendons, aponeuroses, and other dense fibrous connective tissues. While little is known about the continued development of tissue hydration during the minutes and hours after tissue elongation, findings suggest that during this recovery period a gradual rehydration of the tissue can be expected, which tends to be associated with a gradual regain of the initial tissue stiffness [42]. Future in vivo studies are needed to determine whether and under what circumstances the in vitro changes in tissue hydration reported in that study also occur in living bodies. Such studies could have implications for a better understanding of the effects of various stretching routines as well as of myofascial manipulation on viscoelastic tissue properties.

The present study has identified several factors as independent predictors for symptoms of CPP. The authors have not, however, reached final conclusions about the causal relationship between these factors and CPP. A thorough investigation and other different types of studies are necessary to corroborate these results.

The CPPQ-M questionnaire is not a diagnostic tool for CPP. It is, however, useful to detect patients with CPP in primary care and refer them sooner for specialized care and appropriate diagnosis and treatment.

**Study limitations**

The questionnaire was self-administered. This has the advantages of convenience and low cost, but it also has the disadvantage of not being able to determine the actual reaction to each question or detect any doubts or misunderstandings, although the participants were able to consult any specific doubt and receive clarification about any particular question. Additionally, as the authors did not have direct access to the official medical history (and could not therefore confirm the information provided by the patients), they may have underestimated the true incidence, as it is not normal to state one has this sort of disease when it is not the case. Nor was a physical examination performed or objective diagnostic tests done to determine agreement or otherwise between the clinical findings and the score obtained on the CPPQ-M.

**Conclusion**

The prevalence of CPP symptoms is high (26.8%) in Spanish women. This is related with several risk factors (moving and/or lifting heavy loads, laxatives, and/or fiber-rich diet, recurrent urogenital infectious disease, pelvic trauma, irritable bowel disease, anal fissure, and uterine prolapse).

The lack of an international agreement about CPP and its definition limits an epidemiological study as there is no clear, well-defined objective in order to be able to compare the results. Thus, it is necessary to join forces to establish an agreement and, consequently, enable studies to be reproduced in other populations. Only after further studies will it be possible to determine the true situation with regards to CPP, and then design multidisciplinary preventive, diagnostic, and therapeutic strategies to deal with such an incapacitating syndrome.

**References**


Reproductive outcome and fetal karyotype of couples with recurrent miscarriages

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Summary

Purpose: The purpose of this study was to evaluate the relationship between fetal karyotype and parental chromosomal abnormalities, and assess the long-term reproductive outcomes in couples with recurrent miscarriages (RM).

Materials and Methods: The reproductive outcomes of 34 couples with abnormal karyotypes and RM were investigated. Ultrasound examinations were performed during pregnancy, fetal karyotypes were determined following miscarriages, and successful pregnancy outcomes were recorded. Results: Of the 34 couples, 20 individuals presented with chromosomal abnormalities, specifically in nine females and 11 males (45% vs 55%, χ² = 0.2833, p > 0.05). Fifteen couples (44.1%) possessed karyotype polymorphisms, of which the most common variant was a long Y chromosome in males. The reproductive outcomes of subsequent pregnancies consisted of 25 live births of phenotypically normal infants (73.5%), one infant with multiple malformations (2.9%), and eight RM (23.6%). With regards to karyotypes, 69.2% (9/13) of couples had inversions and 73.3% (11/15) had karyotype polymorphisms that resulted in live births of phenotypically normal babies. Fetal karyotyping was performed in a total of 29 cases. Normal karyotypes were present in 48.3% (14/29) of cases, whereas 41.4% (12/29) had abnormalities (either numerical or structural), and 10.3% (3/29) has a karyotype polymorphism. Conclusions: There is a positive correlation between chromosomal abnormalities and spontaneous miscarriages. A complete evaluation and special treatment should be provided to couples with a history of recurrent miscarriage(s) during a subsequent pregnancy, particularly when one partner is a carrier of chromosome abnormalities (i.e., inversions of chromosome 9 and long Y chromosome in males). Prenatal diagnosis is necessary in carrier couples suffering from more than two miscarriages.

Key words: Fetal karyotype; Parental karyotype; Recurrent miscarriage; Reproductive outcome.

Introduction

Spontaneous abortion is one of the most common complications of pregnancy, which can be classified as either sporadic or recurrent. Recurrent miscarriage (RM) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation, according to the guidelines of the American Society of Reproductive Medicine, and affects about one to three percent of the child-bearing population [1-5]. RM causes significant psychosocial morbidity and also presents couples with a challenge of having a family successfully. The causes of repeated pregnancy loss is multifactorial, including antiphospholipid syndrome, thrombophilies, infections, endocrine disorders, uterine structural abnormalities, autoimmune-related disorders, and genetic abnormalities [6]. Moreover, the etiology in 50% of RM cases is unknown. It has been confirmed that there is a relationship between chromosomal abnormalities and RM. Chromosomal abnormalities are identified in more than half of all miscarriages, and these mainly consist of reciprocal translocation, Robertsonian translocations, and inversions [7-9]. Furthermore, in couples with RM, structural chromosomal abnormalities range from 3% to 6% [10-12]. There have been already many reports on chromosomal analyses in couples with RM and the karyotypes of the abortuses. However, in most studies, there is a lack of detailed information on the long-term reproductive outcomes in carrier couples with RM. Furthermore, no previous studies have investigated the relationship between fetal karyotypes and pregnancy outcomes. In this study, the authors prospectively investigated the karyotypes of abortuses and live-born infants of RM couples that underwent appropriate therapy. Additionally, data on the growth and development performance of babies were collected. They aimed to assess the long-term reproductive outcomes of couples with chromosomal abnormalities and RM, as well as determine the relationship between fetal and parental karyotypes.

Materials and Methods

Patient characteristics

The subjects comprised of 34 couples that visited the Department of Obstetrics and Gynecology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, between September 2007 and August 2010. These couples ranged in age between 24 and 49 years, and all of them met the following criteria: 1) all the couples had a history of more than two consecutive RM, 2) abnor-
mal chromosome karyotypes were detected in either of each couple, including numerical abnormalities, structural chromosomal abnormalities, and karyotypic polymorphisms, 3) all couples were non-consanguineous, and the women were not pregnant when they visited the hospital, and 4) systematic examination and appropriate comprehensive treatment had been provided to all 34 couples. All of the couples that participated in this study signed an informed consent form.

Chromosomal analysis
In this study, the authors obtained chromosome preparations from routine peripheral blood lymphocyte, villi, amniotic fluid, and cord blood. Analysis of G-banded metaphase chromosomes was performed on cultured tissue samples using standard procedures. Karyotypes were described according to the 2005 guidelines of the International System for Human Cytogenetic Nomenclature.

Assessment of pregnancy outcomes
Subsequent pregnancy outcomes were followed up prospectively. The following items were recorded in detail, including fetal karyotype, gestational age at the time of pregnancy termination, and primary pregnancy outcome. A successful outcome was defined as the birth of at least one phenotypically normal child. The other reproductive outcomes comprised of miscarriages, stillbirths, and viable offspring with chromosomal abnormalities.

Ultrasound examination
The gestational age was calculated from the last menstrual period (LMP) and confirmed by measuring crown–rump length in transvaginal ultrasound examination. A careful examination for fetal abnormalities was performed in all cases.

Statistical analysis
Statistical analysis was performed using SPSS (version 16.0.). A $p < 0.05$ was considered statistically significant. The t-test, Chi-square test ($\chi^2$), and Fisher Exact test were used, where appropriate.

Results

Chromosomal abnormalities in couples
A total of 34 couples were included in the final analysis. The mean age of the females was $31.76 \pm 4.87$ years and the mean age of the males was $34.65 \pm 4.88$ years. The median number of clinically proven miscarriages was $2.56 \pm 0.93$. After karyotyping, 20 abnormalities were identified, more specifically in nine females and 11 males ($45\%$ vs $55\%$, $\chi^2 = 0.2833$, $p > 0.05$). There was one couple with abnormalities in both the female and male, whereas only one partner was found to carry a chromosomal abnormality in the other 18 cases. These abnormalities included both numerical and structural abnormalities, which consisted of translocations and inversions. In particular, there were four (20%) females with the mosaic Turner syndrome, three (15%) individuals with translocations (i.e., two male and one female), and 13 (65%) individuals with inversions in chromosome 9, where there were no significant differences between males and females ($53.8\%$ vs $46.2\%$, $\chi^2 = 0.0951$, $p > 0.05$).

<table>
<thead>
<tr>
<th>Reproductive outcome</th>
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<th>Karyotype abnormality</th>
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<tbody>
<tr>
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<td>25</td>
<td>46, XX, inv(2)(p12q21)1qh+</td>
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<td></td>
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<td>46, XY, inv(9)</td>
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<td>46, XY, inv(9)(p11q13)</td>
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<td>46, XX, t(1;12)(p13q13)</td>
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<td>45, XX, t(13q:14q)</td>
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<td>45, X[4]/46, XX[94]/47, XXX[2]</td>
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<td>45, X[5]/46, XX[100]/47, XXX[5]</td>
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<td>46, XY, inv(9)(p11q13)</td>
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<td>46, XY, small Y chromosome</td>
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<td>46, XX, 22pstk+</td>
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<td>46, XY, Y&gt;18</td>
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<td>46, XY, Y&gt;18</td>
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| Miscarriage          | 8  | 46, XY, 13pstk+ |
|                      |    | 46, XY, inv(9)(p11q13) |
|                      |    | 45, XY, t(13;14)(p11q11) |
|                      |    | 46, XX, 22pss |
|                      |    | 46, XX, inv(9) |
|                      |    | 46, XX, inv(9)(p11q13) |
|                      |    | 46, XX, inv(9)(p11q13) |

| Induced labor        | 1  | 46, XY, small Y chromosome |
|                      |    | (multiple malformations) |

Chromosomal polymorphisms in couples
Fifteen out of 34 couples (44.1%) presented with a karyotype polymorphism, which was more prevalent in males than females (80% vs 20%, $\chi^2 = 6.9283$, $p < 0.05$). Furthermore, the most common variant in males was the long Y chromosome, which accounted for 40% (6/15) of all polymorphisms.

Reproductive outcomes
After a systematic examination and appropriate treatment, all of the 34 couples became pregnant. Of the 34 pregnancies, 26 resulted in a live birth, where 25 (73.5%) of the offspring were phenotypically normal and one (2.9%) infant had multiple malformations (induced labor). The other eight (23.6%) pregnancies were RM. With regards to karyotypes, 69.2% (9/13) of the couples that had
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an inversion of chromosome 9 gave birth to healthy children, and two of the three couples that had translocations also had successful deliveries after 38 weeks of gestation. Four children were born to Turner syndrome females. It is worthwhile to note that 73.3% (11/15) of the cases with karyotype polymorphisms resulted in live and phenotypically normal births, including 54.5% (6/11) of cases with a long Y chromosome. The karyotypes of the RM couples and their pregnancy outcomes are presented in Table 1.

Fetal karyotypes

In five out 34 pregnancies, karyotyping was not performed because four of these couples decided not to undergo genetic testing and in one case the karyotype was uncertain due to culture failure. A total of 29 fetal karyotypes were included in this analysis. A normal karyotype was found in 14 out of the 29 cases analyzed (48.3%).

Among the 14 pregnancies with a normal fetal karyotype, ten (71.4%) of the cases ended in the birth of healthy infants, whereas one case resulted in induced labor due to multiple malformations and three pregnancies were terminated via abortions. The remaining 15 karyotypes were abnormal. Of these, 41.4% of the karyotypes (12/29) had abnormalities and 10.3% (3/29) displayed a karyotype polymorphism.

The fetal karyotype abnormalities included both numerical and structural chromosomal abnormalities. Four cases of numerical chromosomal abnormalities were observed in this study and all of the embryos terminated in the first trimester. Seven healthy children were born with structural karyotype abnormalities, and the most frequent of these were inversions, with only one abortus detected with a karyotype abnormality. Moreover, three children with a karyotype polymorphism was born phenotypically normal. The pregnancy outcomes according to fetal karyotype are listed in Table 2.

Discussion

Approximately ten to 15 percent of clinically diagnosed pregnancies end via spontaneous miscarriages [13]. Unfortunately, in many couples suffering from RM, the causes of this condition are unknown, partly because they differ between cases. While an increasing number of studies suggest that most RM are due to the presence of parental karyotype aberrations, according to literature, the incidence of chromosome abnormalities in couples that experience a spontaneous abortion is three to 11 percent [14-17]. In the present study, the proportion of parental chromosomal abnormalities among couples was 55.9% (19/34), which is much greater than that reported by others. The authors believe that the higher percentage may be because their subjects were selected from RM couples that had chromosomal aberrations. Further, the incidence of abnormal karyotypes was not significantly different between males and females ( \( p > 0.05 \)). With respect to the reproductive outcome, 73.5% of these couples had a live birth of a healthy infant in subsequent pregnancies. Thus, the authors suggest that karyotype analysis should be an integral part of diagnostics in both spouses with RM.

With regards to karyotype abnormalities, in the present study there were 20% of cases with numerical abnormalities, and 80% of cases with structural rearrangements. The results showed the majority of chromosomal anomalies in cases of structural rearrangements were inversions involving chromosome 9 inversions, which is a rearrangement of a segment that is reversed end to end. Recently, there has been some evidence indicating that an inversion of chromosome 9 leads to an increased risk of miscarriage in about 30% of affected couples [18-22]. In the present study, the authors identified 13 cases with an inversion involving chromosome 9, accounted for 19.1% of the examined patients. The incidence is much higher than that found in the general population, which was reported to be about one percent to 1.65%. It may be that inversions of chromosome 9 are one of the major chromosomal aberrations leading to RM. Furthermore, after a comprehensive evaluation and specific treatment paradigm, nine of these couples with

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<th>Reproductive outcome</th>
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<th>Offspring karyotype</th>
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<td>Healthy birth</td>
<td>20</td>
<td>46, XX</td>
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<td>46, XY, small Y chromosome</td>
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<td>46, XX, dup(1)(q12q21)</td>
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<td>46, inv(9)(p11q13)</td>
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<td>46, XX, 13pstk+</td>
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<tr>
<td>Loss of pregnancy</td>
<td>9</td>
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<td>47, XX, +16</td>
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<td>47, XY, +19[78] / 47, XY, +19,inv(4)[20]</td>
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<td>69, XXX</td>
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<td>46, XX, inv(9)(p11q13)[80] / 92, XXXY, inv(9)(p11q13)[20]</td>
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<td>46, XX</td>
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chromosomal inversion gave birth to healthy children. The authors suggest that chromosomal examination, as well as close monitoring and supportive care, should be provided to couples with chromosomal inversions.

Chromosomal polymorphisms refer to microscopically visible minor differences in chromosome morphology among human groups. Via traditional genetic points-of-view, it is believed that karyotype polymorphisms have no obvious clinical phenotypic or pathological significance. However, a growing number of reports have shown that chromosomal polymorphisms may produce clinical effects and lead to various adverse reproductive outcomes, for instance, infertility, recurrent spontaneous miscarriages, fetal malformations, etc [20]. In this study, the authors found that 15 out of 34 couples had a chromosomal polymorphism, more specifically, in 12 males and six females, which was significantly different. Moreover, chromosomal polymorphisms were primarily of the long Y chromosome type, which accounted for 40% of the cases. The long Y chromosome is a common type of chromosomal polymorphisms, which refers to an increase in the distal sites of the long arm of the Y chromosome. It has been reported that this variation is related to a repeated duplication of heterochromatin or changes in the extent of chromosomal spiralization. According to previous statistics, the long Y chromosome accounts for 2.18% to 30.20% of reproductive abnormalities in male patients [23, 24]. Rodriguez et al. suggested that the variability in the length of the Y chromosome is a polymorphism in human males that is unassociated with reproductive problems. Furthermore, Verma et al. observed that a long Y chromosome in fathers may be unrelated to fetal loss [25-26]. Conversely, Yan et al. suggested that an increasing in the number of DNA repeats in the Y chromosome may influence the development of the nervous system during the early stages of fetal development, and leads to a stillbirth or abortion [23]. According to our observations, all of the female partners of the six males with a long Y chromosome had successful deliveries after 38 weeks of gestation. The authors suggest that a comprehensive etiological screening and a specific treatment paradigm should be performed in all couples with a karyotype polymorphism and RM. Additionally, owing to the risk of abnormal fetal development, they advise that such couples accept a prenatal diagnosis in subsequent pregnancy.

Aside from parental chromosomal aberrations, fetal chromosomal abnormalities are also a major cause of RM. Goddijn et al. suggested that fetal chromosomal abnormalities account for about 50% of first-trimester pregnancy losses [22]. Similarly, Carp et al. observed that embryonic chromosomal aberrations have been found in 29% to 60% of RM. Furthermore, most of these abnormalities were numerical abnormalities (86%) and a low proportion were caused by structural abnormalities (6%) or other genetic mechanisms [22, 27-29]. According to our data, 51.7% (15/29) of fetal karyotypes were abnormal, which is in accordance with previous research. Among these, numerical abnormalities accounted for one-third of the karyotype abnormalities, including trisomy, triploid, and tetraploid, and all of these embryos did not grow beyond the first trimester. In the current study, inversions turned out to be due to abnormalities in fetal karyotypes, which accounted for 66.7% of the cases. The authors believe the higher incidence may be attributable to the small sample size and effective treatment, which increased survival.

It is well known that the causes leading to offspring karyotype abnormalities are very complicated, and may be due to various causes, such as an error in mitosis during early pregnancy, maternal age, various environmental exposures, etc. Furthermore, half of the structural abnormalities may be inherited from a parent carrying a balanced chromosome translocation or inversion. Goddijn et al. reported that a subsequent pregnancy may result in a child with an unbalanced structural chromosomal abnormality due to a parental structural chromosomal abnormality [22]. Consequently, this child may have multiple congenital malformations and/or a mental handicap. Couples with RM are more likely to produce chromosomally abnormal embryos than those without RM. While parental karyotyping is part of the standard management of RM, it is rarely a measure of the fetal karyotype. Hence, in the authors’ opinion, fetal karyotyping should be determined directly via an embryonic biopsy or pre-gestational diagnosis.

In conclusion, there is a positive correlation between chromosomal abnormalities and spontaneous miscarriages. The clinical effects of chromosomal polymorphisms need to be recognized better, in particular, inversions of chromosome 9 and the long Y chromosome in males. Prenatal diagnosis should also be implemented in carrier couples suffering more than two miscarriages. Moreover, the prognosis for a subsequent pregnancy may be affected by other factors, such as antiphospholipid syndrome, thrombophilias, infections, endocrine disorders. Hence, in couples with a history of RM, where one of the partners is a carrier of chromosomal abnormalities, an evaluation, special treatment, supportive care, and close monitoring is associated with a marked improvement in subsequent live birth rates.

Acknowledgments

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References


C-Met expression pattern in uterine leiomyoma

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Summary

Aim: Growth factors take place in the formation and growth of uterine leiomyomas (LMs). Transforming growth factor beta (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, and insulin-like growth factor (IGF) contribute to the pathophysiology of LMs when they bind with a specific membrane receptor and transmit a signal into the cell. Little is known about hepatocyte growth factor (HGF) and its receptor system c-Met in formation and growth of uterine LMs. The aim of this study was to evaluate the c-Met receptor expression on human myometrium and uterine LMs. Expression of c-Met receptor was evaluated by immunohistochemical analysis. Results: Overexpression of c-Met was found in all LM cases and in none of normal myometrium samples c-Met overexpression was seen. Conclusion: HGF and c-Met receptor complex seem to have role in development of uterine LMs.

Key words: Hepatocyte growth factor; c-Met receptor; Uterine leiomyoma.

Introduction

Uterine leiomyomas (LMs) are benign monoclonal tumors arising from the smooth muscle cells of the myometrium. The pathogenesis of LMs is multifactorial. Genetic predisposition, steroid hormones, and growth factors which are important in fibrotic processes and angiogenesis take place in the formation and growth of uterine leiomyomas [1]. Transforming growth factor beta (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and insulin-like growth factor (IGF), which are involved in fibrotic processes and angiogenesis, may also contribute to the pathophysiology of LMs [2-3]. Growth factors may foster LM growth through local paracrine and/or autocrine mechanisms [4].

The hepatocyte growth factor (HGF)/ receptor system has multifunctional properties, such as cell proliferation, cell movement, and morphogenesis [5-6]. The receptor for HGF is a protein product of a proto-oncogene c-Met which encodes a transmembrane tyrosine kinase (P190 c-Met) with structural and functional features of a growth factor receptor [7-9]. Autophosphorylation of this receptor by ligand binding stimulates its intrinsic tyrosine kinase activity with resultant changes in cellular morphology, motility, and growth. Overexpression of this oncogene was shown in different human solid tumors such as hepatomas, carcinomas of colon, rectum, stomach, pancreas, thyroid, kidney, ovary, endometrium, bladder, breast, and prostate [10-22].

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munoreactivity was evaluated according to the number of the stained cells and the intensity of staining. Positive immunostaining was localized to cytoplasm and membrane. Prostate tissue was used as positive control. Extensive and intense immunoreactivity was observed in epithelial component (Figure 1). Immunoreactivity was evaluated according to the number of the stained cells and the intensity of staining. Extensiveness of staining was scored as 0 = 0%, 1 = 1-30%, 2 = over 30%. Intensity was scored as 1 (mild), 2 (moderate), and 3 (intense). A numerical value is gained by product of these two scores. A final score between 0-3 is accepted as negative, a score greater than 3 was accepted as overexpression.

Results

The indication for operation for all patients was abnormal uterine bleeding. Mean age at the time of surgery was 40.6 ± 6.5 years (range 28 - 52). None of the patients were postmenopausal. All of the patients were evaluated before surgery to exclude any other gynecological pathology. The ones that were using oral contraceptives were operated after three months from cessation of the pills. None of the patients were on any other hormonal medications.

Results of immunohistochemical analysis are summarized in Table 1. Extensiveness and intensity scores were calculated as previously defined for all samples. Product of the extensiveness and intensity score yielded a final score for every patient. When final staining score was evaluated, there were no significant difference in this score with respect to patient age and size of the LM (Figure 2).

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Extensiveness</th>
<th>Intensity</th>
<th>Final score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LM</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LM</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>LM</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>LM</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>LM</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>LM</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>LM</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>LM</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>LM</td>
<td>2</td>
<td>2</td>
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<tr>
<td>10</td>
<td>LM</td>
<td>2</td>
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<tr>
<td>11</td>
<td>LM</td>
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<td>12</td>
<td>LM</td>
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<tr>
<td>13</td>
<td>LM</td>
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<tr>
<td>14</td>
<td>LM</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
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<td>15</td>
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<td>2</td>
<td>2</td>
<td></td>
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<td>16</td>
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<td>2</td>
<td></td>
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<tr>
<td>20</td>
<td>LM</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>22</td>
<td>M</td>
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<tr>
<td>23</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

LM = Leiomyoma
M = Normal myometrium.

Figure 2. — a: Evaluation of final staining score according to patient age. b: Evaluation of final staining score according to leiomyoma size, *p >0.05.
Hence, c-Met activation is a late event that aggravates the abundant inflammatory cytokines or pro-angiogenic factors that are important for inducing self-repair responses in numerous org-ans. c-Met is transcriptionally induced by hypoxia and structural and functional features of other growth factor receptors [10]. It is known that, endogenous HGF is important for inducing self-repair responses in numerous organs. c-Met is transcriptionally induced by hypoxia and inflammatory cytokines or pro-angiogenic factors that are abundant in the reactive stroma of full-blown tumours. Hence, c-Met activation is a late event that aggravates the intrinsic malignant properties of already transformed cells by conveying proliferative, anti-apoptotic, and promigra-tory signals [35]. To the authors’ knowledge; although c-Met overexpression has been demonstrated in many human solid tumors, there is no study examining c-Met expression in uterine LM.

It is apparent from this study that normal myometrium has no c-Met overexpression detected by immunohistochemical analysis. It has been demonstrated that uterine LM has c-Met overexpression, but it is still not known which factors induce HGF/ c-Met complex. Sozen et al. stated that steroid hormones (estrogen, progesterone, and glucocorticoid) stimulates VEGF and FGF secretion and activation [36]. Steroid hormones can also be the promoter of HGF/ c-Met complex.

Measuring HGF content and c-Met receptor expression by both immunological and polymerase chain reaction (PCR) techniques both myometrium and LMs can give the exact results. The authors only analysed Met overexpression by immunohistochemical analysis. This is the limitation of this study.

In conclusion, HGF and c-Met receptor complex seem to have role in development of uterine smooth muscle tumors and especially LMs.

**Acknowledgement**

The authors would like to acknowledge the Pathology Laboratory for its valuable support in this research.

**References**


Sexual function in women after vaginal surgery with synthetic mesh material

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Summary

Objective: To prospectively assess the impact of surgery for stress urinary incontinence (SUI) and pelvic organ prolapse (POP) with vaginal synthetic mesh on female sexual function and satisfaction. Materials and Methods: Forty-four women treated by vaginal surgery for SUI and POP between October 2009 and October 2011 were asked to fill in questionnaires at baseline and at six months after surgery to determine the impact on their sexual function and satisfaction. The questionnaires used for assessment were the 19-item Female Sexual Function Index (FSFI) and the Dutch Seksueel Functioneren Algemeen (General sexual function) or SFA-questionnaire. Results: The questionnaire was completed by 27 patients (61.4%) at baseline; Nineteen reported being sexually active and eight were not. At six months follow-up, the questionnaire was returned by eight patients. In the studied population, an overall improvement of sexual function at six months follow-up was found. All six FSFI-domains: desire, arousal, lubrication, orgasm, satisfaction, and pain seemed to slightly improve. Conclusion: The authors found that there was an overall improvement of sexual function after vaginal surgery with synthetic mesh for POP and SUI.

Key words: Vaginal surgery; Pelvic organ prolapse; Stress urinary incontinence; Sexual function; Questionnaire; Tension-free vaginal tape; Vaginal mesh.

Introduction

Women’s sexual health is determined by different factors: anatomical, physiological, medical, psychological, and social. Numerous problems concerning these factors can attribute to sexual dysfunction. Sexual dysfunction is a frequent problem occurring in 50% of the population [1-3]. Both pelvic organ prolapse (POP) and stress urinary incontinence (SUI) are common in women. The mean prevalence of POP is 19.7% and prevalence rates for SUI are 16-36% in women aged 40-59 [4, 5]. Different types of surgery using incisions in the vaginal wall have proven to be safe and efficient for treatment of both conditions. Because of this vaginal incision questions may arise regarding its impact on sexual satisfaction and function.

POP and SUI due to weakness of the pelvic floor are known to negatively affect sexual function and satisfaction of women [6]. Therefore treatment may also improve sexual function.

Over the last few years several studies addressing the impact of the surgical treatment for pelvic floor weakness and POP on sexual function have been published. Both improvement and deterioration were reported. When improvement was found, this often seemed to be caused by the disappearing of coital incontinence. Dyspareunia seemed to be the main reason for deterioration of sexual function. The mean prevalence of de novo dyspareunia after the procedures was 20% [7-11].

The objective of this study was to prospectively assess the impact of surgery for SUI and POP on female sexual function and satisfaction. To be eligible for this study, patients had to be scheduled for anterior or posterior prolapse repair or surgery for SUI.

Materials and Methods

This prospective study was conducted at the University Hospital of Antwerp in Belgium, Department of Gynecology and Obstetrics. The study was approved by the Ethics Committee of the University Hospital of Antwerp. Written informed consent was obtained from all subjects prior to surgery.

Women who underwent surgery for POP and/or SUI at the University Hospital of Antwerp between October 1, 2009 and October 1, 2011 were asked to fill in a questionnaire prior to and six months after surgery. Twenty-seven women, with age varying from 33 to 81 years (mean age 58.08 ± 12.66 years) were eventually enrolled in this study. Exclusion criteria were patients who underwent a concurrent hysterectomy as hysterectomy by itself has been proven to influence sexual function [12] and patients who were to undergo an abdominal approach for the repair of prolapse like the Burch colposuspension technique.

All operative procedures were conducted by a vaginal approach. Surgery for SUI included the tension-free-vaginal method (TVT, TVT-O, TVT-S) and transobturator or TOT procedure (for prolapse repair the method of choice was the prolift posterior and/or anterior procedure).

For the evaluation of sexual function the authors used a validated Dutch translation of the Female Sexual Function Index (FSFI) (http://www.seksueledisfuncties.nl/lijsten/FSFI.PDF). The FSFI, developed by Rosen in 2000, is a 19-item questionnaire that evaluates six domains of sexual function: sexual desire, arousal, lubrication, orgasm, satisfaction, and pain during sexual intercourse. All items were scored from 0-5, with 0 meaning sexually inactivity.

In addition of the questions of the FSFI questionnaire, patients were asked to fill in a non-validated Dutch questionnaire: Seksueel Functioneren Algemeen (general sexual function) or SFA
questionnaire. With the SFA sexual active patients were asked additional questions about the presence of dyspareunia, coital incontinence, and penile pain during intercourse.

Data concerning postoperative complications and adverse effects were collected from the hospital file.

Statistical analysis was performed with SPSS version 19.0. For comparison of pre- and postoperative FSFI-scores a paired t-test was used. A $p < 0.05$ was considered to be statistically significant.

Three questions of the SFA were categorical and were analyzed with a McNemar-Bowker test. Four questions of the SFA were based on an ordinal scale and were analyzed with a Wilcoxon signed ranks test. Again, $p < 0.05$ was considered to be statistically significant.

**Results**

Forty-six patients were asked to participate in the present study of which 19 refused to enrol. Reasons given by the women for refusal varied from old age ($n = 11$) to lack of comprehension of the Dutch language ($n = 8$). Finally 27 patients were included. The sample consisted of 19 (66%) preoperatively sexually active women and eight (44%) preoperatively sexually inactive women, age ranging from 33 to 81 years (mean age 58.08 ± 12.66), parity from 0-6 (mean 2.53 ± 1.55) and BMI from 17.4 - 35.4 (mean 26.48 ± 4.78). Reasons stated for sexual inactivity were old age and partner-related problems.

Eleven (40.7%) and 12 (44.4%) patients were undergoing surgery for SUI and POP respectively. Four (14.8%) were undergoing surgery for POP and SUI simultaneously.

Nine patients did not complete the questionnaire six months after the surgical procedure and were lost to follow-up.

Several patients had comorbid conditions; four suffered from hypertension and two patients were previously diagnosed with irritable bowel syndrome. Four patients had a positive history of depression and were taking antidepressants. Eight patients (29.6%) had undergone a previous hysterectomy. Four of the included patients were regular smokers. Two women were on hormone-replacement therapy. The clinical and demographic characteristics of the women included in this study are shown in Table 1.

There was a significant improvement of urinary symptoms after surgery. At six months follow-up only one patient had developed de novo urge incontinence and one experienced difficulty in voiding. There was one case of mesh-erosion for which the patient had to undergo surgical repair.

Among women who reported they were sexually active before and after surgery.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Preoperative score</th>
<th>Postoperative score</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desire</td>
<td>2.74 ± 1.29</td>
<td>3.56 ± 1.28</td>
<td>0.099</td>
</tr>
<tr>
<td>Arousal</td>
<td>2.49 ± 1.77</td>
<td>3.81 ± 1.83</td>
<td>0.018</td>
</tr>
<tr>
<td>Lubrication</td>
<td>2.36 ± 2.43</td>
<td>4.16 ± 2.14</td>
<td>0.050</td>
</tr>
<tr>
<td>Orgasm</td>
<td>2.17 ± 2.42</td>
<td>3.54 ± 2.50</td>
<td>0.101</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>2.97 ± 1.97</td>
<td>4.29 ± 2.04</td>
<td>0.095</td>
</tr>
<tr>
<td>Pain</td>
<td>2.74 ± 2.78</td>
<td>4.97 ± 1.63</td>
<td>0.069</td>
</tr>
<tr>
<td>Total</td>
<td>15.47 ± 11.13</td>
<td>24.33 ± 9.58</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD.

At baseline but was at follow-up complained of dyspareunia. One patient complained of narrowing of the vagina after surgery. She said not to be disturbed by this narrowing.

When analyzing the SFA, an improvement was found in the occurrence of coital incontinence, but this was not of statistical significance ($p = 0.317$). Two patients suffered from coital incontinence preoperatively. Only one of these two patients was cured from coital incontinence.

No significant ($p = 0.392$) improvement was found in the occurrence of pain during intercourse; this resembled the results of the FSFI (Table 2).

The SFA revealed two cases of narrowing of the vagina, interfering with intercourse. However this increase did not seem to be of statistical significance, with $p = 0.088$.

Before surgery two patients stated to be inhibited in their sexual activity by fear of urine loss, after surgery they no longer had this fear. The inhibition or reduction of sexual activity due to fear of urine loss did not change statistically significantly ($p = 0.180$).

Four patients were avoiding intercourse because of a feeling of swelling in the vagina or bulging of the vagina before surgery. After surgery they did not avoid intercourse because of this reason. The avoidance of intercourse because of a bulging feeling did not change statistically significantly ($p = 0.414$).

There was no change in negative thoughts or feelings such as fear, disgust, shame or guilt associated with sexual activity before and after surgery.

In the SFA there were two questions that were partner-re-
lated. No difference was found in influence of erectile dysfunction or premature ejaculation before and after surgery.

Discussion

There is a significant improvement of urinary and pelvic symptoms after vaginal surgery.

In the studied population the authors found an overall improvement of sexual function at six months follow-up. All six FSFI-domains: desire, arousal, lubrication, orgasm, satisfaction, and pain seemed to slightly improve.

Literature shows that when deterioration of SF was found, this was caused by dyspareunia. Azar et al [13] studied SF in women after surgery for pelvic organ. They found an improvement of general SF and a deterioration in pain-free intercourse. The present authors also found an improvement of general SF. They did not find a deterioration of pain-free intercourse. Instead they found an improvement, however it was not significant.

The present study showed an improvement of coital incontinence after surgery. Jha et al studied sexual function following surgery for SUI they also found an improvement of SF and a reduction of coital incontinence [14]. Similar results were found by Glavind et al [15] and Bekker et al [16].

There are several limitations to the present study. Firstly sexual function is affected by multiple factors including anatomy, physiology, and psychology. In addition to the FSFI the SFA questionnaire addresses some of these factors, but still gives only a glimpse of all the possible problems related to women’s sexual health.

The authors also found that recruitment of patients in their centre was often particularly difficult, due to patients refusing participation when asked directly about sexual health. This limits the present data very much due to the very small sample size.

The lack of cooperation the authors encountered raised questions about the experience of gynecologists and urologists, regarding the influence of vaginal surgery on women’s sexual function. They therefore created another questionnaire-based study to objectively evaluate the way the physicians of gynecology and urology of Antwerp look upon the impact of vaginal surgery on women’s sexual function. For this evaluation the authors used a self-administered nine-item questionnaire. All gynecologists and urologists (n = 235) of the Antwerp region were sent a questionnaire. Response rate was very low (n = 48, 20.4%). The physicians that returned the questionnaire had very different opinions about influence of vaginal surgery for SUI or POP on sexual function. One-third believes there is a positive influence, one-third believes there is no influence, and one-third believes there is a negative influence. Most physicians believe there is no influence whether or not a mesh is used. The physicians that returned the questionnaire stated that they often receive questions of patients regarding sexual function after vaginal surgery. However they believe there is still a taboo concerning this topic.

What this study adds

In conclusion, the results of the present study indicate that vaginal surgery for women with symptomatic SUI or POP positively affects women’s general sexual function. Both patients and physicians show little interest in the subject of sexual function after vaginal surgery for SUI and POP.

References


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Microarray analysis of differentially expressed genes in preeclamptic and normal placental tissues

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Summary
Purpose of investigation: To detect the candidate genes for preeclampsia (PE). Materials and Methods: The gene expression profiles in preeclamptic and normal placental tissues were analyzed using cDNA microarray approach and the altered expression of important genes were further confirmed by real-time RT-PCR (reverse transcription polymerase chain reaction) technique. Total RNA was extracted from placental tissues of three cases with severe PE and from three cases with normal pregnancy. After scanning, differentially expressed genes were detected by software. Results: In two experiments (the fluorescent labels were exchanged), a total of 111 differentially expressed genes were detected. In placental tissue of preeclamptic pregnancy, 68 differentially expressed genes were up-regulated, and 44 differentially expressed genes were down-regulated. Of these genes, 16 highly differentially expressed genes were confirmed by real-time fluorescent quantitative RT-PCR, and the result showed that the ratio of gene expression differences was comparable to that detected by cDNA microarray. Conclusion: The results of bioinformatic analysis showed that encoding products of differentially expressed genes were correlated to infiltration of placenta trophoblastic cells, immunomodulatory factors, pregnancy-associated plasma protein, signal transduction pathway, and cell adhesion. Further studies on the biological function and regulating mechanism of these genes will provide new clues for better understanding of etiology and pathogenesis of PE.

Key words: Microarray analysis; Gene expression profiles; Preeclampsia; Placenta.

Introduction
Preeclampsia/eclampsia (PE) is one of common and severe maternal complications of pregnancy and also the main cause of diseases and death in pregnant women and perinatal infants, with the incidence rate being 7-10% [1]. Since its pathophysiological changes are correlated to multiple systems and organs, and its etiology and pathogenesis have not been fully understood, PE is attracting much interest in maternity studies. Placenta is a unique critical organ that is essential for normal pregnancy maintenance and is responsible for supplying all nutrients for fetal growth and development. Abnormal pathophysiological changes of placenta will cause various maternal and fetal diseases including PE. It has been widely accepted that the clinical symptoms of PE such as hypertension and proteinuria are induced by pathological changes occurring during the development of placenta, leading to damage of vascular endothelial cells.

PE has a family genetic predisposition, which is mainly characterized by maternal inheritance. Chesley et al. [1-3] have found that the incidence rate of PE in daughters of women with PE (26%) was far beyond that (8%) in their brother’s and sister’s daughters. Pedigree analysis has indicated that PE is closely correlated to genetic factors [4-7]. Reincidence rate of PE in women with multiple pregnancies, who had changed their sexual partners, would be increased obviously, especially when the mother of their new sexual partners had a history of PE [8].

On the basis of predisposing genes of PE, the following chromosome segments have been found to be correlated to PE: chromosome 6, chromosome 17 (includes gene of angiotensin-converting enzyme), chromosome 21 (includes gene of superoxide dismutase), and chromosome 3 (includes gene of angiotensin II type 1 receptor) [9]. Presently PE is considered to be one of polygenic inheritance diseases associated with multiple predisposing factors [9]. To date, only a small number of candidate pathogenic genes of PE has been identified, including blood pressure regulatory gene, thrombosis gene, vascular endothelial injury gene, lipid metabolism gene, immune related genes, and mitochondria related genes [10-18].

Most previous studies on pathogenic genes of PE have been focused on maternal body, and the main purpose was to detect maternal gene abnormalities. These studies neglected one of the important characteristics of PE that it is one of diseases related to products of conception produced during special periods of pregnancy. The incidence, development, and turnover of PE are closely related to the placenta. Patients with preeclampsia recover from clinical symptoms of the disorder only after the delivery of the fetus and placenta. Since placenta is one of fetal appendices and is obviously different from maternal tissues, genetic research of PE should not ignore the placenta. There

* The first two authors equally contributed to this work

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is an emerging consensus that the placenta plays an essential role in the etiology of PE. Expression profiling using microarray has proved to be a powerful tool for studying complex disorders such as cancer, diabetes, hypertension, and PE. To date, there have been several reports that have addressed the gene expression profiles of the placentas from preeclamptic women. These studies have shown that genes such as the obesity-related genes, cytokine-receptor genes, host-pathogen interaction genes, lipid metabolism genes, carbohydrate metabolism genes, and -related genes are essential for or strongly associated with the development of PE [19-22].

The scarcity of early biomarkers for PE has hindered our ability to take effectively preventive and therapeutic measures to manage this dangerous disease in a timely manner. Thus, it is of clinical importance to compare the expression profiles between this disease and normally pregnant women; and d) pregnant women without any complications of genital diseases, and other internal or surgical diseases of pregnant women; and d) pregnant women without any complications of pregnancy and labor, including placenta previa, placental abruption, fetal distress, hydramnios or oligohydramnios, intrauterine growth retardation, large for date infant, fetal anemia, and congenital diseases. Women were confirmed with preeclamptic pregnancy according criteria of pregnancy-induced hypertension syndrome by the American College of Obstetricians and Gynecologists [1], and patients who met the PE criteria were included. Informed consent was obtained from each patient. The study was approved by the Ethics Committee for Human Studies at Fudan University.

**Materials and Methods**

**Human subjects**

Placental tissues were collected from three cases with severe PE and from three women with normal pregnancy, which underwent cesarean section delivery at the Affiliated Obstetrical and Gynecological Hospital of Fudan University (Shanghai, China) in August, 2005, and were well matched according to age, parity, and gestation. The including criteria for normal control group were: a) women with singleton pregnancy; b) women with 28-40 weeks of gestation; c) pregnant women without any medico-surgical complications such as diabetes, primary hypertension, nephritis, heart diseases, anaemia, hepatitis, intrahepatic cholestasis during gestational period, sexually transmitted diseases, and other internal or surgical diseases of pregnant women; and d) pregnant women without any complications of pregnancy and labor, including placenta previa, placental abruption, fetal distress, hydramnios or oligohydramnios, intrauterine growth retardation, large for date infant, fetal anemia, and congenital diseases. Women were confirmed with preeclamptic pregnancy according criteria of pregnancy-induced hypertension syndrome by the American College of Obstetricians and Gynecologists [1], and patients who met the PE criteria were included. Informed consent was obtained from each patient. The study was approved by the Ethics Committee for Human Studies at Fudan University.

**Placental tissue biopsy collection**

All of the placental biopsies both from preeclamptic and normal pregnancy were obtained after cesarean sections. To avoid the effect of labor on the expression profile, only placental samples that were obtained from the women who did not undergo labor were included in the study. After placenta expulsion, the maternal decidua and amnionic membranes were removed and about one cm³ of placental tissue was cut from the center of placental maternal surface between basal and chorionic plates. After being rinsed with saline, they were transferred into diethylpyrocarbonate (DEPC)-treated Eppendorf tubes, and frozen with liquid nitrogen and stored at -80°C until analysis.

**Extraction and purification of RNA from placental tissues**

Total RNA was extracted from 400 mg placental tissue using trizol and then precipitated with isopropyl alcohol and purified with RNeasy mini-kit. Both quality and quantity of extracted total RNA samples were examined by loading five μg of each sample on a denaturating agarose gel and staining with ethidium bromide. The RNA was quantified and evaluated for purity by UV spectrophotometry. To further evaluate the quality of the RNA, all specimens were tested by expression analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using RT-PCR. To test for possible contamination by maternal blood cells, the expression level of leukocyte-specific gene/leukocyte common antigen (LCA) was also examined using conventional semi-quantitative RT-PCR. The PCR products were electrophoresed in 2% agarose gel containing ethidium bromide, which were then visualized with ultraviolet.

**cDNA microarray analysis**

BioStarH140s cDNA microarray used in this study contained 14,112 human clones, mainly consisting of the following genes: 1) proto-oncogenes and anti-oncogenes; 2) cell signal transduction protein genes; 3) cyclin genes; 4) outer stress response protein genes; 5) cell regulatory protein genes; 6) apoptosis related protein genes; 7) DNA synthesis, repair, and recombination protein genes; 8) DNA binding, transcription, and transcription factor genes; 9) cell receptor genes; 10) cell surface antigen and attachment protein genes; 11) ion channel and transport protein genes; 12) metabolism genes; and 13) house-keeping genes.

To perform fluorescent labeling, the cDNA was reversely transcribed from ten μg total RNA primed by T-Oligo(dT)15, and then was purified with a PCR purification kit. An in vitro transcription was performed to produce biotin labeled cRNA from the cDNA. To normalize for fluorescent labeling, mRNA isolated from preeclamptic and normal placental tissues was firstly labeled with Cy3-dUTP and Cy5-dUTP, respectively; and then exchanged the labels with mRNA isolated from preeclamptic and normal placental tissues being labeled with Cy5-dUTP and Cy3-dUTP, respectively. A total of three placetas from women with preeclampsia and three from normal subjects were used as test samples in the hybridizations. Glass slides containing the above labeled cDNA was transferred into 35 μl hybridization solution (3% sodium chloride sodium citric acid (SSC), 0.2% sodium dodecyl sulfate (SDS), 5% Denhart’s, and 25% formamide solution) and incubated at 42°C overnight. The glass slides were rinsed with 2% SSC solution containing 0.2% SDS at 42°C for five minutes, and then rinsed with 0.2% SSC solution at room temperature for five minutes and dried while shaking.

The dried glass slides were scanned with a cDNA microarray dual channel laser scanner. The value and ratio of fluorescence intensities of Cy3 and Cy5 of each spot in the cDNA microarray were analyzed with GenePix Pro 4.0 and normalized by Lowess method. The average signal intensities were corrected for median background intensity and transferred with GenBank descriptors to a Microsoft Excel data spreadsheet. Those spots showing more than a twofold difference between the Cy5 and Cy3 signals, i.e., the ratio of Cy5 to Cy3 fluorescence intensity was no less than 2.0 or no more than 0.5, were considered as dif-
Microarray analysis of differentially expressed genes in preeclamptic and normal placental tissues

16 highly differentially expressed genes identified by cDNA microarray were confirmed by real-time fluorescent quantitative RT-PCR, using 18S house-keeping gene as calibration gene. Primer sequence and product sizes of these 16 genes were listed in. All of the primers were provided by the Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. The total RNA isolated from preeclamptic and normal placental tissues was digested with RNA free DNase I at 37°C for 30 minutes. Then phenol/chloroform, 1/10 × 3 mol/l sodium acetate, and 2.5 × cold dehydrated alcohol were added in turn to the mixed solution to precipitate RNA, which was washed with 70% ethanol, and then was dried and re-dissolved in DEPC water solution. The first cDNA was reversely transcribed from 2.5 g total RNA using reverse transcriptase and then amplified by a PCR method. The PCR product was electrophoresed on 1% agarose gel. The 18S band was observed, which indicated that the total RNA was qualified for reverse transcription (Figure 1).

Amplification was performed on a real time-PCR machine. The analyses were carried out using a Light Cycler fast start DNA master SYBR green I kit. Data analysis and processing were made by using Rotor-Gene 5.0 and Excel 7.0 to obtain Ct values for each gene in each sample. In an effort to compare the differences at transcriptional level, Ct values of each target gene were normalized to the Ct value of a house-keeping gene using the following equation: ΔCt = [Ct (target gene)] − [Ct (house-keeping gene)]. In a typical reaction, the PCR product was produced exponentially, i.e., in a 2ⁿ way (where n is the number of cycles). The concentration of the original template is 2⁻ΔCt.

Gene nomenclature

The human genes investigated in this study were named in accordance with the guidelines provided by Human Genome Organization (HUGO) Nomenclature Committee (http://www.gene.ucl.ac.uk).

Results

Hybridization maps and scatter plot of gene expression profiles

Figure 2A shows the overlapping between hybridization map of Cy3 labeled cDNA prepared from preeclamptic placental tissues and that of Cy5 labeled cDNA prepared from normal placental tissues. If the Cy3 signal of a spot was more intensive than its Cy5 signal and the spot was green, the spot represented a gene with a down-regulation tendency; in contrast, if the Cy5 signal of a spot was more intensive than its Cy3 signal and the spot was red, the spot represented a gene with an up-regulation tendency. Figure 2B shows the overlapping between hybridization map of Cy3 labeled cDNA prepared from normal placental tissues and that of Cy5 labeled cDNA prepared from preeclamptic placental tissues.

Scatter plot of microarray hybridization can directly reflect the differences of gene expression profiles of two different samples. The X-axis refers to the intensity of Cy3 signal, while the Y-axis refers to the intensity of Cy5 signal. Each data point represents the hybridization signal of one gene. If the data point is red and the ratio of its X value over its Y value is between 0.5 to 2.0, it represents a gene without expression differences; in contrast, if the data point is yellow and the ratio of its X value to its Y value is no more than 0.5 or no less than 2.0, it represents a gene with marked expression difference (Figure 3).

Profiles of differentially expressed genes

The cDNA microarray used in this study consists of most of the following genes: proto-oncogenes, anti-oncogenes, ion
channel and transport protein genes, cyclin genes, cytoskeleton and movement protein genes, apoptosis genes, DNA synthesis and repair genes, transcription factor genes, cell receptor genes, immune related genes, cell signal transduction genes, metabolism genes, and development genes. Microarray hybridization results showed that the exchange of fluorescent labels for cDNA prepared from preeclamptic and normal placental tissues could effectively eliminate false positive rate caused by nonspecific adsorption characters of fluorescent labels. In two experiments with fluorescent labels being exchanged, 382 and 394 differentially expressed genes were detected, respectively, with the number of genes detected in both of the experiments being 111, which accounts for 29.1% and 28.2% of total detected genes, respectively. Of these 111 genes, 68 were up-regulated with the ratio being no less than 2.0 and 44 were down-regulated with the ratio be no more than 0.5 in preeclamptic placental tissue.

Of 111 differentially expressed genes detected in this study, many of them are: 1) genes relating to infiltration of placenta trophoblastic cells such as latent transforming growth factor binding protein 2 (LTBP-2) gene (NM_000428), and insulin-like growth factor binding protein 1 (IGFBP1) gene (NM_000596); 2) immune related genes such as interferon (a, b and W) receptor 1 (IFNAR1) gene (NM_000629), and mannose receptor C1 (MRC1) gene (NM_002438); 3) pregnancy associated plasma protein (PAPP) and pregnancy specific glycoprotein (PSG) genes including PAPPE/PAPPA2 (NM_021936), PSG1 (NM_006905), and PSG4/PSG9 (NM_002780); the ratio of expression differences of PPAP-A detected by cDNA microarray technique was 12.406; 4) genes relating to cell signaling pathway such as expression of tissue factor pathway inhibitor-2 (TFPI-2) gene (NM_006528) and serine (or cysteine) proteinase inhibitor 1 (SERPINT1) gene (NM_001235); TFPI-2 gene was up-regulated 12.6 times; and 5) other genes such as keratin 15/type I cytoskeletal 15 (KRT15) gene (NM_002275), fragile X mental retardation (FMR1) gene (NM_002024), and cytochrome P450 (CYP19A1/aromatase) gene (NM_031226).

On the basis of previous reports and the results of our previous experiments, 16 key candidate genes were screened from numerous differential expressed genes of preeclamptic placental tissues according to the following criteria: 1) expression differences can be seen during positive and negative detecting process; 2) gene expression differences and protein differences were similar and significant; 3) genes relating to placental development, angiogenesis, and immune response; 4) bioinformatics information indicated that the protein of the candidate genes can interact with proteins relating to pathogenesis of hypertension; 5) the expression products of the candidate genes are transcription factors or the important regulators for other genes. Among the above criteria, the first one is essential, and the other ones are auxiliary and supportive (Table 1).

Gene expression profiles as determined by real-time quantitative RT-PCR

The differences (determined as differences of Ct value) of gene expression profiles between preeclamptic and normal placental tissues were analyzed using a Rotor Gene RG-3000 Real Time PCR machine. They showed that among the 16 highly differentially expressed genes, three were down-regulated, and 13 were up-regulated. The results from real-time RT-PCR were well correlated to those from microarray analysis, i.e., the two techniques showed not only the same change direction of gene expression, but also comparable magnitudes in altered gene expression for most genes screened (Figure 4). Among differentially expressed genes detected in this study, LTBP-2 gene was down-regulated in preeclamptic placental tissue (the ratio of expression differences was 0.324). The expression differences were confirmed by real-time RT-PCR and tissue microarray immunohistochemical analysis.
Discussion

Analysis of differentially expressed genes between preeclamptic and normal placental tissues and the significance

To date, the etiology and pathogenesis of PE is unclear. PE is considered a polygenic trait disease with impaired immune function, infiltration capacity of placenta trophoblastic cells, and placental blood supply. The activated trophoblastic cells and other effector cells will release cytotoxic factors and vasoactive substances, which will induce the damage of vascular endothelial cells and arteriolar spasm [23]. It can be concluded that the etiology and pathogenesis of PE are correlated to changes of a multitude of genes and proteins, rather than abnormalities of a single gene or protein.

The technique of cDNA microarray was developed in the mid-1990s and has been widely used for gene analysis and detection [24]. Presently it was mainly used for high throughput analysis of gene expression and gene polymorphism. It can be used to compare gene expression profiles in certain tissue cells under different physiopathological states, which will benefit for the analysis of different characters and law of certain gene group under different physiopathological states, and understanding of intergenic relationships, the biological functions of genes and their encoding proteins, especially understanding of pathogenesis of certain disease at gene level, which will provide new treatment method for the disease. BioStarH140s cDNA microarray used in this study contains 14,112 human clones. Differentially expressed genes between preeclamptic and normal placental tissues detected using the microarray include most of genes related major cell functions of placental tissue. The analysis of these differentially expressed genes selected from hundreds of genes, and studies on changes of cell proliferation, immunity, apoptosis, signal transduction, transportation and metabolism, and gene synthesis, repair, and regulation, will provide new clues and routes for elucidation of pathogenesis and pathophysiological changes of PE. In this study, some of differentially expressed genes detected by cDNA microarray were confirmed by real-time fluorescent quantitative RT-PCR.

Table 1. — Primer sequence, product sizes, coding proteins, and gene ontology of 16 genes confirmed by real-time fluorescent quantitative RT-PCR.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Genbank No. &amp; mRNA No.</th>
<th>Primer (5’→3’)</th>
<th>Position</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP19A1</td>
<td>NM_031226</td>
<td>5’-CTTGGTGTTGGAATTATGAGG-3’</td>
<td>358</td>
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<td></td>
<td></td>
<td>5’-GAGCGTGGTGGAGGTGTC-3’</td>
<td>856</td>
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<tr>
<td>ELTD1</td>
<td>NM_022159/BC025721</td>
<td>5’-TGGGTGTCTCATCACAACAAGGG</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-TTCCGTGCTAAGGCAACACT</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>FMR1</td>
<td>NM_002024/BC067272</td>
<td>5’-TCCCTACAGCCTATTGGACACG</td>
<td>692</td>
<td></td>
</tr>
<tr>
<td>IFNAR1</td>
<td>NM_000629/AK123813</td>
<td>5’-AGAGCCATCAGTCCGATTACC</td>
<td>742</td>
<td></td>
</tr>
<tr>
<td>IGFBP1</td>
<td>NM_000596/CR595377</td>
<td>5’-CAGAGATGATGTCGTCCT</td>
<td>145</td>
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<tr>
<td>KRT15</td>
<td>NM_002275/AK122864</td>
<td>5’-ACAGGGCACAGGAAAGGT</td>
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<td>302</td>
</tr>
<tr>
<td>LTBP2</td>
<td>NM000428</td>
<td>5’-CCCTACGGAGAAAAATCAAAGAG</td>
<td>504</td>
<td></td>
</tr>
<tr>
<td>MRC1</td>
<td>NM002438/BU198859</td>
<td>5’-AGAGCCATCAGTCCGATTACC</td>
<td>582</td>
<td>118</td>
</tr>
<tr>
<td>PAPP</td>
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<tr>
<td>PAPPE/PAPPA2</td>
<td>NM_021936/AF342989</td>
<td>5’-ATGGGTCTGGGCACTCTG-3’</td>
<td>1,185</td>
<td>350</td>
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<td>Predicted protein PP1665</td>
<td>BC033391</td>
<td>5’-TATCTCCCCGTCGGTAGCACTGT</td>
<td>647</td>
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<tr>
<td></td>
<td></td>
<td>5’-CAGGGGGAGGCTGAGTC-3’</td>
<td>1,105</td>
<td>404</td>
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<td>PSG1</td>
<td>NM_006905/M23575</td>
<td>5’-AGGCCCTGCAAGGCTGCT</td>
<td>347</td>
<td></td>
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<tr>
<td>PSG4/PSG9</td>
<td>NM_002780/BC063127</td>
<td>5’-GAGGTCATCACCACCAAGG</td>
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<td>438</td>
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<td>PSG6/PSG10</td>
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<td>SERPINH1</td>
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<td></td>
<td></td>
<td>5’-CAGCCGATATGCTGAAAAGC-3’</td>
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<td>496</td>
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Table 2 shows that the ratios of gene expression differences between preeclamptic and normal placental tissues detected by the two techniques are comparable, indicating that the result of cDNA microarray analysis was accurate and reliable.

In this study, the expression profiles of differentially expressed genes known in previous studies, and also many unknown differentially expressed genes in preeclamptic placental tissue were detected. Many genes are found to be up- or down-regulated in the placental tissue in PE. LTBP-2 gene and insulin-like growth factor binding protein gene were down-regulated in preeclamptic placental tissues, probably resulting in the decrease of the adhesion of placenta trophoblastic cells to extracellular matrix, and the decrease of invasion and migration of placenta trophoblastic cells. In this study, it was found that mannose receptor gene was down-regulated, and interferon receptor gene was up-regulated. This may induce immunologic regulation disorder, and the increase of activities of lymphocytes and inflammatory cytokines, causing the damage of blood vascular endothelium. The expression of TFPI-2 gene, and TFPI-2 has significant inhibitory effects on four important matrix hydrolases including fibrinolysin, trypsin, and matrix metalloproteinases (MMP-2 and MMP-9) [25]. When these matrix hydrolases are inhibited, the invasion of placenta trophoblastic cells into helicine arteries would be inhibited. Pregnancy related protein genes and cytochrome P450 19A1 (CYP19A1/aromatase) gene may affect the pathophysiological process of PE by regulating immunity and mitochondria metabolism, respectively.

Expression of latent transforming growth factor b binding protein 2 (LTBP-2) gene in preeclamptic placental tissue and the significance

LTBP2 was identified in 1994 from human platelet and was mapped to chromosome 14q24. Having a size of 112,148 bp, the gene encodes a protein with 1,812 amino acids. Structural analysis showed that the gene has several repeat sequences such as epidermal growth factor (EGF)-like repeats and a repeat containing eight cysteine residues. Analysis of in situ hybridization showed that the gene is highly expressed in the lung, placenta, heart, liver, and muscle tissue [26]. To date, four isoforms of the gene were found, including LTBP-1,2,3 and 4, which were assigned to different chromosomes [27,28]. Previous studies have indicated that LTBP genes may play important roles in the following ways [29]: 1) regulating intracellular biosynthesis of TGF-β precursor; 2) promoting the binding of TGF-β precursor to specific connective tissues, and thus being named as “matrix receptor”; 3) inactivating TGF-β precursor and releasing mature TGF-β; and 4) maintaining the basal activity of TGF-β precursor on the cell surface. However, LTBP2 gene was not coordinately expressed with TGF-β gene [30], instead, it had no direct relationship with TGF gene during embryonic implantation. LTBP-2 may be one of components of important extracellular matrixes, or has regulatory effects on cell signal transduction [31-33]. In addition, LTBP-2 can promote migration of Bowes cells and adhesion of melanoma cells [34].

LTBP genes may play a critical role in the regulation of TGF-β. TGF-β is one of polypeptide growth factors and is composed of three isoforms in mammals, i.e., TGF-β1, TGF-β2, and TGF-β3. TGF-β is very important for the development of many tissues, since it is involved in regulation of cell proliferation, differentiation, and metabolism. TGF-β in placental tissue is involved in infiltration and differentiation of placenta trophoblastic cells, immunoregulation, embryonic development, and the development of placental blood vessels [35]. It has been reported that TGF-β1, TGF-β receptor 1, and TGF-β receptor 2 genes are expressed in trophoblastic cells of placental villi and decidual cells, and the expression level in preeclamptic placental tissue is higher than that in normal placental tissue [36,37]. However, a very low expression level of TGF-β1 and TGF-β2 in uterine and placental tissue are also reported [38]. Thus, the role of TGF-β in the pathogenesis of PE is unclear.

The expression of LTBP-2 gene is down-regulated in preeclamptic placental tissue, and the gene may affect the pathological process and development of PE in various ways. The incidence of PE is closely correlated to shallow invasion of placenta trophoblastic cells into deciduomata.
Table 2. — Differences (ΔCt) of gene expression profiles between preeclamptic and normal placental tissues.

<table>
<thead>
<tr>
<th>GENBANK No.</th>
<th>Gene/Gene product (Protein)</th>
<th>PE-Ct</th>
<th>N-Ct</th>
<th>-ΔCt</th>
<th>2^-ΔCt</th>
<th>Microarray analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_000428</td>
<td>LTBP2/Latent transforming growth factor β binding protein 2</td>
<td>32.83</td>
<td>31.34</td>
<td>-1.29</td>
<td>0.409</td>
<td>0.324</td>
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<tr>
<td>NM_002438</td>
<td>MRC1/Mannose receptor C type 1</td>
<td>31.22</td>
<td>29.70</td>
<td>-1.32</td>
<td>0.401</td>
<td>0.41</td>
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<tr>
<td>BC025721</td>
<td>ELTD1/EGF, latrophilin and seven transmembrane domain containing 1</td>
<td>23.88</td>
<td>21.73</td>
<td>-1.95</td>
<td>0.259</td>
<td>0.342</td>
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<tr>
<td>NM_000629</td>
<td>IFNAR1/Interferon (α, β and Ω) receptor 1</td>
<td>18.65</td>
<td>20.41</td>
<td>1.98</td>
<td>3.89</td>
<td>2.689</td>
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<tr>
<td>AK122864</td>
<td>KRT15/Keratin 15, type I cytoskeletal 15</td>
<td>21.42</td>
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<td>4.40</td>
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<td>3.363</td>
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<td>AF342989</td>
<td>PAPPE/Pregnancy-associated plasma protein E1</td>
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<td>6.63</td>
<td>28.26</td>
<td>4.019</td>
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<td>CR595377</td>
<td>IGFBP1/Insulin-like growth factor binding protein 1</td>
<td>26.23</td>
<td>31.81</td>
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<td>Hypothetical protein PP1665</td>
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<td>5.12</td>
<td>17.39</td>
<td>8.57</td>
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<td>M23575</td>
<td>PSG1/Human pregnancy-specific β-1 glycoprotein</td>
<td>28.96</td>
<td>31.01</td>
<td>3.15</td>
<td>4.76</td>
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<td>BC012609</td>
<td>SERPINH1/Serine (or cysteine) proteinase inhibitor</td>
<td>24.30</td>
<td>30.29</td>
<td>4.99</td>
<td>6.19</td>
<td>7.301</td>
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<tr>
<td>BC067272</td>
<td>FMR1/Fragile X mental retardation protein</td>
<td>20.74</td>
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<td>3.81</td>
<td>8.416</td>
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<tr>
<td>BC063127</td>
<td>PSG4/Pregnancy specific β-1-glycoprotein 4</td>
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<td>3.06</td>
<td>7.78</td>
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<td>NM_031226</td>
<td>CYP19A1/Cytochrome P450 19A1</td>
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<td>NM_002581</td>
<td>PAPPA/Pregnancy-associated plasma protein A</td>
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<td>TFPI2/Tissue factor pathway inhibitor 2 precursor</td>
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<td>26.22</td>
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<td>12.04</td>
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<td>BC020652</td>
<td>PSG6/Pregnancy specific b-1-glycoprotein 6</td>
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<td>2.96</td>
<td>7.78</td>
<td>10.982</td>
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<tr>
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<td>18s ribosomal RNA</td>
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<td>16.62</td>
<td>0.10</td>
<td>1.39</td>
<td>0.91</td>
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</table>

Microarray analysis of differentially expressed genes in preeclamptic and normal placental tissues.

Table 2. — Differences (ΔCt) of gene expression profiles between preeclamptic and normal placental tissues.

Differences (ΔCt) of gene expression profiles between preeclamptic and normal placental tissues.

Microarray analyses

Microarray analyses

Microarray analyses

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Microarray analyses

Microarray analyses
expression of IGFBP-1 gene can be regulated by insulin, adrenocorticotropin, cAMP, cytokines, and estrogen [44-48]. As an important multifunctional protein with significant biological meanings, IGFBP-1 can: a) regulate the transportation and metabolism of IGF-1 by binding to IGF-1; influence the transportation of IGF across vascular endothelial cell; b) prolong the half life of IGF-1, and regulate the clearance rate of IGF; c) determine tissue specificity of IGF by regulating the distribution of IGF in specific tissues and cells; d) regulate the interaction between IGF and its receptor, and thus regulate the biological activity of IGF-1; and e) inhibit metabolism, promote cell proliferation, and induce the increase of blood sugar through IGF-independent pathways.

IGFBP-1 is one of the proteins in decidua that have the highest expression level, indicating that it has regulatory effects on part of maternal-fetal interaction process. Abnormal expression of IGFBP-1 gene will induce a series of pregnancy-associated diseases. Giudice et al. [47] showed that the concentration of IGFBP-1 in serum of women with PE was five times of that of normal control group, and the concentration was correlated to the severity of PE. Angiopasm and the decrease of blood flow can be observed in preeclamptic placental tissue results in insufficient nutrient supply, which will cause hypoglycemia and hypoinsulinemia. Large scale biosynthesis and secretion of IGFBP-1 in decidua and liver can be induced by hypoinsulinemia. Other studies showed that unbalance between stimulating factors (e.g., adrenocorticotropin, progesterone, and cAMP) and inhibitory factors (e.g., insulin, IGF, and IL-1β) relating to the release of IGFBP-1 will increase the level of IGFBP-1. The increase of progesterone and antagonists of IL-1 receptor in pre-eclamptic placental tissue may increase the level of IGFBP-1. IGFBP-1 can bind to placenta trophoblastic cells at RGD-independent integrin binding site (α5β1), resulting in inhibition on the invasion ability of placenta trophoblastic cells. Paul et al. showed that the invasion of placenta trophoblastic cells into decidua in IGFBP-1 transgenic pregnant mice was obviously shallower than that in wild-type pregnant mice, indicating that the overexpression of IGFBP-1 may inhibit invasion process of placenta trophoblastic cells. IGFBP-1 can also inhibit the invasion of placenta trophoblastic cells into decidua by inhibiting the expression of MMP29 gene and inducing the expression of TIMP-1 gene. IGF-I cannot only promote the proliferation, differentiation, and migration of placenta trophoblastic cells, but also the protease secretion and blastocyst implantation. IGFBP-1 can bind to IGF-I, inhibit the biological activity of IGF-I, resulting in invasion disorder of placenta trophoblastic cells, placenta shallow implantation, and finally the incidence of PE [49].

Shang et al. [50] analyzed IGFBP-1 in serum and placental tissue of preeclamptic and normal pregnancy by enzyme linked immunosorbent assay (ELISA) and immunohistochemical analysis, showed that IGFBP-1 level in serum and the positive rate of IGFBP-1 were higher in preeclamptic placential tissue than in normal placential tissue, and the level of IGFBP-1 in serum and placental tissue was positively correlated to the incidence and development of PE. In this study, the results of cDNA microarray analysis and real-time RT-PCR showed that the expression level of IGFBP-1 was obviously higher in pre-eclamptic placential tissue than in normal placential tissue, indicating that IGFBP-1 gene may be involved in the incidence and development PE during gene expression process and metabolism process, and IGFBP-1 level in serum can objectively reflect placental functions and may be used in the clinic.

IGFBP-1 can regulate the activity of IGF-1 via self phosphorylation. After dephosphorylation, the affinity of IGFBP-1 to IGF-1 is decreased, and then the activity of IGF-1 is increased. On the contrary, phosphorylated IGFBP-1 will inhibit the activity of IGF-1. Therefore, further studies on expression regulation, post-translational modification of IGFBP-1 gene, and its action mechanism in the pathogenesis of PE may benefit for better understanding of the change of ischemia and metabolism in preeclamptic placenta trophoblastic cells, and open up a broad prospect for studies on etiology, prevention, diagnosis, and treatment of PE.

Expression of pregnancy specific proteins in preeclamptic placental tissue and the significance

Pregnancy specific glycoproteins (PSGs) include pregnancy-associated plasma protein A (PAPP-A), PAPP-B, and PAPP-C. PAPP-A is synthesized by syncytiotrophoblast and decidual cells and then is secreted in blood circulation [51]. Cultures of placenta trophoblastic cells and decidual cells cannot synthesize PAPP-A by themselves unless pregnancy serum is added into, indicating that pregnancy serum may have a special inducer that can induce the production of PAPP-A in placenta trophoblastic cells and decidual cells [52]. PAPP-A gene was mapped to chromosome 9q33.1. It has a molecular weight of 750~820 kD, and is stable at pH 4-10, but will be fully damaged at pH<2 or pH>10. It has regulatory effects on activity of cell factors. Lawrences et al. [53] showed that PAPP-A is one of proteases relating to IGFBP-4. Intact IGFBP-4 can inhibit the activity of IGFs, but degraded IGFBP-4 loses the inhibitory effect, indicating that IGFBP-4 can be used as one of positive regulators of active IGFs strain. PAPP-A is one of special proteins of pregnancy serum that can be detected in four to six weeks after last menstrual period. PAPP-A level in pregnancy serum is increased more obviously than human chorionic gonadotropic (hCG) along with the increase of pregnant weeks, and will reach the peak at term (at that time, the PAPP-A level in serum is ten times of that in amniotic fluid). After childbirth, PAPP-A level is decreased gradually, with the half life being three to four days. Six weeks after childbirth, PAPP-A in serum can no longer be detected. The change rule of PAPP-A in amniotic fluid is sim-
ilar to that in maternal blood. PAPP-A cannot be detected in cord blood and the body of fetus. Since it does not secrete in kidney, PAPP-A cannot be detected in pregnancy urine throughout the whole duration of pregnancy.

PAPP-A level in pregnancy serum may have relationships with placental functions and maturity. Therefore, detection of PAPP-A level in pregnancy serum can be directly used to monitor placental maturity, and indirectly reflect fetal growth and development. PAPP-A level in pregnancy serum presents a high value in twin pregnancy [54], and presents a low value in the condition of spontaneous abortion, ectopic pregnancy, intrauterine growth retardation, fetal death, fetal anomaly, and pregnancy associated with diabetes because the synthesis of PAPP-A is inhibited by placenta insufficiency [54-56]. Hence, detection of PAPP-A level in maternal serum can be used as an auxiliary index to determine placental functions and maturity. It was demonstrated that PAPP-A level in pregnancy serum could be detected as early as when any overt symptoms could be observed and other proteins in pregnancy serum were still not affected by the increase of PAPP-A level, and the increase amplitude of PAPP-A level was positively correlated to illness degree [57,58]. Therefore, some researchers proposed that detection of PAPP-A level in maternal serum during early pregnancy can be used to reflect placental functions and predict and monitor the pathogenesis of PE.

In this study, the expression of PAPP-A gene was obviously up-regulated in preeclamptic placental tissue. As one of endocrine organs, placenta can produce PAPP-A, which will then enter into maternal and fetal blood circulation, and have various effects on metabolic and immune system. As a protease inhibitor, PAPP-A can benefit for maintenance of placental barrier through its activation effect on complements, and its inhibitory effect on proteolysis of maternal phagocytes [59]. Placental ischemia and hypoxia, and placenta shallow implantation induced by the incidence of PE, and response of placenta trophoblastic cells to placental hypoxia will increase the expression level of PAPP-A, which will prevent fetus away from immune rejection through its immunosuppressive action. Simultaneously increased PAPP-A in maternal serum may reflect the degree of placental function decrease and the severity of hypoxia. Therefore, the high expression level of PAPP-A in maternal serum may reflect the degree of placental function decrease.

In conclusion, differentially expressed genes in preeclamptical placental tissue were analyzed by cDNA microarray technique, and many genes are likely related to the pathophysiology of PE. The gene expression profile of preeclamptical placental tissue is obviously different from that of normal placental tissue, indicating that neutrophilic granulocytes and monocytes in placenta-site of decidua were activated during late pregnancy, the incidence of PE, or in the condition of placental hypoxia, which would cause up-regulation of PSG in placental trophoblastic cells and the increase of PSG excretion, probably resulting in inhibition of maternal immune rejection, prolongation of pregnancy time, and the improvement of fetal survival. However, the regulating mechanism and the structure and functions of PSGs should be further studied.

Conclusions

In conclusion, differentially expressed genes in preeclamptical placental tissue were analyzed by cDNA microarray technique, and many genes are likely related to the pathophysiology of PE. The gene expression profile of preeclamptical placental tissue is obviously different from that of normal placental tissue, indicating that the pathophysiology of PE is very complicated and is involved in a wide range of multiple changes relating to cell metabolism, cell cycle, gene regulation, and cell signal transduction. The result of this study showed that during late period of PE high level of gene expression in placental trophoblastic cells, which are the immunologic barrier between pregnant women and fetus, is the response to maternal immune rejection, which will keep fetus away from maternal immune rejection and improve the survival of fetus. Further studies on the biological function and regulating mechanism of these genes will provide new clues for better understanding of etiology and pathogenesis of PE.
Acknowledgments

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References


Microarray analysis of differentially expressed genes in preeclamptic and normal placental tissues


Effects of laparoscopic ovarian endometriosis cystectomy combined with postoperative GnRH-a therapy on ovarian reserve, pregnancy, and outcome recurrence

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Summary
Objective: The aim of this study was to investigate the impacts of laparoscopic ovarian endometriosis cystectomy combined with postoperative GnRH-a therapy on ovarian reserve, pregnancy outcome and recurrence. Materials and Methods: This was a prospective control study. The experimental group: 63 patients with combinations of laparoscopic bilateral ovarian endometrial cystectomies and gonadotropin-releasing hormone agonist (GnRH-a) treatment for three months. Control group: 62 patients with laparoscopic bilateral ovarian endometrial cystectomies. Benchmarks: the changes of follicle stimulating hormone (FSH) and FSH / luteinizing hormone (LH), etraadiol (E2) in preoperative and postoperative three months or menstrual two to three days after surgery, natural pregnancy, and cyst recurrence in 18th month during postoperative follow-up. Results: In experimental group after six months, the percentage of returned FSH accounted for 95.3% of normal range, in the control group it was 82.2%, and the difference was significant (p < 0.05). The natural pregnancy rate of preoperative infertility patients (57.1%) was higher than the control (36.8%) (p < 0.05). The recurrence rate of preoperative infertility patients (12.7%) was lower than the control (27.4%) (p < 0.05). Conclusion: After bilateral laparoscopic ovarian endometrial cystectomy, an implement of GnRH-a therapy can improve the postoperative pregnancy rate, which changes with clinical stage and patient age, reduces ovarian recurrence, and its influence on ovarian reserve is lesser.

Key words: Laparoscopic ovarian endometrial cystectomy; Ovarian reserve; Sex hormone; Pregnancy outcomes; Recurrence.

Introduction
With the rapid development of laparoscopic surgery, the incidence of endometriosis (EM) has increased annually, has become a common gynecological disease, and has been referred to as “benign cancer” and “the pelvic sandstorm”. The chronic pelvic pain, dysmenorrhea, and infertility caused by EM impact on women’s health and quality of life seriously. Its pathogenesis, metastasis, invasion, and recurrence are unclear; after years of researches, there are still many unanswered questions, so it has become an intractable disease. Ovarian endometriosis cysts are the most common type in EM (about 17%~44% of pelvic endometriosis) [1]. For EM patients in childbearing age, the principles of laparoscopic conservative surgery surrounding clear diagnosis, reductive lesions, reductive pain, and promotive fertility have been widely accepted and became a preferred option. However, there have been uncertainties regarding laparoscopic ovarian endometrial endometriosis ovarian cystectomy, including its impact on residual endocrine function, the possibilities of residual ovarian syndrome and premature failure, and continuous drug treatment for easy recurrence. Ovarian reserve of women in childbearing age has especially attracted attention in recent years. At present, there are a large number of studies that have shown that after laparoscopic ovarian EM cystectomy, there was a decline in ovarian reserve and even the risk of ovarian senility, recurrence, infertility, and other related problems; therefore in recent years it has drawn many attention with the rapid evolution of laparoscopic surgery.

Ovarian reserve, including the number and quality of remaining follicles, reflects female fertility. The decreased functional reserve is caused by the consumption of follicle and decline of their quality. A number of studies have shown that the basic hormones: follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) are important indicators to evaluate ovarian reserve. During the second or third day of menstruation, FSH < 20 mIU/ml, FSH / LH < 3.6, and E2 < 80 pg/ml are regarded as normal [2]. Gonadotropin-releasing hormone agonist (GnRH-a) is an internationally recognized prevention for EM recurrence and is the most effective drugs, and plays an important role in reproduction.

Therefore, this study was designed to observe laparoscopic bilateral ovarian endometrial cystectomy combined with postoperative GnRH treatment for three months, and there set two groups (the combined treatment; the control), the differences of FSH, FSH/luteinizing hormone (LH), and estradiol (E2) changes between the two groups preoperatively and postoperatively three months or during the second or third day of menstruation, menstrual two to three
Materials and Methods

Objects
There were 125 childbearing cases with laparoscopic bilateral ovarian endometrial ectopic cystectomies from April 2008 to May 2010 in the present hospital. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Changji No 2 People’s Hospital. Written informed consent was also obtained from all participants. All patients with married, with normal preoperative menstrual cycles experiencing surgeries at menstrual seven to ten days, had preoperative infertility, and pelvic pain were recorded. According to the revised American Fertility Society (AFS) staging, bilateral ovarian endometriosis lesions were divided into II (36 cases), III (59 cases), and IV (18 cases). According to AFS staging, all cases were divided into two groups, 63 patients in the experimental group combined with GnRH-a treatment for three months; 62 patients in control group without special treatment. The two groups’ ethnicities, ages had no statistical differences.

Exclusion criteria: before surgery the patient had taken hormone drugs within three months and patients with malignant or suspicion of malignancy.

Ovarian endometriosis cystectomies were implemented under anesthetia and the intraoperative vital signs were monitored by conventional laparoscopy.

Postoperative treatment
All patients in the experimental group took 3.6 mg GnRH-a every 28-35 days / times according to patient body weight (body weight < 55 kg: every 35 days / times; weight 55 kg ~ 65 kg: every 30 days / times; weight 65 kg: every 28 days / times) for three months continuously after five to eight days after surgery. If some low-estrogen indications emerged, such as hot flashes, sweating, irritability, and others, a detection of the levels of estrogen were assessed; if necessary add-back therapy was applied, that is, oral tibolone one tablet (2.5 mg) daily at the same time every day continuously, until hot flashes and other symptoms were relieved to maintain the basis of estrogen hormone levels at 30-50 (pg / ml) window dose. The control group did not take any medication.

Detections
At menstrual two to three days FSH, LH, and E2 values were regarded as indicators of ovarian reserve status [3, 4]. In preoperative menstrual two to three days, postoperative three months or at menstrual two to three days, and postoperative six months menstrual two to three days, FSH, LH, and E2 fluctuating levels were detected, there were follow-ups regarding natural pregnancy and recurrence which lasted 18 months.

Serum was collected by centrifugation of blood samples and stored at -20°C. (FSH, FSH / LH, E2 double-antibody radioimmunoassay kits were provided, including SN2682-type of γ-counter (intra block error < 5%, error between groups < 10%).

Statistical analysis
Preoperative and postoperative levels of sex hormones were measured by t-test (SPSS17.0).

Results

General data

The basic characteristics of the two groups with ovarian endometriosis endometriosis are shown in Table 1. There were no significant differences between age and body mass index (BMI) of these two groups.

Sex hormone

The sex hormone level changes before surgery, at postoperative three and six months are shown in Table 2. The FSH, LH, and E2 levels of experimental group at postoperative three months were significant lower (p < 0.05, Table 2) compared with the preoperative ones. In the control group at postoperative three months, FSH and LH levels were significant higher (p < 0.05, Table 2), and E2 was lower (p < 0.05, Table 2) than the preoperative level. The basal FSH level of experimental group restored to 95.3% of normal range after postoperative six months, while that of the control recovered to only 82.2%, and the difference was statistically significant (p < 0.05, Table 2).

Pregnancy outcome

The pregnancy outcome and recurrence after postoperative 18 months, after drug-discontinuation 32-68 days included 63 patients that experienced menstrual back tides in follow-up experimental group, with an average of 42.8 days, as shown in Table 3. After six months the basal FSH level of experimental group recovered to 95.3% of normal range, while in control group it recovered to 82.2%, and the difference was significant (p < 0.05, Table 3). The number of pregnancies within postoperative 18 months accounted for 19 patients (47.51% of the preoperative infertilities). The spontaneous pregnancy rate of preoperative infertility patients was 57.1%, and was higher than that in the experimental group, which was 36.8% (p < 0.05, Table 3). The recurrence rate of the experimental group was 12.7%, compared with the 27.4% of control group and the difference was statistically significant (p < 0.05, Table 3).

Discussion

The combination of laparoscopic ovarian endometrial cystectomy and postoperative GnRH-a therapy can inhibit FSH and LH secretion, regulate pituitary function and ul-

Table 1. — The preoperative characteristics of two groups.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n)</td>
<td>63</td>
<td>62</td>
</tr>
<tr>
<td>&lt; 35years old</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>&gt; 35 years old</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.10 ± 1.33</td>
<td>20.13 ± 1.07</td>
</tr>
</tbody>
</table>

Table 2. — The sex hormone level changes before surgery, at postoperative three and six months.

<table>
<thead>
<tr>
<th></th>
<th>Preoperative</th>
<th>Postoperative three months</th>
<th>Postoperative six months</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/ml)</td>
<td></td>
<td>7.2 ± 3.1</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td></td>
<td>0.8 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td></td>
<td>15 ± 5</td>
<td>9 ± 3</td>
</tr>
</tbody>
</table>

Table 3. — The pregnancy outcome and recurrence after postoperative 18 months.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancies</td>
<td>19 (47.51%)</td>
<td>4 (12.7%)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>11 (36.8%)</td>
<td>8 (25.8%)</td>
</tr>
</tbody>
</table>

Xin-hua Yang, Fei Ji, AiXingZi, AiLi, HaNiKeZi, TuerXun, Yan He, Yan Ding
Laparoscopic ovarian endometriosis cystectomy combined with postoperative GnRH-a therapy on ovarian function.

Ovarian endometriosis cystectomy has short- and long-term impacts on ovarian function to varying degrees. Postoperative combined treatment inhibited the function of pituitary gland, leading to transient decrease of postoperative FSH, LH, and E2 levels. The majority of patients during follow-up had gradually recovered, with no significant impact on ovarian function, which was consistent with clinical studies to a certain extent.

Laparoscopic ovarian endometrial cystectomy combined with GnRH treatment can cause temporary ovarian dormancy, reduce pelvic and uterine blood supply, reduce secretion of inflammatory cytokines in postoperative wound [12, 15], reduce the infiltration of residual lesions in the ovarian cortex, and reduce the invasion of residual lesions to ovulation hole. This is conducive to postoperative ovary restoration, and is a preferred treatment mode for ovarian cyst combined with infertility [16]. Preoperative infertility cases were 28, including 13 cases with primary infertility and 15 cases with secondary infertility: the average of years of infertility was 3.20 ± 0.68. The results showed that, compared with the preoperative situation (36.8%), postoperative pregnancy rate (57.1%) of infertile patients in the experimental group was significantly higher (p < 0.05), suggesting that combined treatment can improve postoperative pregnancy rate significantly, but the effects were related to clinical stage and patient age. Endometriosis with infertility treated with postoperative Zoladex can improve the pregnancy rate significantly. The majority of pregnancies occur at one postoperative year, especially within six months after GnRH-a drug discontinuance, which is a very important period for guided pregnancy. If after 18 months there were no pregnancies, assisted reproductive technology was recommended as soon as possible [17, 18].

Hyperplasia, infiltration, and diffusion are characteristics of endometriosis, an extensive pelvic adhesion and ambiguity of the lesions, surrounding tissues and organs are visible in severe uterine endometriosis, the pelvic anatomical

Table 2. — The serum FSH, FSH / LH, and E2 level changes in two groups before surgery, and at three and six months postoperatively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Menstrual duration (d)</th>
<th>Menstrual cycle (d)</th>
<th>Menstrual duration (d)</th>
<th>Menstrual cycle (d)</th>
<th>Infertility before surgery</th>
<th>Natural pregnancy after surgery (cases)</th>
<th>Pregnancy (%)</th>
<th>Interval duration (d)</th>
<th>Recurrence cases after 18 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>63</td>
<td>5.0</td>
<td>30.0</td>
<td>5.1</td>
<td>29.5</td>
<td>21</td>
<td>12</td>
<td>57.1</td>
<td>7.0 ± 1.0</td>
<td>8</td>
</tr>
<tr>
<td>Control group</td>
<td>62</td>
<td>4.8</td>
<td>0.3</td>
<td>5.0</td>
<td>28.0</td>
<td>19</td>
<td>7</td>
<td>36.8</td>
<td>9.0 ± 1.2</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3. — The menstrual back tide, natural pregnancy and recurrence at 18 months postoperatively.
structure is destroyed, affecting the pick-up and tubal transport, and could cause infertility. The restoration of the anatomical structure must rely on surgery, in separation process there is some risk of lesion rupture, unresectable invisible lesions in the rear pelvic cavity, and deep infiltration, uncompleted reductive surgery caused by the closed uterine rectal fossa, and is a hormone-dependent disease; residual disease may continue growth under ovarian hormones, so its recurrence rate is very high. Jee et al. [19] performed a retrospective cohort study to observe the recurrence of ovarian endometrioid cyst after conservative surgery (four groups: postoperative GnRH-a treatment for three, four, and six months; expectation therapy without medication), found that GnRH-a treatment for six months can better control the rate of recurrence. This study included EM cases with or without laparoscopic ovarian cystectomy women combined with postoperative GnRH-a treatment, their recurrences, and curative effects were assessed by follow-up for 18 months. Compared with preoperative phase, the recurrence rate (12.7%) of the experimental group was significantly lower than that of the control group (27.4%) (p < 0.05). It demonstrated that combined treatment can reduce the recurrence rate significantly, and is related to clinical stage and patient age. The recurrence rates and recurrence intervals had no significant difference (p > 0.05).

Recently some scholars [20, 21] have not supported the protective ovarian effect of GnRH-a, owing to the unclear mechanism of this protective function (a large component of human ovaries include primordial follicles, which are not subject to the effect of gonadotropin). Therefore, further researches are required to explore GnRH-a’s protective function on ovary.

References


Ectopic pregnancy and laparoscopy

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²University of Belgrade, Clinic of Gynecology and Obstetrics “Narodni Front”, Belgrade (Serbia)

Summary

Purpose: The aim of this study was to compare the success of surgical procedures performed by laparoscopy and laparotomy in the treatment of tubal ectopic pregnancy. Materials and Methods: In this prospective study, there were 57 women who were operated due to tubal ectopic pregnancy. Laparoscopic surgery was performed in 36 women (study group). Conventional abdominal surgical treatment was performed in 21 women (control group). Results: Among 36 women from study group, a laparoscopic linear salpingotomy was performed in 69.44% cases, salpingectomy in 13.88% cases, and milking of tube in 16.66% cases. In the control group, linear salpingotomy was performed in 57.14% cases, salpingectomy in 28.57% cases, and milking of tube in 14.28% cases. Patent ipsilateral fallopian tube at three months after surgery was 66.66% in the study group and 52.38% in the control group. The intrauterine pregnancy rate was 19.44% in the study group and 19.05% in the control group. Conclusion: The percentage of tubal patency and intrauterine pregnancies after laparoscopic surgical treatment was not higher than after conventional surgical treatment by laparotomy.

Key words: Ectopic pregnancy; Laparoscopy; Laparotomy; Surgery.

Introduction

The frequency of ectopic pregnancy has increased to a large extent over the past 20 years, both in developing as well as in developed countries. The incidence of ectopic pregnancy in the United States is 0.64%, and in the United Kingdom it is 1.11% [1, 2]. In order to preserve the fertile capability of women, the prime importance is to diagnose ectopic pregnancy as soon as possible. Ectopic pregnancy can be treated either medically or surgically. Surgical treatments may be radical or conservative and they may be performed by laparoscopy or laparotomy [3]. The possibilities of the conservative laparoscopic surgical treatment are great, if the ectopic pregnancy is discovered in intact stage. Laparoscopy is cost-effective and is the preferred surgical approach [4]. The reproductive potential of women after ectopic pregnancy is significantly decreased. One previous ectopic pregnancy increases the possibility of its recurrence by 10% [5]. The use of transvaginal ultrasound, the determination of serum beta-human chorionic gonadotropin (β-hCG), and progesterone concentrations raises the suspicion of an ectopic pregnancy at the early stage, while laparoscopy enables the precise onset of the final diagnosis, as well as estimation of the severity and operability of the pathological changes in the pelvis. The application of the operative laparoscopic techniques in haemodynamically stable women with ectopic pregnancy, enables the preservation of the fertile capability of any woman. Women with a first-recorded ectopic pregnancy have a significantly lower long-term delivery rate and a manifold increased risk of further ectopic pregnancies [6]. The risk of recurrence of ectopic pregnancy is approximately ten percent among women with one previous ectopic pregnancy and at least 25% among those with two or more previous ectopic pregnancies [5]. The aim of this study was to compare the success of surgical procedures performed by laparoscopy and laparotomy in the treatment of tubal ectopic pregnancy.

Materials and Methods

In this prospective study, the authors analyzed the success rate of the application of few operative laparoscopy techniques in 36 women (study group) treated for tubal ectopic pregnancy compared to the success rate of the conventional abdominal surgical treatment in 21 women (control group) in the Department for Laparoscopic Surgery, Gynecology-Obstetrician Clinic “Narodni Front” in Belgrade. The diagnosis of ectopic pregnancy was based on the anamnesis information, transvaginal color Doppler ultrasound examination, values of serum β-hCG, while the laparoscopy enabled the final diagnosis.

The patients were treated by laparoscopy or laparotomy, based on their haemodynamic status, experience of the surgeon, and the availability of endoscopic equipment. To remove an ectopic pregnancy, the following surgeries were performed on the fallopian tubes: salpingotomy, salpingectomy, and extirpation of tubal pregnancy through the fimbrial end. Laparoscopy operations were performed using a Harmonic scalpel. It works on the basis of breaking hydrogen bonds on the molecular level in human cells using high frequency vibrations (up to 50,000/min). It performs cutting and coagulation on its tip with only a local effect and temperatures not higher than 90°C. Laparoscopic salpingotomy was performed in women who had the desire for future pregnancies, in haemodynamically stable women, no severe adhesions in the tubal wall, in the case of absence of pathology of the contralateral tube, or in cases with size of ectopic pregnancy less than five cm, and where the gestational sac was located in the ampulla, infundibulum or isthmic portion.

During laparoscopic salpingotomy, linear incision on the antimesenteric tubal wall was made at the site of maximum distension, extending from one to two cm. Product of conception was removed from the tube using laparoscopic atraumatic forceps or...
hydrometra. Laparoscopic salpingectomy was performed in women who had the desire for future pregnancies or in the case of tubal rupture, in a previously reconstructed tube, in the case of recurrent tubal pregnancy in the same fallopian tube, and in the case of tubal pregnancy greater than five cm. It was executed by progressively coagulating and cutting the mesosalpinx, beginning with the fimbrial end to proximal portion. Excised tube was removed intact or in sectioned part or placed in an endobag and removed. Extirpation of tubal pregnancy through the fimbrial end, ie, milking of ipsilateral tube was performed in the case where the product of conception was located on fimbrial end or distal tubal segment. This was accomplished by aspiration or use of gently grasping forceps which removed product of conception. In some cases, tubal abortion had already occurred. Laparotomy was performed through a Pfannenstiel incision and standard surgical techniques. Laparotomy was performed in women with extensive intraperitoneal bleeding with tubal rupture or poor visualization of the pelvis at the time of laparoscopy. Salpingotomy was performed using monopolar needle. With salpingotomy, the mucosal margins were then closed with interrupted sutures. The seromuscular sutures were placed using delayed absorbable material. Salpingitis in the anamnesis was reported in three (14.28%) women. Two (9.52%) women gave birth previously and three (8.33%) women had abortions earlier. The diagnosis of the ectopic pregnancy in both group was linear salpingotomy. Salpingectomy was performed frequently in the control group (p < 0.05). Size of tubal pregnancy and surgical time did not differ between the study and control groups (p > 0.05). Hospital stay was significantly shorter in the study group (p < 0.05). Estimated blood loss was significantly lower in the study group (p < 0.05). Size of tubal pregnancy and surgical time did not differ between the study and control groups (p > 0.05). Estimated blood loss was significantly lower in the study group (p < 0.05). Size of tubal pregnancy and surgical time did not differ between the study and control groups (p > 0.05).

Results

Out of 57 women with tubal ectopic pregnancy, 36 were treated with laparoscopy and 21 women by laparotomy. There was no conversion to laparotomy in the women treated by laparoscopy in this study. Out of 36 women with tubal ectopic pregnancy from the study group, two (5.55%) women were previously operated due to tubal gravidity. Three (8.33%) women were operated earlier due to a tubal infertility factor. Salpingitis in the anamnesis history was reported in four (11.11%) women. Five (13.88%) women gave birth previously and three (8.33%) women had abortions earlier. There were 21 women in control group. Among them, two (9.52%) women were operated earlier due to tubal gravidity and one (4.76%) due to tubal infertility factor earlier. Salpingitis in the anamnesis was reported in three (14.28%) women. Two (9.52%) women gave birth previously and three (14.28%) women had abortions earlier. The average age of the study group was 32.5 ± 3.9 years and 33.7 ± 2.6 years in the control group. These differences were not statistically significant (p > 0.05). The diagnosis of the ectopic pregnancy in study and control group was set from 35 to 56 days after the last menstruation. The most frequent location of the tubal ectopic pregnancy in both groups was in the distal half of the fallopian tube. The most frequent types of the surgical procedures ectopic pregnancy in both group was linear salpingotomy. Salpingectomy was performed frequently in the control group (p < 0.05). Size of tubal pregnancy and surgical time did not differ between the study and control groups (p > 0.05).

Table 1. — Surgical outcome in the study and control groups.

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>3 8.33</td>
<td>2 9.52</td>
</tr>
<tr>
<td>Ruptured tubal pregnancy</td>
<td>3 8.33</td>
<td>5 23.81</td>
</tr>
<tr>
<td>Unruptured tubal pregnancy</td>
<td>30 83.33</td>
<td>14 66.66</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>1.93 ± 0.6</td>
<td>4.18 ± 1.21</td>
</tr>
<tr>
<td>Surgical time (min)</td>
<td>35.20 ± 18.50</td>
<td>43.10 ± 15.30</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>55.5 ± 72.5</td>
<td>145.5 ± 125.5</td>
</tr>
<tr>
<td>Patency of contralateral tube</td>
<td>4 80.00</td>
<td>4 66.66</td>
</tr>
<tr>
<td>Occlusion of contralateral tubal patency</td>
<td>1 16.66</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>5 100.00</td>
<td>6 100.00</td>
</tr>
</tbody>
</table>

Table 2. — Hysterosalpingographic findings three months after surgery.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral salpingotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral tubal patency</td>
<td>20 40.00</td>
<td>6 50.00</td>
</tr>
<tr>
<td>Patency of ipsilateral tube with contralateral tubal occlusion</td>
<td>9 36.00</td>
<td>3 25.00</td>
</tr>
<tr>
<td>Occlusion of ipsilateral tube with contralateral tubal patency</td>
<td>6 24.00</td>
<td>3 25.00</td>
</tr>
<tr>
<td>Total</td>
<td>25 100.00</td>
<td>12 100.00</td>
</tr>
<tr>
<td>Unilateral salpingotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patency of contralateral tube</td>
<td>4 80.00</td>
<td>4 66.66</td>
</tr>
<tr>
<td>Occlusion of contralateral tube</td>
<td>1 20.00</td>
<td>2 33.33</td>
</tr>
<tr>
<td>Total</td>
<td>5 100.00</td>
<td>6 100.00</td>
</tr>
<tr>
<td>Total</td>
<td>24/36 (66.66%)</td>
<td>11/21 (52.38%)</td>
</tr>
<tr>
<td>Occlusion of ipsilateral tube</td>
<td>8/36 (22.22%)</td>
<td>4/21 (19.05%)</td>
</tr>
</tbody>
</table>
procedures, or in the postoperative period due to the residual trophoblast. All patients from study group were discharged between the first and third postoperative day. One (4.76%) woman from control group had a wound infection in the postoperative period, after the third postoperative day. On analysis of the pathologic changes of ectopic trophoblastic tissue, it was found that 37 (64.91%) out of 57 specimens were degenerated products of conception and 20 (35.08%) specimens of trophoblastic tissue included hemorrhage. These results are shown in Table 1.

Hysterosalpingography was performed at three months after surgery. In the study group, 24 (66.66%) out of 36 women had a patent ipsilateral fallopian tube. In the control group, 11 (52.38%) out of 21 women had a patent ipsilateral fallopian tube. The difference was not statistically significant \((p > 0.05)\). These results are shown in Table 2.

All operated women were scheduled for medical control in the period from 12 months after surgery. Nine (25.0%) out of 36 women from study group became pregnant. In seven (19.44%) women, the pregnancy was intrauterine and in two (5.55%) women it was ectopic. Five (23.81%) out of 21 women from control group became pregnant. In four (19.05%) women the pregnancy was intrauterine and in one (4.76%) woman it was ectopic. The difference was not statistically significant \((p < 0.05)\). Six (25.00%) out of 24 women from study group with patent ipsilateral tube became pregnant. In five (20.83%) women, the pregnancy was intrauterine and in one (4.16%) woman it was ectopic. Three (27.27%) out of 11 women from control group with patent ipsilateral tube became pregnant. In two (18.18%) woman the pregnancy was intrauterine and in one (9.09%) woman it was ectopic. The difference was not statistically significant \((p > 0.05)\). These results are shown in Table 3.

Discussion

In the last period, the frequency of ectopic pregnancy shows a permanent increase. Ampulla is the most frequent implantation site in the fallopian tube, with approximately 73.3%, then isthmus 12.5%, fimbrial 11.6%, and interstitial 26.1% [7]. In the present study group, the ampullar location of ectopic pregnancy was represented in 28 (77.77%) cases and in 16 (76.19%) cases from the control group. The early onset of the diagnosis enables the application of the conservative laparoscopic or conventional surgical treatment. The conservative surgical treatment often includes linear salpingotomy with the removal of the gestational products. In the present study, salpingotomy was performed in 25 (69.44%) patients from the study group and in 12 (57.14%) patients from the control group. Other authors have performed laparoscopic salpingostomy in 66.5% and in 84.9% patients with ectopic pregnancy [8, 9]. Radical surgical treatment or salpingectomy was performed in five (13.88%) patients with tubal ectopic pregnancy from the present study group and six (28.57%) patients from the control group. Other authors have performed laparoscopic salpingectomy in 80% of patients with ectopic pregnancy [10]. All patients were offered hysterosalpingography at three months postoperatively. The tubal patency rate of the treated side, i.e. ipsilateral tube in the present patients from the study group was 66.66% and 52.38% patients from the control group. Other authors suggest that the tubal patency rate of the treated side was 90% in patients with linear salpingotomy with suturing and 94% without suturing [11]. Tubal patency of the treated tube was demonstrated at hysterosalpingography in 55% that underwent laparoscopic surgery [12]. Tubal patency after laparoscopic salpingotomy and salpingectomy by laparotomy in patients with a small unruptured tubal patency was 73% and 83%, respectively [13]. Nine (25%) women from the study group and five (23.81%) women from the control group became pregnant during 12 months after surgery. The percentage of intrauterine pregnancies in patients with patent ipsilateral tube after laparoscopic surgery was 20.83% in the present study group and 18.18% in the control group. The rate of recurrent ectopic pregnancies in women in the present study group was 5.55%, while in women of the control group it was 4.76%. Other authors suggest that the subsequent spontaneous intrauterine pregnancy rate was 62% after laparoscopic salpingotomy in patients with non-ruptured tubal patency and the ectopic pregnancy rate was 17.3% [14]. Intrauterine pregnancy rates up to 24 months were established as 65.2% in salpingectomy and 60.1% in the salpingostomy groups [15]. The 24-month cumulative rate of intrauterine pregnancy was 67% after laparoscopic salpingectomy and 76% after laparoscopic salpingostomy in the treatment of tubal ectopic pregnancy [16]. The rate subsequent ectopic pregnancy was 15% after laparoscopic salpingostomy and ten percent after laparoscopic salpingectomy [5]. The rates of subsequent intrauterine pregnancy were 74% in the laparoscopy group and 61% in the laparotomy group and the rates subsequent to ectopic pregnancy were four percent in the laparoscopy group and ten percent in the laparotomy group [3]. Subsequent intrauterine pregnancy rates in patients with a small unruptured tubal pregnancy after salpingotomy by laparoscopy and salpingectomy by laparotomy was 57% and 53%, respectively, while the recur-

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>IUP</td>
<td>7</td>
<td>19.44</td>
</tr>
<tr>
<td>EP</td>
<td>2</td>
<td>5.55</td>
</tr>
<tr>
<td>Total</td>
<td>9/36</td>
<td>25.00</td>
</tr>
<tr>
<td>IUP in women with patent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ipsilateral tube</td>
<td>5</td>
<td>20.83</td>
</tr>
<tr>
<td>EP in women with patent</td>
<td>1</td>
<td>4.16</td>
</tr>
<tr>
<td>ipsilateral tube</td>
<td>6/24</td>
<td>25.00</td>
</tr>
</tbody>
</table>

rent ectopic pregnancy rate was seven and 14 percent, respectively [4]. No serious complications occurred during the surgical procedures or during the postoperative period. One (4.76%) woman from the present control group had a wound infection in the postoperative period.

One of the most common complications of laparoscopic tubal salpingotomy in the treatment of tubal ectopic pregnancy is incomplete removal of products of conception as an persistent ectopic pregnancy requiring additional therapy. In the present study, there were no cases of persistent ectopic pregnancy. One population-based study found that the failure rate of laparoscopic salpingotomy was 6.6% [9]. The literature data state that the percentage of intraoperative laparoscopic complications ranges from zero to eight percent, with the average being two percent and the percentage of postoperative complications ranged from zero to 15%, with the average being nine percent [17]. In the present study, the authors conclude that laparoscopic treatment of tubal ectopic pregnancy is not more successful than conventional surgical treatment by laparotomy. The percentage of tubal patency and intrauterine pregnancies after laparoscopic surgical treatment was not higher than after conventional surgical treatment by laparotomy. Laparoscopy has a shorter duration of surgical time and hospital stay, compared with laparotomy.

References


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Amniotic fluid amino acid concentrations in fetal skeletal dysplasia

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¹Kocaeli Derince Education and Research Hospital, Department of Biochemistry
²Kocaeli Derince Education and Research Hospital, Department of Obstetrics and Gynecology, Kocaeli (Turkey)

Summary

The authors’ objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with skeletal dysplasia in the second trimester of pregnancy. Eighteen pregnant women who had fetuses with skeletal dysplasia detected by ultrasonography (skeletal dysplasia) in the second trimester and 35 women who had abnormal triple screenings indicating an increased risk for Down syndrome were included in the study. Amniotic fluid was obtained by amniocentesis. The chromosomal analysis of the study and control groups was normal. Levels of free amino acids and non-essential amino acids were measured in amniotic fluid samples using GC/FID free (physiological) amino acid kit by gas chromatography. The mean levels of essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) in amniotic fluid were found to be significantly lower in fetuses with skeletal dysplasia than in the control group (p < 0.05). The detection of significantly lower amino acid concentrations in the amniotic fluid of fetuses with a skeletal dysplasia compared to healthy fetuses suggests amino acid deficiency may play an etiological role in the pathogenesis of skeletal dysplasia.

Key words: Amino acids; Skeletal dysplasia; Amniotic fluid.

Introduction

Skeletal dysplasias are a group of congenital abnormalities of the bone and cartilage that are characterized by short stature. Skeletal dysplasia, sometimes called dwarfism, is a disorder of short stature defined as height that is three or more standard deviations below the mean height for age, race, and gender. Skeletal dysplasias involve disproportionately short stature, there are many other associated conditions such as small arms and truck, bowlegs, skull malformations, such as a large head, and cloverleaf skull. Maternal hydramnios is the most significant event associated with fetal skeletal dysplasia during pregnancy, and fetal hydrops is frequently observed. Fetal activity may be decreased in the lethal types of skeletal dysplasia [1].

The skeleton’s mass is made up of non-living bone matrix and many tiny bone cells. Half of the bone matrix’s mass is water, while the other half is collagen protein and solid crystals of calcium carbonate and calcium phosphate [2-3].

The aim of the present study was to determine the concentrations of amino acids in amniotic fluid of pregnant women whose fetuses were diagnosed to have skeletal dysplasia in the second trimester of pregnancy. The authors hypothesized that the concentrations of amino acids may be decreased in fetuses with skeletal dysplasia.

Materials and Methods

The study was performed at the Prenatal Diagnosis Unit of Dicle University Hospital between January 2010 and January 2013. The study was approved by the institutional review board and Ethics Committee of the university hospital, and written informed consent was obtained from all participants. All pregnant women who had a fetus with skeletal dysplasia (n = 18) in the second trimester were included in the study. The first 35 women who attended the present clinic and had abnormal triple screens indicating an increased risk for Down syndrome were included in the study as the control group (n = 35). Mean maternal age was 27.5 ± 2.3 years for the skeletal dysplasia group and 28.1 ± 3.4 years for the study group. The mean gestational age at sampling was 18.2 ± 1.1 weeks for the skeletal dysplasia group and 19.1 ± 1.3 weeks for the study group. Maternal body mass index was 29.2 ± 1.0 kg/m² in skeletal dysplasia group and 27.8 ± 1.2 kg/m² in the study group. Five women in the skeletal dysplasia group and seven in the control group were nulliparous (Table 1).

The authors evaluated biometric parameters and ultrasonography (U/S) findings consistent with the diagnosis of skeletal dysplasia. The femora, humeri, tibia, and ulna were symmetrically shortened. U/S scan performed and a decreased rate of development of the femora (femur length [FL] < 5th centile).

Obese patients and those with any systemic or endocrine disorder were excluded from the study. All pregnancies were accurately dated by the last menstrual period and by first-trimester U/S investigation. Amniotic fluid samples were obtained by routine transabdominal amniocentesis and collected into 10-ml dry tubes. All amniotic fluid samples were free of blood contamination. Venous blood samples were taken within 10 minutes after amniocentesis from the pregnant women and collected into Ethylenediaminetetraacetic acid (EDTA)-containing tubes. All samples were immediately centrifuged at 3,000 g for 10 minutes and stored at -20°C until assayed. Levels of free amino acids (essential amino acids: histidine, leucine, lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, and valine) and non-essential amino acids (alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamic acid, glutamine, glycine, ornithine, and proline) were measured in plasma and amniotic fluid samples using GC/FID free (physiological) amino acid kit by gas chromatography (focus GC-

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

<table>
<thead>
<tr>
<th></th>
<th>Skeletal dysplasia group (n = 18)</th>
<th>Control group (n = 35)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>27.5 ± 2.3</td>
<td>28.1 ± 3.4</td>
<td>0.376*</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>5 (9.4%)</td>
<td>7 (13.2%)</td>
<td>0.805*</td>
</tr>
<tr>
<td>Gestational age</td>
<td>18.2 ± 1.1</td>
<td>19.1 ± 1.3</td>
<td>0.447*</td>
</tr>
<tr>
<td>Maternal body mass index</td>
<td>29.2 ± 1.0</td>
<td>27.8 ± 1.2</td>
<td>0.702*</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD. There were no statistically significant differences between groups (Student t-test, p > 0.05).

Table 2. — Concentrations of 20 amino acids in amniotic fluid samples of fetuses with skeletal dysplasia and controls.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control group (n = 35) (μmol/l)</th>
<th>Skeletal Dysplasia Group (n = 18) (μmol/l)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>162.9 ± 49.2</td>
<td>158.2 ± 35.5</td>
<td>0.315</td>
</tr>
<tr>
<td>Asparagine</td>
<td>22.8 ± 3.9</td>
<td>23.5 ± 4.1</td>
<td>0.217</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.2 ± 1.2</td>
<td>8.1 ± 4.6</td>
<td>0.701</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>2.6 ± 1.0</td>
<td>2.5 ± 1.4</td>
<td>0.439</td>
</tr>
<tr>
<td>Cysteine</td>
<td>29.5 ± 2.7</td>
<td>28.7 ± 3.3</td>
<td>0.101</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>33.5 ± 2.0</td>
<td>32.5 ± 6.3</td>
<td>0.605</td>
</tr>
<tr>
<td>Glutamine</td>
<td>42.0 ± 5.7</td>
<td>38.2 ± 9.9</td>
<td>0.508</td>
</tr>
<tr>
<td>Glycine</td>
<td>145.4 ± 41.9</td>
<td>142.3 ± 40.8</td>
<td>0.178</td>
</tr>
<tr>
<td>Histidine</td>
<td>57.3 ± 13.2</td>
<td>21.5 ± 4.8</td>
<td>0.002*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>18.2 ± 3.5</td>
<td>11.2 ± 1.1</td>
<td>0.013*</td>
</tr>
<tr>
<td>Leucine</td>
<td>75.1 ± 9.5</td>
<td>49.2 ± 8.8</td>
<td>0.019*</td>
</tr>
<tr>
<td>Lysine</td>
<td>92.5 ± 17.0</td>
<td>33.4 ± 5.5</td>
<td>0.002*</td>
</tr>
<tr>
<td>Methionine</td>
<td>29.8 ± 1.2</td>
<td>8.8 ±1.3</td>
<td>0.011*</td>
</tr>
<tr>
<td>Ornithine</td>
<td>20.1 ± 3.0</td>
<td>19.2 ± 2.9</td>
<td>0.197</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>41.3 ± 7.6</td>
<td>22.6 ± 4.9</td>
<td>0.012*</td>
</tr>
<tr>
<td>Proline</td>
<td>214.4 ± 28.8</td>
<td>218.5 ± 29.6</td>
<td>0.882</td>
</tr>
<tr>
<td>Threonine</td>
<td>132.6 ± 8.5</td>
<td>58.3 ± 10.7</td>
<td>0.015*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>18.7 ± 2.3</td>
<td>5.2 ± 1.2</td>
<td>0.018*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>55.2 ± 9.3</td>
<td>53.1 ± 7.0</td>
<td>0.778</td>
</tr>
<tr>
<td>Valine</td>
<td>171.4 ± 9.2</td>
<td>103.1 ± 11.4</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD. *p < 0.05 for the skeletal dysplasia group compared to control (Student t-test).
stricting the growth of long bones and the resulting small thoracic cage causes a mechanical restriction of lung growth. This may be lethal at birth, or death may occur in early infancy. In general, the limb bones are markedly shortened and in perinatal lethal disease are often below the 5th percentile. The diagnosis of a lethal skeletal dysplasia can be assessed prenatally based on the short femur and small thorax [1].

The estimated frequency of lethal skeletal dysplasias is 1.5 per 10,000 births [2]. The most common lethal groups are osteogenesis imperfecta, thanatophoric dysplasias, achondrogenesis, and short ribbed polydactyly [3].

In many cases, there will already be a prenatal or neonatal diagnosis of a lethal skeletal dysplasia made before the autopsy is begun. The cornerstone of diagnosis is the radiograph. To an expert radiologist, the radiographs will usually establish the diagnosis of a specific type of skeletal dysplasia. In some cases, a molecular diagnosis will clarify doubts about the radiological diagnosis and render genetic counseling more secure [1-3].

Prenatal screening and diagnosis are primarily performed in the second trimester, but late first trimester fetal structural assessment is becoming more common with advances in transvaginal ultrasound imaging and the widespread use of first trimester screening for Down syndrome. Increased nuchal translucency can be associated with skeletal dysplasia; when this association is present, approximately 85 percent of cases are lethal skeletal dysplasias [4].

The abnormality seen in the bone of patients with skeletal dysplasia is failure of endochondral ossification. Intramembranous and periosteal ossification are undisturbed. Histologic studies have shown disarray of the chondrocytes, with loss of columnization and loss of normal chondrocyte proliferation. Endochondral growth is disturbed and the bones remain short [5].

Amino acids are small biological molecules that, when linked together in a long chain and folded into a globular structure, form a protein. Proteins serve both structural and physiological functions in the body. Of the 20 amino acids found in proteins, nine are essential to diet because body cannot produce them. When recovering from a bone injury, intake of essential amino acids becomes especially important. The development of in vitro cell culture methods has made it possible to study bone cell metabolism and growth and obtain a deeper insight into the pathophysiology of skeletal diseases. The studies support that amino acids has a positive effect on osteoblast proliferation, activation, and differentiation. Therefore, administration of amino acids may be useful in clinical treatment and prevention of skeletal disorders [6].

The present authors found lower levels of amniotic fluid essential amino acids in the skeletal dysplasia group than in the control group which might explain amino acid deficiency in fetuses with skeletal dysplasia. They speculate that replacement of essential amino acids might improve the outcome of infants with skeletal dysplasia during the neonatal period. This is a preliminary study on amniotic fluid amino acid concentrations conducted on a small patient series. They believe that it would be beneficial to conduct further studies with larger groups to determine the amino acid levels of fetuses with skeletal dysplasia.

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The routine value of anogenital distance as an anthropometric measurement in newborns

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Summary
Objective: Anogenital distance (AGD) is a sexually dimorphic trait; AGD is an external marker of sexual differentiation at birth in animals. Study Design: The present study used a prospective, cross-sectional design. Data were collected from newborn infants that were delivered at hospital in Bucheon between March and September 2009. The mothers of the infants provided sociodemographic information and obstetric history. The relationships between AGD and other anthropometric measures (i.e., weight, height, and head circumference) were analyzed. Moreover adjusted AGD values were compared with obstetric characteristics, including number of deliveries, abortion history, preterm history, and duration of gestation. Results: Significant correlations were noted for all anthropometric measures in male and female newborns ($p < 0.0001$). The adjusted AGD values in males and females were also significantly associated with the duration of gestation ($p = 0.0008$ and $p = 0.0148$). Moreover, the adjusted AGD in female newborns, but not in males, was significantly associated with maternal abortion history ($p = 0.0242$). Conclusion: The AGD is a valuable anthropometric marker in term infants.

Key words: CRH; Progesterone; Phases of labor; Term labor; Latent phase of labor; Active labor; Postpartum.

Introduction
The anogenital distance (AGD) is an external marker of sexual differentiation at birth. Specifically, the AGD is a measure of the caudal border of the genital swelling [1]. During the embryonic stage, the genital swelling differentiates into the labia majora in females and the scrotum in males [1]. AGD measurements are routinely used as an indicator of endocrine function in animal studies. The United States Environment Protection Agency (USEPA) recommends measuring the AGD to determine reproductive toxicity and fertility effects. However, very few human studies have used AGD measurements.

Anthropometric measurements are easy to measure and represent an infant’s nutritional status (i.e., body weight, height, and head circumference). Knowing the endocrine status of a newborn immediately after delivery would be advantageous. The AGD is a representative measurement of androgen action during the embryonic stage. The present study aims to confirm that AGD is an easy measurement and a valuable anthropometric marker for endocrine problems in newborn infants. The AGD in infants has not been published in the Republic of Korea. Therefore, the aim of the present study is to provide data on AGD as a reference value of Korean newborns.

Materials and Methods
Study subjects and measurements
Data were collected from newborn infants that were born at a hospital in Bucheon. A cross-sectional study was conducted from March to September 2009. The present study was approved by the Ethics Committee for the Protection of Persons in Biochemical Research at the Institute of Medical Science of Soonchunhyang University Bucheon Hospital. One hundred and sixty pregnant women were enrolled in the study. A questionnaire was administered to the mothers regarding sociodemographic characteristics and maternal obstetric history. Maternal and infant body weight and height were measured.

The adjusted AGD values were classified into four groups: short (adjusted AGD < 25th percentile), first intermediate (25th percentile ≤ adjusted AGD < 50th percentile), second intermediate (50th percentile ≤ adjusted AGD < 75th percentile), and long (adjusted AGD ≥ 75th percentile). The proportion of mothers in each of the four groups that had a history of abortions was calculated, as well as the duration of gestation.

Physical examination
All AGD measurements were taken by a trained nurse. A detailed examination of the anthropometric measurements was performed under the direction of a trained obstetrician. Anthropometric items were measured in the infants (i.e., body weight, length, head circumference, and AGD) ($n = 61$ females and $n = 99$ males). The protocol for AGD measurements was based on a previously published study [1]. The AGD was measured as follows: 1) the newborn infant was placed in the dorsal decubitus position with both hips flexed; 2) light pressure was exerted on the infant’s thighs until the examiners hand touched the subject’s abdomen; 3) measurements were made with Vernier calipers; 4) the distance was measured from the center of the anus to the posterior convergence of the fourchette (where the vestibule begins) in female newborns [2] and from the center of the anus to

* These authors contributed equally to this work and should be considered co-corresponding authors.

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the junction of the smooth perineal skin with the skin of the scrotum in male newborns (Figure 1).

**Statistical analysis**

AGD measurements were divided by body weight (mm/kg). Preterm (gestation < 37 weeks) and low-birth weight (birth weight < 2,500 g) newborns were not excluded from the analysis. Correlations were calculated between the AGD and the other anthropometric measures (i.e., weight, height, and head circumference). Moreover, the adjusted AGD was compared with the obstetric characteristics of the mother. Specifically, the number of deliveries, abortion history, preterm history, and duration of gestation were recorded. The adjusted AGD in newborns was classified into the following three groups: short (adjusted AGD < 25th percentile), intermediate (25th percentile ≤ adjusted AGD < 75th percentile), and long (adjusted AGD ≥ 75th percentile). The proportion of male and female newborns in each of the four groups was also calculated, as well as the maternal history of abortions and the duration of gestation. The differences among the variables were determined using STATA version 10. Statistical significance was set at *p* < 0.05.

**Results**

**General characteristics**

The age of the mothers in the present study ranged from 16–44 years, with a median age of 32 years (Table 1). The median body mass index (BMI) was 21.7 kg/m². The majority of the mothers (67.6 %) had other children. One hundred nine of the subjects (68.1%) lived in a rural area. Nearly one-half (46.1%) of the subjects graduated from a college or university. A history of preterm births was not reported by most of the mothers (89.4 %). Moreover 65.0% of the mothers reported never having an abortion. Cesarean section (58.1%) was the main method of delivery and most of the subjects were > 37 weeks gestation (66.2 %).

**General and anthropometric characteristics in newborns**

AGD measurements were obtained from 160 newborns (males = 99; females = 61). The characteristics of the newborns are listed in Table 2. No neurological or behavioral disorders were noted in the newborns with genital measurements. The average AGD in male infants was 19.8 ± 3.2 mm and 15.1 ± 3.1 mm in female infants. The AGDs in males were greater than females (*p* < 0.0001; Figure 2). Specifically, males displayed a 1.3-fold greater AGD as compared with females. No significant differences were noted in body weight, height, head circumference, Apgar scores, or gestational duration between male and female infants.

**Correlations between AGDs and anthropometric measures**

The associations between the AGDs and anthropometric measures in newborns are shown in Table 3. In males, the AGD was correlated with height (*r* = 0.328), weight (*r* = 0.3564), and head circumference (*r* = 0.2124). The AGD in females was only correlated with weight (*r* = 0.2929). Moreover, AGD was positively correlated with weight in male and female infants. These results are consistent with previous studies that have demonstrated that body weight is a known predictor of AGD [3]. As a result, AGD values were adjusted according to weight in the present study. Significant correlations were noted for all of the anthropometric measurements in males and females (*p* < 0.0001).
Adjusted AGD and maternal obstetric characteristics

The adjusted AGD (mm/kg) was examined in relation to a previous maternal history of preterm delivery and abortion, as well as the current delivery type and duration of gestation (Figure 3). In male infants, a significant difference was noted between the adjusted AGD and the duration of gestation. The adjusted AGD in males born at < 37 weeks gestation was longer ($p < 0.0001$) as compared with term infants. These differences were also noted in female infants ($p = 0.464$). In addition, the adjusted AGD in females was significantly different in mothers with a history of abortions ($p = 0.0028$). The adjusted AGD in female newborns born at > 37 weeks gestation was not significantly different as compared with female infant’s born to mothers with a history of abortions (data was not shown). Therefore, these results suggest that the duration of gestation may be impacted in female infants born to mothers with a history of abortions.

Statistical analyses were used to determine the relationship between abortion history and duration of gestation. Newborns were classified into three groups. The short adjusted AGD group consisted of infants with AGD measurements below the 25th percentile. The long adjusted AGD group included infants with AGD measurements in the 75th percentile. The intermediate group included infants with AGD measurements between the 25th and 75th percentile. The intermediate adjusted AGD group and the long adjusted AGD group were compared to the short adjusted AGD group.

Abortion frequency in the intermediate and long adjusted AGD groups was compared with the short adjusted AGD group (Table 4). Significantly differences were noted in females in the long adjusted AGD group as compared with females in the short group ($p = 0.088$). No significant differences were noted in males. The frequency analysis of gestational duration was also analyzed (Table 5). Significant differences were noted in the long adjusted AGD group as compared with the short adjusted AGD group in males and females ($p = 0.0008$ and $p = 0.0126$, respectively). Differences were noted in AGD values between preterm and term delivered infants.

Discussion

The aims of the present study were to determine the anthropometric value of AGD in Korean infants. The authors hypothesized that fetal weight gain occurs late in gestation after sexual organ development, which occurs during the early fetal period. They found that AGD was correlated with height, weight, and head circumference in term infants. Therefore, AGD is a valuable anthropometric measurement in term newborns, but not premature infants.

### Table 4. — A comparison of adjusted anogenital distance (mm/kg) according to experience of abortion.

<table>
<thead>
<tr>
<th>Adjusted AGD (mm/kg)</th>
<th>Males (n = 99)</th>
<th>Females (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
<td>Yes (%)</td>
</tr>
<tr>
<td>Short</td>
<td>12 (16.2)</td>
<td>13 (37.1)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>35 (54.7)</td>
<td>15 (42.9)</td>
</tr>
<tr>
<td>Long</td>
<td>17 (26.6)</td>
<td>7 (20.0)</td>
</tr>
</tbody>
</table>

AGD: anogenital distance.

### Table 5. — A comparison of adjusted anogenital distance (mm/kg) according to duration of gestation in newborns.

<table>
<thead>
<tr>
<th>Adjusted AGD (mm/kg)</th>
<th>Males (n = 99)</th>
<th>Females (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
<td>Yes (%)</td>
</tr>
<tr>
<td>Below 25%</td>
<td>4 (10.8)</td>
<td>21 (33.9)</td>
</tr>
<tr>
<td>25-50%</td>
<td>18 (48.7)</td>
<td>32 (51.6)</td>
</tr>
<tr>
<td>50-75%</td>
<td>15 (40.5)</td>
<td>9 (14.5)</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.
Moreover, the authors found that their AGD measurements in males were consistent with other reports, but their AGD measurements in females were longer than previously reported [1]. This difference in AGD values in the present study provides a reference sample for Korean infants. Furthermore, they found that the adjusted AGD in females was statistically significant in mothers with a history of abortions. While the underlying cause for this result is unknown, it may be due to a maternal hormonal effect. Future studies should evaluate the relationship between previous abortions and AGD values. AGDs are sexually dimorphic in animals, with longer AGDs reported in males as compared with females. This difference suggests the action of androgens on the undifferentiated tissues during fetal developmental. The relationship between endocrine disruptors and AGDs in male animals has been previously studied. In male rats, perinatal administration of vinclozolin and ethane dimethane sulphonate (EDS) induced female-like AGD values [4, 5]. Moreover, anti-androgen action in male animals was significantly correlated with AGDs. AGD was the most sensitive parameter when anti-androgenic properties were measured in pregnant rats [6]. Female rats that were exposed to polychlorinated biphenyls (PCBs) displayed significantly increased AGDs, which may be due to androgen responsiveness in female rats during fetal developmental [7]. A decrease in the AGD and an increase in the incidence of undescended testes have been reported following exposure to monobenzyl phthalate (MBE) [8]. AGDs may be reflective of prenatal androgen or anti-androgen endocrine disruptors in both sexes. Endocrine disruptors may interrupt sexual organ differentiation. Therefore, AGDs represent the effect of endocrine disruptor exposure during the prenatal period. In human studies, the anogenital ratio is a useful method to diagnose androgen-induced labioscrotal fusion in premature and full-term female infants [2]. A decrease in size, feminization of the AGD, and incomplete testicular descent is associated with anti-androgen action, including phthalate levels in the mother’s urine. In another study, AGD measurements were not correlated with intrauterine exposure to dichlorodiphenyldichloroethylene (DDE), an androgenic compound [10].

Figure 3. — Comparisons of adjusted anogenital distance in male and female newborns according to maternal obstetrical characteristics: (A) experience of abortion, (B) experience of preterm, (C) type of delivery and (D) duration of gestation (*p < 0.05, **p < 0.005).
Physical exams in male newborns should be required within 24 hours of birth (i.e., testis position, penile length, and AGD to genitalia length) [11]. Previous studies have reported that phthalate levels were higher in males with short AGDs [12]. Thus, effects of endocrine disruptors should be evaluated after delivery.

Salazar-Martinez et al. [1] and Callegari et al. [3] reported that the AGD in females was shorter than what was reported in the present study; however, the AGD values in males were consistent between studies. In the previous study, the AGD measurement was two-fold greater in males than in females. The present data were inconsistent with this finding [1]. Therefore, the data in the present study may be specific to the Korean population. Moreover, differences in the AGD were noted in the present study between preterm and full-term newborns. Based on the present study, the adjusted AGD in full-term newborns was shorter as compared with premature newborns, indicating that full-term infants have increases in weight rather than other anthropometric factors. As a result, AGD is a good anthropometric method that is easily measured in full-term infants. The present data showed that the adjusted AGD in females was associated with maternal abortion history. Hormone levels were not evaluated in the present study. Interestingly, there are no previous reports on the relationship between adjusted AGD in females and maternal abortion history. The limitation of the current study is that there was a small sample from one hospital. However, this is the first report in the Republic of Korea in which AGD was assessed in newborns. The present study is the first evaluation of AGD that took into account the obstetrical history of the mothers. The aim of the present study was to provide information regarding a new anthropometric method for infants. This method should be further evaluated in a larger sample of newborns.

The AGD data in the current study represents an anthropometric index for AGD. Newborns in the present study did not have any congenital sexual organ issues. Endocrine disruptors in relation to AGD were not evaluated. If congenital sexual organ anomalies were present, endocrine disruptor levels and AGD values should be measured.

Acknowledgment

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References

Treatment of polycystic ovarian syndrome with insulin resistance by insulin-sensitizer

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Summary

Objective: The aim of this study was to observe clinical curative effects of combination application of dimethylbiguanide and pioglitazone and single application of pioglitazone in patients with polycystic ovarian syndrome (PCOS) complicated with insulin resistance (IR). Materials and Methods: Forty cases of patients with PCOS complicated with IR were investigated, and 20 cases of infertile women without PCOS were taken as the control group. PCOS group was divided into group A and group B according to body mass index (BMI) to detect glucose and lipids metabolism indicators, C reactive protein (CRP), etc. There were 20 cases in group A (Pioglitazone) and 20 cases in group B (dimethylbiguanide and pioglitazone). After treatment for 12 weeks, changes of the above various indicators were compared. Results: After treatment, insulin resistance index and serum testosterone (T) of two groups patients with PCOS significantly reduced (p < 0.05). Compared to before treatment, BMI of group B significantly reduced (p < 0.05 < 0.05). For INS at two hours after treatment, group B reduced more significantly (p < 0.05). Conclusion: The combination of dimethylbiguanide and pioglitazone was more effective for the treatment of PCOS complicated with IR than simple pioglitazone; chronic inflammation occurrence was possibly one of reasons for insulin sensitivity reduction of patients with PCOS.

Key words: Polycystic ovary syndrome; Insulin resistance; Hyperinsulinemia; Insulin-sensitizer.

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common reproductive endocrine disorders for women in adolescence and childbearing age, and its main clinical features are chronic anovulation, hirsutism, obesity and infertility. Among women in childbearing age, its incidence rate is 6% to 10% [1], and it occurs in 75% of patients with anovulatory infertility [2]. Also, it is the main pathogeny for anovulatory infertility. PCOS pathogeny is still unclearly elucidated. Since Burghen et al. [3] firstly proposed that insulin resistance (IR) was involved in the pathophysiology process of PCOS in 1980, researches on PCOS-IR have been deepened. In recent years, more and more researches show that PCOS patients all present different extents of IR regardless of obesity or not, and the incidence rate can reach 70% [4]. IR and hyperinsulinemia (HI) play an important role in PCOS pathogenesis, and they are closely related to its long-term metabolic complications. Since this disease was initially treated by Stein and Leventhal in 1935, it has lasted for 77 years to now, but a more ideal treatment method has been not found. Therefore, scholars are exploring a new treatment scheme in recent years. As a symptomatic treatment for IR, application of insulin-sensitizer in PCOS is a newer method. Especially, clinical data of thiazolidinediones (TZDs) Pioglitazone used for treating PCOS are less, and studies suggest that dimethylbiguanide and pioglitazone have a similar role in insulin sensitivity and high androgen [5]. Studies in recent years [6, 7] suggest that IR of patients with low-grade chronic inflammation and PCOS are closely associated with metabolic syndrome (MS). Insulin-sensitizer can reduce C-reactive protein (CRP) level, which further suggests that IR of PCOS is possibly an inflammatory reaction [8]. This study aimed to prospectively observe the improvement situations of endocrine, metabolic and reproductive functions of patients with PCOS complicated with IR, in case of combination application and simple application of two drugs, providing a basis for seeking the best treatment scheme of PCOS complicated with IR, and investigate the relationship of CRP with IR.

Materials and Methods

Patients

Forty cases of patients with PCOS complicated with IR and (or) HI treated in endocrine metabolism departments of Child Health Hospital of Jiangxi and the present hospital From May 2010 to June 2011 were selected. Their ages were between 18 and 34 years, and mean age was 26.04 ± 3.868 years. Among them, infertility duration of patients in childbearing age was one to eight years, and mean duration was 3.98 ± 1.97 years. In addition, 20 cases of non-PCOS infertile women were taken as the control group. For PCOS diagnostic code, the diagnostic code prepared in Rotterdam Conference of American Society for Reproductive Medicine in 2003 [9], and hyperprolactinemia, thyroid disease, Cushing’s syndrome, diabetes mellitus and other endocrine diseases were excluded. Also, all patients had no chronic disease, and they did not smoke and drink and did not administer hormones for treatment in the prior three months.
diagnostic code of IR, the upper 1/4 position value of Homa model insulin resistance index in normal control group (HOMA-IR) was used for judgment. If HOMA-IR ≥ 1.66 [10], fasting insulin (FINS) >15 mIU/L and (or) insulin at two hours after dining > 80mIU/L, it was diagnosed as HI. According to body mass index (BMI), 40 cases of patients with PCOS complicated with IR and (or) HI were divided into to group A (non-obesity group, BMI < 25 kg/m², 20 cases) and group B (obesity group, BMI ≥ 25 kg/m², 20 cases). For group A, Pioglitazone treatment was conducted, and combination treatment of pioglitazone and dimethylbiguanide was conducted in group B. This study was conducted in accordance with the Declaration and with approval from the Ethics Committee of the third Hospital affiliated Nan-Chang University. Written informed consent was also obtained from all participants.

**Observation methods and indicators**

1. General indicators: Height (m) and bodyweight (kg) of patients were measured to calculate BMI: BMI = bodyweight (kg) / height² (m²), and the work was carried out by the specially-assigned person.
2. Serologic indicators: 1) reproductive hormone: After fasting for 12 hours, phlebotomizing was conducted and examined for all patients in the 3rd day of menses or amenorrhea period (B ultrasound examination showed no dominant follicle). Chemiluminescence immunoassay was used to detect follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T). 2) Glucose and lipids metabolism indicators: After fasting for eight to 12 hours, fasting elbow vein blood was drawn the next morning to detect fasting blood-glucose (FPG), fasting insulin (FINS), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and liver and kidney function. In addition, oral glucose tolerance test and insulin release test (blood glucose and insulin at one and two hours after administering glucose) were carried out. The full automatic biochemical analyzer was used to detect blood lipids and liver and kidney functions; Glucose oxidase method was used to detect blood glucose; radioimmunoassay was used to detect insulin. 3) CRP: detected with the full automatic biochemical analyzer. (3) B ultrasound: abdominal B ultrasound (liver, gallbladder, spleen, pancreas) examinations: observe fatty liver situations of the patients. (4) IR indicators: HOMA model was used to calculate insulin resistance index (HOMA – IR = FPG × FINS / 22.5) and insulin sensitivity index [ISI = 1 / (FINS × FPG)] for evaluating IR extent. In addition, blood glucose was re-examined in every month. If fasting blood-glucose ≥ 3.6 mmol/l, the original treatment was maintained. After treatment for 12 weeks, phlebotomizing was carried out to re-examine glucose and lipids metabolism and reproductive hormone indicators.

**Drug administration method**

For all patients, on the basis of constant diet control and exercise amount, group A orally administered Pioglitazone, 30 mg daily. As group A, group B additionally administered dimethylbiguanide 500mg/time after meals, three times daily. Also, the two groups of patients continuously administered drugs for 12 weeks. Before drug administration, liver and kidney functions were normal. All patients were asked to conduct contraception during drug administration and record menstrual changes and drug side-effects. At the same time, follow-up visit was carried out once every four weeks to observe menstrual changes and drug side-effects of the patients, and liver and kidney functions and blood glucose were detected regularly. If patients conceived, drug administration was immediately stopped.

**Statistical analysis**

SPSS19.0 software was used for statistical analysis. Firstly, normality test was carried out for measurement data. For non-normal distribution data, logarithmic transformation was carried out to convert them into normal distribution data and then analyze them. They were expressed as ‘x ± s. For comparison between two groups, t test was used. Analysis of covariance was used for comparison between two groups after treatment. In addition, t test of paired samples was used for comparison between before and after treatment; Pearson or Partial correlation analysis was used for analysis of correlation, and HOMA-IR and CRP were respectively used as dependent variables to conducting multivariate stepwise regression analysis by combining other independent variables. For rate comparison between two groups, Fisher exact probability method was used. If p < 0.05, there was a significant difference.

**Results**

In group B, drug treatment was stopped in two cases due to pregnancy during drug administration. In the early treatment, one case in group B (namely dimethylbiguanide compatibility group) presented superior abdominal discomfort, but was tolerable. There was no apparent nausea and vomiting symptoms. Also, discomfort sense was gradually and naturally relieved. No anemia and edema occurred. During the whole treatment process, no severe side-effects such as lactacidosis occurred. Before and after treatment, liver and kidney function of all patients had no apparent change.

**Comparisons of various indicators before treatment between PCOS group and the control group**

With regards to age, there was no significant difference between PCOS group and the control group, and two groups had comparability. FINS, 2hPG, lh INS, 2hINS, TG, TC, LDL-C, and IR indicator HOMA-IR of PCOS group all were significantly higher than those of the control group (p < 0.01), and ISI was significantly lower than that of the control group (p < 0.01). Also, reproductive hormone (T, LH), LH/FSH and inflammatory factor were also significantly higher than those of the control group (p < 0.01), and there were significant differences. In addition, BMI, FPG, and 1hPG of PCOS group were significantly higher than those of the control group (p < 0.05), and FSH was significantly lower than that of the control group (p < 0.05). The differences all had statistical significances. HDL-C of PCOS group was slightly lower than that of the control group (p > 0.05) and there was no significant difference (Table 1).

**Comparisons of various indicators before treatment between group A and group B**

Before drug administration, FINS, 2hINS and 2hPG of group B were significantly higher than those of group A (p < 0.05), and BMI, HOMA-IR, TG, and inflammatory factor (CRP) were significantly higher than those of group A (p < 0.01). The differences all had statistical significances. In addition, HDL and ISI group B were significantly lower than those of group A (p < 0.01), and there were significant dif-
Treatment of polycystic ovarian syndrome with insulin resistance by insulin-sensitizer

Comparisons of various indicators between group A and group B between before and after treatment (x ± s).

<table>
<thead>
<tr>
<th>Item</th>
<th>Non-obese group (group A)</th>
<th>Obese group (group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>20.48±2.41</td>
<td>20.52±2.59</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>4.90±0.48</td>
<td>4.86±0.30</td>
</tr>
<tr>
<td>1hPG (mg/L)</td>
<td>9.98±2.11</td>
<td>9.27±1.28</td>
</tr>
<tr>
<td>2hPG (mg/L)</td>
<td>6.95±1.02</td>
<td>6.35±0.64**</td>
</tr>
<tr>
<td>FINS (mIU/L)</td>
<td>13.17±8.26</td>
<td>6.79±6.79**</td>
</tr>
<tr>
<td>1hINS (mIU/L)</td>
<td>109.11±44.81</td>
<td>59.82±25.89**</td>
</tr>
<tr>
<td>2hINS (mIU/L)</td>
<td>88.85±50.68</td>
<td>53.70±30.33**</td>
</tr>
<tr>
<td>Homa-IR</td>
<td>2.94±2.03</td>
<td>1.46±0.89**</td>
</tr>
<tr>
<td>LnSI</td>
<td>-3.97±0.73</td>
<td>-3.34±0.58**</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.06±0.36</td>
<td>1.00±0.33*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.27±0.73</td>
<td>4.21±0.51</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.67±0.75</td>
<td>2.35±0.59*</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.39±0.19</td>
<td>1.47±0.17</td>
</tr>
<tr>
<td>T (ng/dl)</td>
<td>75.01±28.57</td>
<td>43.56±17.10**</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>13.32±6.08</td>
<td>5.80±4.14**</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>5.17±1.87</td>
<td>4.94±2.04</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>2.84±1.45</td>
<td>1.21±0.68**</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.42±1.23</td>
<td>1.96±1.26$</td>
</tr>
</tbody>
</table>

Note: Comparison of group A and B before treatment, $ p < 0.05, $$ p < 0.01; vs before treatment,* p < 0.05, ** p < 0.01. Comparasion between two groups after treatment, # p < 0.05.

Comparisons of various indicators of group A and group B after treatment significantly decreased and HDL-C and ISI significantly increased, and the differences all had a statistical significance (p < 0.01). Also, TC also decreased (p < 0.05), and there was a significant difference. For FSH, there was no significant difference between before and after treatment (Table 2).

Comparisons of various indicators after treatment between group A and group B

Analysis of covariance was used to control influences of BMI and pretherapy indicators. After treatment, 2hINS and T of the obesity group (group B) decreased more significantly than the non-obesity group (group A) (p < 0.05), and there were significant differences. After influence of pretherapy level of BMI was controlled, BMI of group B decreased more obviously. In the improvements of LH, FSH, and blood glucose and blood lipids metabolisms, there was no significant difference in the curative effect between the two groups (p > 0.05).
Regression analysis

Pearson correlation analysis showed that HOMA-IR was respectively and positively related to BMI, FINS and 1hINS \((r = 0.54, 0.59, 0.58, p < 0.01)\), positively related to CRP \((r = 0.43, p < 0.05)\), and negatively related to LnSI \((r = -0.9, p < 0.01)\), and it had no significant correlation with other indicators. CRP was respectively and positively related to TG, TC, LDL-C and BMI \((r = 0.56, 0.51, 0.53 and 0.67, p < 0.01)\), positively related to FINS and Homa-IR \((r = 0.42 and 0.43, p < 0.05)\), negatively related to HDL-C \((r = -0.5, p < 0.01)\) and negatively related to LnSI and LH/FSH \((r = -0.48 and -0.39, p < 0.05)\), and it had no significant correlation with other indicators. Multivariate stepwise regression analysis was further carried out respectively with HOMA-IR and CRP as independent variables, and the result showed that BMI and FINS were independent related factors of HOMA-IR, and BMI was the independent related factors of CRP.

Regular menses recovery and pregnancy rate improvement after treatment

In group A, 12 cases after treatment restored regular menses (66.67%). In the follow-up period of half a year to one year, three cases were pregnant. In group B, 15 cases after treatment restored regular menses (83.33%). In the follow-up period of half a year to one year, five cases were pregnant. For the curative effect, there was no significant difference between the two group \((p > 0.05)\).

Discussion

PCOS is a heterogeneous disease, and its pathogenesis is still unclear. Recent studies show that IR and HI are not only the conventional manifestations, but also its important pathophysiological basis. Also, incidence risk of type II diabetes mellitus, arteriosclerosis and cardiovascular disease increases for PCOS patients complicated with IR and HI [11, 12] and obesity will quicken the process of glucose metabolism disorder [13]. The results of this study also found that obesity extent of PCOS patients was positively associated with IR, and FPG, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR and TG of PCOS patients in the obesity group were significantly higher than those of PCOS patients in the non-obesity group. Also, the incidence rate of glucose tolerance reduction (IGT) of PCOS patients in the obesity group was significantly higher than that of PCOS patients in the non-obesity group, indicating that the extents of IR and glucose and lipid metabolism disorder of fat PCOS patients were more serious than those of PCOS patients in the non-obesity group. It is speculated that obesity can aggregate IR.

As IR and HI play an important role in PCOS incidence, it is the key for treating PCOS to improve IR and HI. Dimethylbiguanide and pioglitazone are clinic common insulin-sensitizers, and they cannot only improve IR of PCOS patients, but also increase their ovulation rate and conception rates. For the former dimethylbiguanide, there are more reports on PCOS treatment. A majority of studies suggested that dimethylbiguanide could improve IR of PCOS, reduce androgen level and reduce blood lipids and blood glucose levels [14, 15]. The mechanism of the later improving IR is mainly to activate receptor-\(\gamma\), increase insulin sensitivity, enhance conduction insulin signal system, enhance glucose transport of peripheral tissues, improve pancreatic islet beta cell function and regulate fat cells in order influence expressions and secretions of fat-derived cytokines for eliminating IR and realizing hypoglycemic effect by activating peroxisome proliferator. There are still few studies on Pioglitazone used for PCOS treatment. In this study, after PCOS patients in the non-obesity group were treated with Pioglitazone for 12 weeks, compared with pretherapy, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR, TG, LDL, sex hormone (T, LH), and LH/FSH all significantly reduced, and ISI significantly increased, suggesting that pioglitazone could reduce androgen level, restore regular menses, increase conception rate, and improve glucose and lipid abnormalities of patients with PCOS complicated with IR and reduce incidence risks of metabolic complications such as diabetes mellitus, hypertension, coronary heart disease, etc. After drug administration, BMI of the patients had no apparent change. For PCOS patients in the obesity group, after dimethylbiguanide and pioglitazone were jointly administered for 12 weeks, compared with pretherapy, their BMI, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR, ISI, TG, TC, LDL, sex hormone (T, LH), and LH/FSH all significantly reduced, and ISI significantly increased. Studies suggest that combination of dimethylbiguanide and pioglitazone have a synergistic effect of improving IR. For the patients insensitive to dimethylbiguanide additional application of pioglitazone will improve curative effect. How is the curative effect of combination of dimethylbiguanide and pioglitazone used for PCOS treatment? There are still few studies addressing this query. Baillargeon et al. [16] reported that the curative effect of combination of dimethylbiguanide and rosiglitazone used for the treatment of non-obesity PCOS patients wasn’t better than that of simple drug. In the present study, BMI, 2hINS and T of group B (namely combination application group) reduced more significantly than group A (pioglitazone group). Also, FINS and HOMA-IR were more apparently reduced. Although there is no significant difference, it suggests that compared with simple pioglitazone, combination application of two drugs can better reduce androgen level and improve IR of PCOS and thus correct hyperinsulinemia. In addition, LH, FSH, and blood glucose and lipid metabolisms were improved, more obviously than pioglitazone group, but there was no significant difference.

CRP is an inflammatory and acute reactive protein, and its increase is one of important indicators of predicting car-
diovarious disease and type II diabetes mellitus [17, 18]. In recent years, chronic inflammation theories on PCOS are widely concerned. The study conducted by Ruggeri et al. [19] and Catureggi et al. [20] suggested that IR and metabolic syndrome of PCOS patients were closely related to the mild chronic inflammation. In this study, CRP level of PCOS patients was significantly higher than that of the control group, suggesting that chronic inflammatory reaction was involved in the occurrence and development process of PCOS, which was in line with research result of Boulman et al. [21]. Person correlation analysis showed that CRP was positively associated with FINS, HOMA-IR and BMI respectively, but stepwise regression analysis indicated FINS and HOMA-IR were not independent correlation factors of CRP, while BMI was an independent correlation factor of CRP, indicating that serum CRP level and BMI had a correlation, and one reason for insulin sensitivity reduction possibly lays in the chronic inflammatory reaction.

In brief, the time length of this study was short, and sample size was small. It is necessary for observing effects of combination of dimethylbiguanide and pioglitazone on improvements of IR and metabolism and reproductive functions and further solving the infertile problem of PCOS patients to carry out larger sample-size prospective studies. At the same time, it is necessary to carry out a number of clinical studies addressing the side-effects of the two.

References


Uterus retrieval in cadaver: technical aspects

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Summary
The authors describe uterus retrieval in cadavers. Uterine retrieval with its vasculature could be successfully achieved in four of the presented cases. Special attention was given to dissection of bilateral ureters and hypogastric vasculature. Uterine retrieval with its vasculature and supporting sacrouterine, vesicouterine peritoneal folds is an anatomically feasible procedure in preparation for uterus transplantation.

Key words: Uterus retrieval; Uterus transplantation; Cadaver.

Introduction
Although inspiring developments have taken place in assisted reproductive technologies in the last decades, no current approach has been able to treat the problem known as uterine related infertility (URI) resulting from congenital or followed by hysterectomy [1-7]. The only accepted solution to URI to date has been gestational surrogacy which is not acceptable in all parts of the world.

The idea that uterine allotransplantation could be used in URI was supported by several animal studies resulting in healthy offspring [8-11]. The first uterine transplant attempt was performed in a Saudi Arabic woman in 2000 from a live donor [12]. The transplanted uterus had to be removed 99 days later possibly due to the weakness in surgical technique and obstruction of the anastomosed vessels. A good retrieval technique with adequate length of vasculature and width of supporting structures seems to be noteworthy for a successful attempt.

Materials and Methods
The authors report their initial experience in the retrieval of the human uterus from fresh frozen cadavers.

Following, institutional ethics committee approval, four fresh-frozen female cadavers were included in the study. The cadavers were obtained from Anatomy Department of Akdeniz University School of Medicine. All dissections were performed by two gynecologists, one plastic surgeon, and two anatomists. The cadavers were placed in the supine position, and a vertical midline incision was made from pubis to xiphoid process of sternum.

Uterine retrieval consisted of three main phases. First phase: dissection and transection of the bilateral round ligaments were completed to mobilize the bladder anteriorly. Mobilization of the upper vagina was achieved posteriorly by conserving the uterosacral ligaments and opening the peritoneal sheath. Second phase: ureters were dissected bilaterally starting from their course over common iliac bifurcation to their passage under the uterine vessels. Third phase: bilateral internal iliac vasculature and uterine vessels were dissected.

Exploration time ranged from 95 to 150 (mean 110) minutes. Initially, the authors aimed to obtain an adequate exposure in the pelvis. In the first phase of retrieval, they grasped and divided the round ligaments laterally to directly get access to parametrium by blunt dissection. The anterior leaves of the broad ligaments were incised and dissected anteriorly until vesicouterine reflection. Upper vagina was mobilized posteriorly by detaching uterosacral ligaments from the sacrum with overlying peritoneum.

In the second phase, the peritoneum was sharply opened lateral to the infundibulopelvic ligaments. With traction of the infundibulopelvic ligaments, the posterior leaves of the peritoneum were sharply opened and ureters were dissected bilaterally starting from their course over common iliac bifurcation and posterior to ovarian vessels to their passage under the uterine vessels to allow careful preservation of the uterine vessels. The authors tried to dissect and remove the bilateral cardinal ligaments (lateral areolar connective tissue bundles) as wide as possible for the purpose of lateral support during uterus transplantation.

In the third phase, psoas major muscle and external iliac vessels were identified by dissection. After grasping the posterior parietal peritoneum overlying the psoas major muscle (lateral to the external iliac artery) the peritoneum parallel to the external iliac artery was cut. Starting from the bifurcation, internal iliac vessels lying lateral and parallel to bilateral ureters were dissected as entirely as possible distal to the point of origin of its posterior division to maintain the branches to uterus intact and obtain the greatest length of the internal iliac vessels (Figure 1). Right side was easier to isolate than left due to the location of the sigmoid colon.

Finally the uterus, ten cm hypogastric artery, eight cm hypogastric vein, and three cm proximal vagina could be retracted in four cadavers with its vasculature and supporting ligaments (Figure 2). The uterus could not be completely retrieved in one cadaver due to the weakness in surgical technique and obstruction of the anastomosed vessels.

Discussion
Uterine related infertility due to congenital or acquired agenesis of uterus has no treatment with current assisted reproductive technologies [1-6]. Through advances in sur-

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gical techniques, improvements in immunosuppressive agents and experience acquired from solid organ transplantations, and unusual allograft transplantations have gained acceptance especially in the last decades [8-20].

Unlike other organ transplantations, uterine transplantation is not a life-sustaining procedure with potential risks to live donor, recipient mother, and child [21]. Therefore previous experience in gynecologic oncology might be a major advantage in understanding the anatomy and relationships of the retroperitoneal vasculature which is vital in avoiding serious injury.

It is important to preserve vascular supply of the ureter during dissection in a live donor not to cause fistula formation resulting from related thrombosis.

Dissection of internal iliac vessels with its major branches lying in the obturator fossa may be risky and difficult in a live donor related to the numerous and anomalous veins that occupy the lateral floor of the obturator fossa. Pelvic vasculature should be preserved as wide as possible in a live donor not to interfere with other organ function. It may be wise to ligate the hypogastric artery distal to the point of origin of its posterior division.

There are several discussions on the definition of cardinal ligaments (CL) [22]. Fritsch et al. have defined CL as the bundle connecting the pelvic brim and the uterine cervix [23]. American version of Gray’s anatomy defined CL as extension of the perivascular sheath of the internal iliac vessels [24]. Dissection of the cardinal ligaments is quite difficult due to its areolar texture and proximity of the uterus vasculature and ureter.

Although this study is limited in showing the safety of the procedure, it confirms the anatomical feasibility of the uterus retrieval procedure with adequate vascular length and width of supporting ligaments. Cadaver dissection might be quite helpful in establishing a good retrieval technique.
References

Laparoscopic management of ovarian benign masses

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Summary
Purpose: To determine whether the presence of normal ovarian tissue could assist in the diagnosis of large benign ovarian neoplasms in young females and in choosing the laparoscopic treatment. Materials and Methods: A prospective study of 25 patients treated surgically for a cystic ovarian neoplasm measuring diameter more than ten cm or volume more than 500 ml and having normal ovarian tissue or ovarian crescent sign (OCS). Ultrasonography was performed at six weeks, then at three, six, nine, and 12 months postoperatively. Results: The mean age of patients was 15.3 ± 3.6 years, ranging between 6.5 and 19 years. The mean preoperative volume of the ovarian neoplasm was 1,686 ± 1,380 cm³, ranging between 550 and 6,000 cm³. The presence of OCS was visualized by ultrasonography in all 25 patients and serum tumor markers were negative in 22. No borderline tumors or malignancies were identified. There was a statistically significant difference between the volume of the affected ovary and the contralateral ovary during the first six weeks follow-up, but without significant difference after three months. Conclusions: Postoperative ultrasound revealed that the affected ovary resumed its normal volume within three months after surgery, despite the thinned appearance of the ovarian cortex present on ultrasound as the OCS. Laparoscopic ovarian preservation should be the preferred surgical approach for adolescents to ensure the conservation of the entire ovarian tissue.

Key words: Ovarian cysts; Cystectomy; Pelvic ultrasound; Laparoscopy; Adolescents.

Introduction
Laparoscopic treatment of large ovarian cystic neoplasms in young females is a challenge for pediatric gynecologists and pediatric general surgeons. The standard surgical staging is performed when the lesion is highly suspicious of malignancy, but malignant tumors are rare in this population [1]. The use of minimally invasive techniques was reported, but with limited results in preserving ovarian tissue [2, 3]. Even by laparotomy, the ultimate goal is to preserve the tube and the ovary whenever possible, and to maximize the future reproductive potential of these patients [4]. The presence of normal ovarian tissue or the ovarian crescent sign (OCS) depends on whether healthy tissue could be seen adjacent to the tumor within the affected ovary. It seems that the value of the OCS in large cystic ovarian neoplasms within young female population remains poorly defined.

The purposes of this study were: to determine the presence of the OCS, to describe the authors’ experience of laparoscopic tumorectomy for ovarian neoplasms measuring more than ten cm or more than 500 cm³, to document pathologic findings, and to evaluate the postoperative volume of the preserved ovary after removal of the large persistent ovarian neoplasm in pediatric and adolescent population.

Materials and Methods
The study was approved by the Institutional Ethics Committee of the Mother and Child Health Institute of Serbia. It was a prospective study of the patients treated by laparoscopic surgery for cystic ovarian tumors measuring more than ten cm or more than 500 cm³, at the Department of Pediatric and Adolescent Gynecology of the present institution, between June 2006 and January 2012. The preoperative assessment included a complete physical examination, lab analyses, ultrasonography, magnetic resonance imaging (MRI) of abdomen and pelvis and chest X-ray for each patient. Ultrasonographic reports included length, width, and thickness of the neoplasm and of the ovarian crescent sign presence (Figure 1). To eliminate bias, the OCS in all patients was assessed independently by three examiners, two gynecologists, and a radiologist (ZBS, MD, and BL) and their findings did not differ in any of the 33 patients. Ovarian volume was calculated according to the formula (length x height x width x 0.523). Laparoscopic procedure was performed by a single surgeon (ZBS) using a uniform technique, which is given in detail. Postoperative ultrasonography was scheduled to measure the volume of the preserved ovary. Data were collected on demographic characteristics, preoperative evaluation, ultrasonographic and MRI reports, pathology results, hospital course, and complications. Patients were excluded if there was a high suspicion of malignancy based on physical exam, ultrasonographic, and MRI findings. Data were expressed as the mean ± SD. The paired Student’s t-test was used to compare the groups. A p < 0.05 was considered statistically significant.

Informed consent was obtained from the patients and parents before surgery. Twenty-four hours prior to the procedure, careful aspiration of most of the cyst fluid was performed (using a spinal needle, Dahlhausen, size 186 x 1½). Under general endotracheal anesthesia, laparoscopic surgery was performed, using CO₂ distension of the abdominal wall. A ten-mm Optiview port was placed at the superior edge of the umbilicus to introduce the ten-
mm laparoscope. Two other Optiview ports were placed laterally under direct vision: a ten-mm port on the left side and five-mm port on the right side at the level of anterior superior iliac spines.

Firstly, a peritoneal washing was obtained. An abdominal linear incision was made along the antimesenteric border of the ovarian cyst, approximately three cm from the ipsilateral tube, parallel to it. Capsule was stripped from the ovarian stroma using two graspers for traction and countertraction. Bipolar forceps were used to coagulate the bleeding vessels at the base of the capsule; two to five vessels were coagulated per tumor (median 3.3). Attempts were made to remove the whole ovarian neoplasm without opening it. Great care was taken to prevent spillage into abdominal cavity. The authors did not suture any part of the ovary. The excised tissue was placed in an endobag and removed from the body through the left port site or umbilicus. The whole preserved ovary remained as an empty sac within the abdomen. The abdomen was then copiously irrigated. The surgical wound was closed with subcutaneous sutures. Postoperative follow-up of ovarian volume was evaluated at six weeks and three months postoperatively, or more if required.

Results

Thirty-three patients who underwent laparoscopic surgical management for adnexal cysts ≥ ten cm or ≥ 500 cm³ with preservation of the whole affected adnexa were observed. Eight of them with large paraovarian cysts were excluded from this study. The mean age of the remaining 25 patients at the time of surgery was a 15.3 ± 3.6 years, ranging from 6.5 to 19 years, only one patient being under ten years of age. The mean preoperative size and volume of the ovarian neoplasm were 15.0 ± 3.2 cm, (range 10 to 22), and 1,686 ± 1,380 cm³, (range 550 to 6,000 cm³). The indications for the first ultrasound scan were recurrent abdominal pain or discomfort in 16 patients and abdominal swelling in nine patients, including two premenarchal. Preoperatively, all the ovarian neoplasms appeared to be cystic, unilateral masses, without evidence of multiple septations. The presence of normal ovarian tissue was documented for all neoplasms. Serum tumor markers CA-125, beta human chorionic gonadotropin (βhCG), alpha-fetoprotein (AFP), inhibin B, and lactate dehydrogenase, were negative in 22 patients. In three patients, the serum levels of CA-125 were elevated, measuring 45 and 60 U/l. Considering the benign appearance on ultrasonography and presence of the OCS, laparoscopy with tumorectomy was the standard surgical approach. The mean duration of the procedure was 65 min (range 45 to 120). The postoperative hospital stay was from one to four days. Histopathology revealed ten serous cystadenomas, six mucinous cystadenomas, eight mature cystic teratomas, and one endometriosis. No borderline tumors or malignancies were identified. Pelvic washing was negative in all patients. Postoperative ultrasonography measured the volume of both ovaries. At six weeks after surgery, the mean volume of 23 affected ovaries was 17.6 ± 8.0 cm³, and three months after laparoscopy the mean ovarian volume decreased to 8.0 ± 1.3 cm³, which is not significantly different from the mean volume of the contralateral ovary

<table>
<thead>
<tr>
<th>Time after</th>
<th>Affected ovaries</th>
<th>Contralateral ovaries</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>6 weeks</td>
<td>17.6±8.0 (9.0-36.0)</td>
<td>6.5±1.3 (2.0-8.1)</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>3 months</td>
<td>8.0±1.3 (6.0-10.0)</td>
<td>6.4±1.4 (2.0-8.2)</td>
<td>p &lt; 0.258</td>
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</table>

Discussion

Large cystic ovarian neoplasms with the presence of the ‘ovarian crescent sign’ are usually of benign nature [5, 6]. The initial reports showed that the OCS has the potential to become a simple and effective way of excluding ovarian malignancy in adults, as well as in pediatric and adolescent patients [5, 6]. The visualization of healthy ovarian tissue does not require a high level of ultrasound skills and could be successfully included into a routine ultrasonographic practice of all operators dealing with adnexal tumors in young patients. The present study shows that radiologists can identify OCS as accurately as gynecologists can. The absence of the OCS should be an indicator to refer the patient to a gynecologist specialized in practice with young fe-
male population [6]. The only exception may be a large paraovarian cyst, because absence of the OCS around the cyst is expected. A multicentric study reported the OCS presence in 16% of adult ovarian neoplasms with borderline malignancy [7]. However, endoscopic conservative surgery was an acceptable option for women with borderline ovarian tumors who wished to preserve fertility [8]. In two patients of the present series, the serum level of CA-125 was slightly elevated, but in both patients OCS was visualized. Adding a single CA-125 measurement to the ultrasound imaging performed by an experienced examiner does not improve preoperative discrimination between benign and malignant adnexal masses in adult patients [9]. Ovarian cyst aspiration is not a standard procedure. Some authors described a technique of transabdominal drainage under ultrasonographic control or laparoscopic guided aspiration followed by laparoscopic excision of both ovarian and paraovarian cysts [10, 11]. In the present study, a cyst fluid aspiration 24 hours before laparoscopy was performed for two reasons – to decompress slowly and to give the normal ovarian tissue a chance to retract before cystectomy, and it could be performed because the risk of malignancy was minimal in the chosen population. The treatment of ovarian masses in young females depends on the specialty of the operator [12]. Some surgeons insisted on minimally invasive techniques, but the fear of cancer led them to perform eight ovarianectomies or adnexectomies for large benign cystic masses [2]. Other operators successfully preserved all ovaries, through laparotomy or laparoscopy [4, 10]. All studies were limited by a small number of patients. Laparoscopic excision of ovarian cysts may be associated with damage to the ovarian reserve. Electrocoagulation after laparoscopic excision of ovarian cysts may cause reduction in ovarian reserve [13]. Some authors found that ovarian volume was significantly reduced after three months of laparoscopic cystectomy [14]. Reddy and Laufer reported that in nine adolescent patients, after laparotomy, cystectomy, and suturing, it took six weeks for the ovary to reduce to its normal volume [4]. The present authors used electrocoagulation very restrictively and found that after laparoscopic preservation (of whole) ovaries required three months to recover. Further prospective work will show whether the use of the OCS will improve the management of adolescent patients with large cystic adnexal masses.

Conclusions

In the appropriately selected adolescent population, laparoscopic ovarian cystectomy for large ovarian neoplasm should be the preferred surgical approach. It is possible to preserve the whole ovary in most patients with large cystic neoplasms. After maximally conservative procedure, ovarian tissue requires three months to restore its normal volume.

References


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Laparoscopic hysterectomy: really so risky to a vaginal cuff dehiscence?

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Summary

Purpose of investigation: The authors report their experience with vaginal vault suturing procedure in patients that underwent total laparoscopic hysterectomy (TLH) for benign diseases. Vaginal vault colpotomy and closure were only laparoscopically carried out.

Materials and Methods: Longitudinal retrospective study was conducted in 550 patients, affected by gynaecological benign pathologies, that underwent TLH were enrolled. Information about age, body mass index, parity, anticoagulant therapy, previous pelvic surgery, smoking, diabetes, menopausal status, and procedure characteristics (operating time, blood loss, uterus weight, postoperative recovery time, and adverse outcomes) were collected. Postoperative complications and adverse outcomes were recorded. Results: Only one case (0.2%) of vaginal cuff dehiscence (VCD) occurred four weeks after surgery, which was quickly laparoscopically repaired with interrupted intracorporeal knots. The trigger event was sexual intercourse in a patient affected by systemic lupus erythematosus (SLE). No cases of VCD presented in patients with other considered comorbidities. Conclusion: A careful technique could further decrease the incidence of postoperative vaginal cuff dehiscence, regardless of laparoscopic or vaginal suture approach.

Key words: Vaginal cuff dehiscence, Total laparoscopic hysterectomy, Surgical complications, Endoscopic Surgery.

Introduction

Hysterectomy is the most common non–pregnancy-related major surgery performed on women in the world: about 600,000 hysterectomies are annually performed in the United States [1].

The choice of surgical approach (abdominal, vaginal, and laparoscopic) depends on skills of the surgeon, uterine or vaginal size, and availability of an adequate surgical equipment.

Total laparoscopic hysterectomy (TLH) has been described as a beneficial technique as well as vaginal hysterectomy (VH) and more advantageous for patients, if it is conducted with a safe technique [2, 3]. Nevertheless, in literature, a higher incidence of postoperative complications is reported in TLH compared to VH: among these, vaginal cuff dehiscence (VCD) is described to be a rare but severe complication. Its incidence after TLH varies between 0.3% and 3.1% [4, 5]. It can lead to serious life-threatening developments and be complicated by vaginal eversion, intestinal ischemia, and intra-abdominal infection [6]. Recently, some authors suggested that a transvaginal suture is safer than laparoscopic one [4, 7].

In the present study the authors report their surgical outcomes in women that underwent reverse hysterectomy (RH) [8] for benign diseases, in which vaginal closure was laparoscopically carried out.

Materials and Methods

A longitudinal retrospective study was conducted in Minimvasive Surgery and Operative Obstetrics Unit, (Woman and Child Health Departments) of Obstetrics and Gynaecology Clinic at the University of Padua. Patients eligible for inclusion in the study were women who underwent RH for benign uterine disease, during the three-year period from January 2010 to January 2013. In total, 550 women affected by benign uterine pathologies were included: in all women had been subjected to a preoperative PAP test, clinical examination, ultrasound exam to assess the absence of malignant ovarian pathologies, and hysteroscopy to assess the absence of malignant uterine pathologies were performed [9].

Exclusion criteria included malignancy, antithrombotic therapy and cardiopulmonary diseases (defined as a history of cardiac failure, myocardial infarction, unstable angina, acute or recent vascular thrombosis, asthma or pulmonary obstructive disease poorly controlled, or contraindicating prolonged Trendelenburg position).

Clinical and surgical records such as age, menopausal status, parity, body mass index (BMI), smoking, diabetes, corticosteroid therapy, previous pelvic surgery, and procedure characteristics (operating time, blood loss, uterus weight, recovery time) were collected.

Postoperative complications and adverse outcomes were classified using the morbidity scale proposed by Dindo et al. [10]. Patients were postoperatively evaluated by anamnesis and physical examination two months after surgery. Sexually active patients were instructed not to restart sexual intercourse until this evaluation. A descriptive statistical analyses was performed on these data.

Surgical technique

The surgery was performed under general anesthesia with nasogastric tube insertion and a bladder catheter placed immediately before the operation. All patients underwent antibiotic prophylaxis with two g cefazolin 30 minutes before surgery.
Two gynaecologists, experienced in advanced laparoscopic surgery, performed all procedures with similar techniques and instruments over time [8].

During a RH, after exposure of the anterior vaginal fornix, circular colpotomy was performed with a clear cut incision by a Harmonic Ace. Closure began from the distal angle of the vaginal cuff and proceeded to the opposite side, making sure to include the pubocervical fascia, part of the cardinal ligament, and even the vaginal mucosa and the rectovaginal fascia with interrupted sutures of 0 polydioxanone intracorporeal knots on a 36-mm half-circle taper point needle. The vaginal vault was therefore suspended to the pubocervical fascia, part of cardinal ligament, and to the residual uterosacral ligaments.

### Results

A total of 550 laparoscopic hysterectomies were included in the study. Mean age was 52 ± 8.565 years. BMI was 24.85 ± 3.759 g. Two hundred and one women (28.7%) were smokers, 196 (35.4%) patients were in premenopause, while 354 (64.6%) were in menopause. One-hundred-sixteen (21.3%) women were nulliparous, while 132 (24.3%) had one child, 225 (41%) had two children, 40 (7.4%) had three children, 21 (1.9%) had four children, and 16 (1.4%) had five children. One hundred fifty-two patients (78.4%), chronic pelvic pain in 64 (11.6%), endometriosis in 15 (2.7%), and endometrial hyperplasia in 40 (7.3%). The operating time was 112 ± 53.3 min, median blood loss was 85.64 ± 161.47 ml, and mean recovery time was 2.4 ± 1.35 days. The mean uterus weight was 280.59 ± 251.35 g. Postoperative complications are reported in Table 1, according to Dindo et al. classification [10].

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Cumulative percentage</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>510</td>
<td>92.7%</td>
<td>92.7</td>
</tr>
<tr>
<td>Grade I</td>
<td>30</td>
<td>5.5%</td>
<td>98.2</td>
</tr>
<tr>
<td>Grade II</td>
<td>3</td>
<td>0.5%</td>
<td>98.7</td>
</tr>
<tr>
<td>Grade III a</td>
<td>2</td>
<td>0.4%</td>
<td>99.1</td>
</tr>
<tr>
<td>Grade III b</td>
<td>3</td>
<td>0.5%</td>
<td>99.6</td>
</tr>
<tr>
<td>Grade IV</td>
<td>2</td>
<td>0.4%</td>
<td>100.0</td>
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Only one case (0.2%) of vaginal cuff dehiscence occurred with intestinal loops of small bowel protrusion. The patient was readmitted four weeks after surgery because of pelvic pain and poor vaginal bleeding. The trigger event was sexual intercourse and she was affected by SLE. Vaginal cuff dehiscence was quickly laparoscopically repaired with interrupted intracorporeal knots.

### Discussion

VCD is described to be one of the major complication after TLH. Its incidence after total laparoscopic hysterectomy varies between 0.3% and 3.1 %. According to literature, VCD is defined as a full thickness separation, partial or total, of the anterior and posterior edges of the vaginal cuff with or without bowel evisceration. [7].

It usually occurs in the first month after surgery, but it has been described also after several years: median time to dehiscence has been reported from 1.5 to 3.5 months. It should be suspected in patients with vaginal bleeding and profuse purulent discharge [11]. In postmenopausal women, it usually follows surgery for genital prolapse or complicated pelvic operations: high-grade enterocele, vaginal vault prolapse, and severe cuff atrophy contribute to the weakening of the vaginal apex [12]. In premenopausal women, it is commonly associated by intercourse or unusual sexual practices after vaginal surgery. The most frequent surgical prescription to a hysterectomy is fibromatosis, afflicting mostly woman during reproductive age and impacting their quality of life with unpleasant symptoms.

Several factors are involved in the pathogenesis of fibromatosis: leiomyoma growth and development seems to depend on steroid, genetic background, cytogentic anomalies, and inflammatory environment [13].

Less frequent surgical prescriptions are chronic pelvic endometriosis and endometral hyperplasia [14]. Nowadays, the reason for the increased incidence of VCD after TLH is still a matter of concern: the suturing method is mentioned as an etiological intraoperative factor.

In a recent systematic review [4], analyzing data from more than 13,000 total endoscopic hysterectomies (both laparoscopic and robotic) included in series published between 1989 and 2010, Uccella et al. suggested that the modality of cuff closure may play a key role in the genesis of VCD, with transvaginal suture being associated with threefold and ninefold reductions in the incidence of cuff separations respectively compared with laparoscopic and robotic sutures. They concluded that vaginal dehiscence and other complications (such transvaginal bleeding, reintervention, infections) are significantly reduced when the vaginal vault is closed transvaginally. They reported a risk of vaginal dehiscence of 0.64%, vaginal bleeding 0.46%, reintervention 0.66%, infections 0.28% after TLH compared to 0.18%, 0.18%, 0.24% and 0.12 % after LAH or VH, respectively. They hypothesized that this important disparity was related to the use of electrocautery (at the time of colpotomy) and to thermal tissue damages, to the insufficient tissue involved in the suture (due to the magnification of pelvic anatomy by laparoscope), and to the worst reliability of the knots made with laparoscopic instruments.
In 2007, Hur et al. [5, 15] observed a decreasing incidence (1.35%) of VCD in TLH (958) performed from January 2006 to December 2009 in comparison to those (662) performed from January 2000 to March 2005 (4.83%). They attributed the reason of this decrease to the improved surgeon experience.

The incidence of VCD in the present series of cases was 0.2%: this lower value is simply obtained by means of skillful technique, which is the essential condition to perform a TLH.

During a TLH, energy is generally used in dividing the cuff for cutting and performing hemostasis. The degree of tissue injury has relevance on outcome of vaginal cuff closure: the suture must not incorporate injured tissue.

In a recent study [16], assessing the histopathological degree of energy-related injury after a robotic TLH performance in the swine vagina, Gruber et al. observed that tissue injury related to ultrasonic was significant lower than bipolar energy, while monopolar was slightly less than that associated with bipolar energy. In other studies, monopolar energy has been associated with the greatest degree of tissue damage.

Even if ultrasonic energy causes the least tissue damage because of its less thermal spread, tissue necrosis and devascularisation, it may produce histologic effects in adjacent tissues by transferring mechanical energy to tissues resulting in protein denaturation and vaporization of fluids in cells, but postsurgical effects of various energy type were not analysed.

The present authors routinely use Harmonic ACE which allows a good haemostasis and clear cut incision. Through its continuous and homogeneous tension, it ensures tissue quality optimization and concurs to favourable outcome of the suture. [17]

Recent studies [18] reported a significant decrease of VCD employing barbed suture instead of traditional suture: Siedhoff et al. demonstrated the absence of complications after laparoscopic barbed suture (compared to braided suture comprised of polyglycolic acid and Endostitch and to monofilament suture). The authors adjusted the results with patient characteristics and concluded for barbed suture superiority, because of the tension distribution across the length of the suture and less tissue ischemic damage with a significant shortening in procedure time.

Other studies [19] compared laparoscopic suturing of the vaginal cuff with a single-layer unknotted running suture and both laparoscopic and transvaginal closure with knotted interrupted sutures. The authors found a threefold increase (3.3%) of VCD incidence in laparoscopic closure with knotted interrupted sutures (while in transvaginal interrupted suturing was 1.3 % and in laparoscopic running one was 2.4%). There was no statistical difference with regard to VCD between these three groups.

In the present series of laparoscopic vaginal closure with intracorporeal interrupted suture, only one case of VCD out of 550 occurred in a patient with a systemic disease. According to literature, in the present authors’ experience, VCD does not seem attributable to the suture tensile strength but rather to primary healing defects. Routinely, the authors prefer to remove high size uterus through the vagina, after a partial morcellation. For this reason, their preoperative workup includes hysteroscopy with endometrial biopsy in all patients to rule out premalignant and malignant lesions [9].

Several preoperative risk factors have been postulated for VCD: increased age, coitus before healing, trauma or tape, corticosteroid therapy and radiotherapy, use of the Valsalva maneuver, previous vaginoplasty, postoperative infection, hematoma, poor surgical technique, vaginal atrophy, smoking, malnutrition, anaemia, diabetes, menopausal status, and previous pelvic surgery [20].

Although it seems biologically plausible that any condition compromising wound healing increases the risk of vaginal cuff dehiscence, the data on such risk factors are approximate.

According to literature [21, 22], risk factors (such as considered smoke, higher BMI, diabetes, menopausal status, and previous pelvic surgery) did not seem to predispose patients to VCD after laparoscopic surgery.

The inconsistent recording of risk factors in studies, infrequency of the VCD, and the lack of their comparison between women with and without dehiscence in most retrospective studies make it difficult to assess the significance of each of these potential risk factors.

Immunosuppressive status and systemic connective tissue disease (systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, and Raynaud’s disease) is common in young women and predispose them to the development of surgical complications, even in the elective setting [23].

In the present series, the only case of VCD occurred in a patient with SLE, probably consequence of altered inflammatory processes, the past use of immune modulating drugs (steroids, methotrexate, hydroxychloroquine, which are associated to higher risk of infection and poor wound healing), and the microvascular vasospasm of Raynaud’s phenomenon.

The trigger events are those which increase abdominal pressure (along with vaginal atrophy or enterocele after hysterectomy) because of stretching of vaginal wall and shifting of intra-abdominal pressure towards the vaginal apex, such as sexual intercourse [24]. The surgical joining between pubocervical and rectovaginal fascia allows to restore the anatomical and functional conditions of pelvic organs.

Conclusion

In the authors’ experience only one case of dehiscence in a woman with systemic lupus erythematosus and after an cautious sexual intercourse four weeks after surgery occurred. That clinical condition resulted in a VCD and protrusion of small bowel loop, which was quickly laparoscopically re-sutured.
Various methods of suturing of the vaginal vault have been described, with the aim to restore pelvic stability and to avoid the risk of dehiscence of the vaginal vault.

In the early years after the introduction of the laparoscopic technique, many authors have reported an increased incidence of this complication. Today, the experience and the proper equipment has allowed us to perform a suture with excellent results and lower incidence of VCD. The authors believe that laparoscopic surgical procedures may be actually performed involving also cardinal ligaments in the sutures (not only pubocervical and rectovaginal fascia). These landmarks are the main support structures of the apex of the vagina, with an effective pelvic stability. In the authors’ experience, combining these measures with careful technique could further decrease the incidence of this severe complication.

References


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Epidemiologic investigation of polycystic ovarian syndrome (PCOS) in Han ethnic women of reproductive age in Liaoning Province, China

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Summary

Objective: To determine the incidence of polycystic ovary syndrome (PCOS) among Han women of reproductive age in Liaoning Province in Northeastern China, based on the Revised Rotterdam 2003 criteria. Materials and Methods: A retrospective cohort study was carried out on 1,600 women using questionnaires, physical examination, ultrasonography, and biochemical indices (aged = 19 to 45 years; n = 1,600). PCOS patients were identified using the Revised Rotterdam 2003 criteria. Results: A total of 132 Han women of reproductive age were diagnosed with PCOS, with a prevalence of 8.25%. The prevalence of menstrual dysfunction was as follows: 97 patients (73.48%) had abnormal menstruation, three (2.27%) had polymenorrhea, and 94 (71.21%) had oligomenorrhea. Up to 64 patients (48.48%) had androgen excess, 42 (31.82%) had biochemical evidence of androgen excess, and 34 (25.76%) had clinical androgen excess. Up to 34 patients (25.76%) were obese (body mass index [BMI] ≥ 25) and 19 (14.39%) had hirsutism (F-G scoring ≥ 6). A total of 127 patients (96.22%) were diagnosed with PCOS via ultrasonography, 67 of whom (50.76%) had a unilateral polycystic ovary and 60 (45.46%) had bilateral polycystic ovaries. Conclusions: The prevalence of PCOS in this study population was 8.25%, with an infertility rate of 27.8%. The classical manifestation of PCOS is PCO, abnormal menstruation, and obesity. The high-risk factors of PCOS include high free testosterone index, homeostasis model assessment-insulin resistance (HOMA-IR), increased serum testosterone and androstenedione, decreased sex hormone-binding globulin, long history of infertility, menarche later than 16 years old, and failure to have regular menstruation within two years.

Key words: PCOS; Physical examination; Distribution features; Physical examination.

Introduction

Polycystic ovary syndrome (PCOS) is a common complicated endocrine and metabolic disorder that affects five to ten percent of the population [1]. The diagnostic criteria for PCOS remain controversial because of the diversity and heterogeneity of its clinical features [2]. According to the Rotterdam criteria, the prevalence of PCOS in women seeking primary healthcare in Salvador, Brazil, was 8.5% [3]. In a community sample of an Iranian population, the prevalence of PCOS was 7.1% (95% CI: 5.4%–8.8%) using the National Institutes of Health (NIH) definition, 11.7% (95% CI: 9.5%–13.7%) based on the Androgen Excess Society (AES) criteria, and 14.6% (95% CI: 12.3%–16.9%) using the Rotterdam definition [4]. Different ethnic groups have different prevalence rates. The PCOS prevalence rates among Black and among Caucasian American women of reproductive age were 8.0% and 4.8%, respectively [5]. The prevalence of PCOS among Mexican women is approximately 6.0%, similar to that in other populations, but lower than the 12.8% among Mexican-American women [6]. The prevalence of PCOS among unselected women from Southern China is 2.2%, much lower than the average prevalence [7]. However, Southern China and Northeastern China differ greatly in climate, environment, and lifestyle. Thus, the prevalence of PCOS among Han ethnic women of reproductive age in Northeast China may differ from that in Southern China. European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine (ESHRE/ASRM) -sponsored PCOS Consensus Workshop in Rotterdam discussed and recommended the current criteria for PCOS [8]. These criteria emphasise the exclusion of other diseases that can cause androgen excess; thus, it is an exclusion criterion. Although the Rotterdam criteria was established based on ten years of research as a bridge for international science communication, further research should confirm whether it is suitable for Han ethnic women of reproductive age in China.

The etiology and pathogenesis of PCOS is still unclear. At present, PCOS is regarded as a consecutive process, beginning with prepuberty or earlier, during which adult clinical manifestations change with heredity and environment [9]. Up to 50 million of the five billion women of reproductive aged in China will have PCOS, with an incidence rate of five to ten percent. Thus, providing proper diagnosis and treatment, and preventing long-term complications are arduous tasks. Meanwhile, the diverse clinical manifestations and severe long-term complications of PCOS have gradually attracted attention [8,10-13]. Although
drugs effectively prevent type 2 diabetes, hypertension, hyperlipidemia, and cardiovascular events, support from evidence-based medicine is lacking [14]. Presently, changing lifestyles (changing dietary habits, exercise for weight reduction) is an easy, cheap, and efficient therapy [15]. Hence, it is the most efficient, safest, and cheapest management strategy for patients diagnosed with PCOS.

The clinical manifestations of PCOS differ according to race and to age [16-19]. Thus, we should focus on the features of PCOS among the Han population in China to understand PCOS, to diagnose and treat the disease correctly, as well as to prevent its long-term complications. A total of 1,600 Han women of reproductive age who came from four sites in Liaoning Province were investigated via epidemiologic cluster layer sampling.

Materials and Methods

Participants

We surveyed women of reproductive age who were permanent residents in four different areas in Liaoning Province, Northern China, including the two cities Shenyang and Yingkou, and the two towns Benxi and Zhangwu. This sampling frame was chosen because it was the most logistically feasible, maximising the representativeness of geographic position and economic level in presenting prevalence and enabling further research on PCOS. All the Han ethnic women aged 19 years to 45 years were investigated, and finally, 1,600 women participated the study. Suspected PCOS patients were screened using inclusion criteria, and the controls were filtered from the cohort according to 1:1. The PCOS patients were diagnosed according to the diagnostic criteria recommended by the 2003 Rotterdam ESHRE/ASRM. Nest cases control study of diagnosed PCOS patients and normal controls were carried out.

Questionnaire

A total of 1,600 Han women of reproductive age from Liaoning Province were investigated from May to December in 2008. The questionnaire included common status, menstruation history (menarche age, primary or secondary amenorrhea, functionary uterus bleeding, oligo-menorrhea), recent hormone or drug intake, disease and treatment history, birth status, diet and exercise habit, familial disease history (including diabetes, hypertension, cardiovascular events, seborrheic alopecia, and the menstruation history of first and second degree relatives), and childhood history of married women (pregnancy-induced hypertension, gestational diabetes, preterm birth, and macrosomia).

Physical examination

Height and weight were measured (to calculate the body mass index, BMI), as well as abdominal circumference and waistline (to calculate waist to hip ratio, WHR). The subjects were examined for hair distribution (F-G hirsutism score) [20], acne, seborrheic alopecia, lactation, exophthalmos, swollen thyroid, and beast masses, as well for acanthosis nigricans. Blood pressure and pulse rate were recorded, and gynaecologic examinations (vulvae, vagina, cervix, body of uterus, appendix, and rectal examination for unmarried women) were performed.

Ultrasonography

Using an Ssc370 type ultrasound machine with 7.3 Hz, pelvic ultrasonography was performed per vagina for married women and per rectum for unmarried women. The size (length, width, thickness) and position of the uterus and endometrial thickness were measured. The sizes of both ovaries were measured, and the number of two to nine mm antral follicles (on longitudinal and coronal sections of the ovaries) was determined to obtain the mean value and describe special status.

Endocrine and biochemical indexes checking

The fasting blood glucose (FBG) of suspected PCOS patients and matched controls from the cohort were determined. Fasting venous blood samples were collected and sent to the Third Hospital of Beijing Medical University to determine fasting insulin (INS), triglyceride (TG), total cholesterol (TCH), high density lipoprotein (HDL), low density lipoprotein (LDL), thyroid stimulating hormone (TSH), testosterone (T), sex hormone-binding globulin (SHBG), androstenedione (A), as well as to calculate homeostasis model assessment of insulin resistance and the free testosterone index (FAI).

Intake, exclusion, and diagnostic criteria

Healthy Han ethnic women of reproductive age (19 to 45 years) in from Liaoning Province were included in the study. To minimise treatment bias, the authors excluded pregnant women or those suspected of pregnancy, lactating women, and those with endocrine diseases, as well as those on long-term oral contraceptives or subcutaneously injected contraceptive.

PCOS patients were diagnosed using ESHRE/ASRM of 2003 (8). Women who fulfilled any of the three criteria were considered suspected PCOS patients.

Normal women with signs of irregular menstruation, hirsutism, acne, and sebaceous flux were matched by ages (± 2) and BMI (± 2) in the cohort.

Statistical analysis

Descriptive statistics were generated to enable comparisons between groups. Continuous variables were checked for normality, and the means are presented with standard deviations, medians, or interquartile ranges, as appropriate. Distributions were compared using Student’s t-test or Mann–Whitney U as appropriate. Categorical variables were compared using a Pearson’s χ2 test. Differences with p-values < 0.05 were considered significant. All statistical analyses were carried out using SPSS 15.0.0 for Windows.

Results

Common features of researched groups

The age, profession, education degree, average family income, married/unmarried ratio, number of pregnancies and deliveries, BMI, and obesity distribution of the three groups are shown in Tables 1 and 2.

Of the 1,600 respondents, 132 (8.25%) were diagnosed with PCOS using the diagnostic criteria proposed by ESHRE/ASRM in 2003.

Menstruation history

Up to 97 of the 132 (73.48%) diagnosed PCOS patients had abnormal menstruation. Among the 97 cases, three (2.27%) had polymenorrhoea, 94 (71.21%) had oligomenorrhoea, six (4.55%) had secondary oligomenorrhoea; 61 cases (46.9%) had menstrual cycles lasting 35 days to 60 days, whereas 33 cases (35.1%) had menstrual cycles last-
Epidemiologic investigation of polycystic ovarian syndrome (PCOS) in Han ethnic women of reproductive age in Liaoning Province, China

Marking more than 60 days. The mean menarche age was 14.27 years ± 1.64 years old. The menarche ages of 20 cases were older than 16. In 42 cases (31.8%), regular menstruation began more than two years after menarche.

**Androgen excess**

The prevalence of androgen excess was 43.5%, among which biochemical androgen excess was 29.8%, whereas clinical androgen excess was 23.7%. Up to 19 cases (14.4%) exhibited hirsutism (F—G ≥ 6 score), 13 cases had acne (9.95%), eight (6.1%) had sebaceous flux, and two cases (1.5%) had acanthosis nigricans syndrome. Thus, the main manifestations of androgen excess among Han ethnic women of reproductive age in Liaoning Province were acne, hirsutism, sebaceous flux, and thick pores.

Hirsutism was mild in Liaoning province, with only 19 cases (14.39%) with F-G scores more than 6. Total score distribution was as follows: muffle 96 scores (37.20%), lower abdomen 52 score (20.16%), chest 43 score (16.67%), thigh 30 score (11.63%), upper abdomen 19 score (7.36%), mandible 15 score (5.81%), upper arm 1 score (0.39%), back 1 score (0.39%), and hip 1 score (0.39%). The main manifestations of hirsutism among Han women of reproductive age are thin hairs on the upper lip, thick hairs in middle of the lower abdomen, thick pubes extending to the crissum and groin, but seldom male distribution, long hair around the areola, and hairs inside the thigh, mainly near groin.

**Polycystic ovaries**

Polycystic ovaries are prominent among the PCOS patients. A total of 126 cases (96.2%) had polycystic ovaries, 68 of which were bilateral 58 (44.3%) were unilateral. The average number of follicles in the left ovary was 14.05 ± 5.51 and 15.23 ± 6.14 in the right ovary.

### Table 1. — Common features of researched groups.

<table>
<thead>
<tr>
<th>Features</th>
<th>All</th>
<th>Suspected PCOS and controls</th>
<th>PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1,600</td>
<td>966</td>
<td>132</td>
</tr>
<tr>
<td>Age (year)</td>
<td>34.38 ± 5.07</td>
<td>33.17 ± 4.89</td>
<td>31.15 ± 4.89</td>
</tr>
<tr>
<td>Profession (%)</td>
<td>13.6 / 19.1 / 31.6 / 2.8 / 0.2 / 10.6 / 16.0 / 33.7 / 3.7 / 0.3 / 14.1 / 3.3 / 9.1 / 6.4 / 14.0 / 3.8 / 9.7 / 8.1 / 12.1 / 4.5 / 12.1 / 3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cadre/teacher/office clerk/medical staff/student/worker/farmer/business/others</td>
<td>0 / 0.9 / 42.7 / 56.4</td>
<td>0 / 0.6 / 44.4 / 55.0</td>
<td>0 / 0 / 43.6 / 56.8</td>
</tr>
<tr>
<td>Education degree (%) illiterate/elementary school/high school/polytechnic school/junior college and higher</td>
<td>22.5 / 48.6 / 27.9 / 1.1</td>
<td>18.7 / 44.5 / 35.4 / 1.3</td>
<td>18.9 / 50.8 / 28.8 / 1.5</td>
</tr>
<tr>
<td>Average income of the family (%)</td>
<td>1546 / 54</td>
<td>924 / 42</td>
<td>115 / 17</td>
</tr>
<tr>
<td>Married/unmarried ratio</td>
<td>1.84 ± 1.12 / 0.92 ± 0.39</td>
<td>1.82 ± 1.14 / 0.88 ± 0.41</td>
<td>1.72 ± 1.09 / 0.82 ± 0.44</td>
</tr>
<tr>
<td>Menarche (age)</td>
<td>14.39 ± 1.51</td>
<td>14.18 ± 1.44</td>
<td>14.27 ± 1.64</td>
</tr>
<tr>
<td>BMI</td>
<td>22.73 ± 4.60</td>
<td>22.84 ± 3.29</td>
<td>23.27 ± 3.93</td>
</tr>
<tr>
<td>Obesity BMI ≥ 25</td>
<td>285</td>
<td>189</td>
<td>34</td>
</tr>
</tbody>
</table>

### Table 2. — Three groups divided according to age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>19 - 25</th>
<th>26 - 35</th>
<th>36 - 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>65 (40.06%)</td>
<td>810 (50.63%)</td>
<td>725 (45.31%)</td>
</tr>
<tr>
<td>Suspected PCO</td>
<td>50 (5.18%)</td>
<td>581 (60.14%)</td>
<td>335 (34.68%)</td>
</tr>
<tr>
<td>Diagnostic PCOS</td>
<td>16 (12.12%)</td>
<td>92 (69.70%)</td>
<td>24 (18.18%)</td>
</tr>
</tbody>
</table>

**Obesity**

BMI is often used to evaluate obesity. In 2000, the WHO Western Atlantic regional officials, the International Association for the Study of Obesity (IASO), and the International Obesity Task Force (IOTF) set the overweight and obesity cut-offs at BMIs of 23 kg/m² and 25 kg/m². Only 29 cases (21.96%) were overweight and 34 cases (25.75%) were obese. The patients were divided into an obesity group (34 cases, BMI ≥ 25) and a non-obesity group (98 cases). The triglyceride levels, free testosterone index, fasting insulin, constriction blood pressure, and hirsutism in the obesity group were higher than those in the non-obesity group. The levels of high-density lipoprotein and sex hormone-binding globulin were significantly lower than those in the non-obesity group. The other biochemical indices, such as cholesterol, androstenedione, number of follicles, low density of lipoprotein, and diastole blood pressure, did not significantly differ between the two groups (p > 0.05). The incidence rates in the obesity group of silent lifestyle, abnormal menstruation, history of infertility, and interval from menarche to regularisation of menstrual cycle exceeding two years were higher than those in the non-obesity group, but the differences were not significant (the χ² values were 1.64, 0.869, 2.663, 0.625 respectively, p > 0.05, Table 3)

**Age distribution features**

The 132 diagnosed PCOS patients were divided into three groups in terms of age: 19 years to 25 years, 26 years
Table 3. — Clinical and biochemical characteristics of 132 patients in obesity and non-obesity groups.

<table>
<thead>
<tr>
<th>Items</th>
<th>Obesity group</th>
<th>Non-obesity group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol/l)</td>
<td>1.67 ± 1.23</td>
<td>1.09 ± 0.68</td>
<td>3.436</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.24 ± 0.36</td>
<td>1.44 ± 0.31</td>
<td>-3.130</td>
<td>0.002</td>
</tr>
<tr>
<td>FAI</td>
<td>8.91 ± 8.07</td>
<td>4.92 ± 3.95</td>
<td>3.776</td>
<td>0.000</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>33.90 ± 16.68</td>
<td>55.57 ± 22.19</td>
<td>-5.203</td>
<td>0.000</td>
</tr>
<tr>
<td>INS (uU/ml)</td>
<td>7.01 ± 4.21</td>
<td>4.54 ± 6.25</td>
<td>2.140</td>
<td>0.034</td>
</tr>
<tr>
<td>F-G SCORE</td>
<td>3.00 ± 3.45</td>
<td>1.59 ± 2.29</td>
<td>2.689</td>
<td>0.008</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>124.62 ± 25.13</td>
<td>111.04 ± 11.97</td>
<td>4.173</td>
<td>0.000</td>
</tr>
<tr>
<td>LON (number)</td>
<td>14.94 ± 5.34</td>
<td>13.73 ± 5.59</td>
<td>1.098</td>
<td>0.274</td>
</tr>
<tr>
<td>TCH (mmol/l)</td>
<td>11.52 ± 4.13</td>
<td>11.80 ± 4.44</td>
<td>-0.323</td>
<td>0.747</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.88 ± 0.21</td>
<td>1.11 ± 0.16</td>
<td>2.952</td>
<td>0.004</td>
</tr>
<tr>
<td>TCH (mmol/l)</td>
<td>4.75 ± 0.69</td>
<td>4.68 ± 0.38</td>
<td>0.463</td>
<td>0.644</td>
</tr>
<tr>
<td>A (nmol/l)</td>
<td>11.94 ± 5.34</td>
<td>13.73 ± 5.59</td>
<td>1.098</td>
<td>0.274</td>
</tr>
<tr>
<td>RON (number)</td>
<td>16.35 ± 6.39</td>
<td>14.85 ± 6.03</td>
<td>1.235</td>
<td>0.219</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.24 ± 0.59</td>
<td>2.19 ± 0.57</td>
<td>0.437</td>
<td>0.663</td>
</tr>
<tr>
<td>T (nmol/l)</td>
<td>2.28 ± 1.07</td>
<td>2.21 ± 1.06</td>
<td>0.325</td>
<td>0.745</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>84.59 ± 15.38</td>
<td>81.24 ± 74.07</td>
<td>0.261</td>
<td>0.795</td>
</tr>
</tbody>
</table>


to 35 years, and 36 years to 44 years. The 26 years to 35 years old group accounted for 69.7%, less than that in the 36 years old group, which occupies 81.8% (Table 4).

Fertility survey

Of the 132 PCOS patients, 17 were unmarried women and 115 were married women. Among the patients who had been pregnant, 32 took longer than one year to conceive, 17 took longer than one year, six took longer than two years, three took longer than three years, and two took longer than four years. Only 11 patients had histories of pregnancy, but with no live births, and four patients had at most four miscarriages. The control group included five unmarried women and 127 married women. Among those with histories of pregnancy, 15 took longer than one year to conceive, ten took longer than one year, and five took longer than two years. Three had histories of pregnancy, but with no live births and one a history of miscarriage. The clinical criterion for infertility, set by the WHO in 1995, is one year. The prevalence of infertility among the diagnosed PCOS patients was 27.83%, whereas in control group was 11.81%.

Discussion

The prevalence of PCOS in Europe was five to ten percent [20]. The prevalence rates in Jinan City and in Yan Tai City were 6.46% and 7.2%, respectively, as reported by Chen et al. [21]. The prevalence of PCOS in this present epidemiologic search was 8.25%, which is consistent with that in Europe and slightly higher than those in Jinan City and Yan Tai City in China. The prevalence of PCOS is correlated with economic level. However, larger sample size is needed to confirm whether PCOS can be attributed to the difference in quality of life, nutrition, lifestyle, or pressure.

PCOS is a common complicated endocrine and metabolic disorder in women of reproductive age [22]. The symptoms improve with age [23]. PCOS is centralised around the age of 35 years, in accordance with the aforementioned fact [24]. The present study shows that the main clinical manifestation of PCOS in Liaoning Province is abnormal menstruation, with rate reaching 97%, whereas the androgen excess rate was only 23.7%. This result is different from those of a previous European research, wherein the abnormal menstruation rate was 80% and the androgen excess rate was 69% [9]. However, it is similar to the rate in Japan, wherein the abnormal menstruation rate was 92% and the androgen excess rate was 23% [25]. This difference may be because F-G scoring is unsuitable for Asians. Among the 132 matched controls, the correlations between body hair and sex hormone F-G scoring were 71.5%, 85.8%, 93.8%, 97.7%, 98.8%, and 100%, which correspond to scores 0, 1, 2, 3, 4, and 5, respectively. Thus, to determine the normal value from the skewed distribution, the authors set the critical value of F-G scoring to 3. The result is slightly higher than that of Zhao et al. [26], with a critical value of 2. These results show that hirsutism among Han women of reproductive age is slightly lower than that among women in occident countries, which may have resulted from racial differences. This result proves that racial difference affects the clinical manifestations of PCOS; thus, the diagnostic criteria should be established according to the different racial characteristics.

Furthermore, the incidence of obesity in this study differs from that in Europe. The obesity rate is 25.76%, which is clearly lower than the rate in Europe (50%–70%) [27], but similar to that in Japan (20%) [28]. However, the present rate is higher than in the study by Chen in 2004 (8.23%) [29]. The difference may be due to research time, caseload, and quality of life. Oligoovulation and non-ovulation are very common in PCOS; thus, many patients consult at the hospital for infertility [30]. Therefore, more attention should be given to treating infertility in the clinic. In this study, the infertility rate of PCOS in the unselected population was only 27.8%, which is lower than that of the normal group (11.82%). Therefore, infertile PCOS patients should initially be observed for endocrinal and metabolic disorders. Drugs to stimulate ovulation are unnecessary during the beginning of treatment.

Significantly more women in the PCOS group required more than two years to have regular menstruation than that

Table 4. — Age distribution of 132 PCOS patients.

<table>
<thead>
<tr>
<th>Group (age)</th>
<th>Cases</th>
<th>Proportion (%)</th>
<th>Accumulative proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 25</td>
<td>16</td>
<td>12.1</td>
<td>12.1</td>
</tr>
<tr>
<td>26 - 35</td>
<td>92</td>
<td>69.7</td>
<td>81.8</td>
</tr>
<tr>
<td>36 - 44</td>
<td>24</td>
<td>18.2</td>
<td>100.0</td>
</tr>
</tbody>
</table>


307
in the control group \((p < 0.05)\). The authors defined menstrual delay as the occurrence of menarche later than 16 years. Significantly more women had menstrual delay in the PCOS group than that in the control group \((p < 0.05)\). Thus, patients with delayed menarche and delayed regular menstruation, especially oligomenorrhea, have higher risk of PCOS. Therefore, patients who experience delayed menarche or long menstrual cycles, fail to establish regular menstrual cycles within two years after menarche, and those with complications that cause androgen excess, such as obesity and polycystic ovaries, should be examined for PCOS. These patients should be diagnosed earlier to receive the appropriate intervention.

The etiology of PCOS is still unclear. Recent studies have linked increased androgen and insulin resistance with PCOS [31, 32]. The study showed that free androgen index (FAI), homeostasis model assessment-insulin resistance (HOMA-IR), increased serum testosterone, and androstenedione, and decreased SHBG are high-risk factors for PCOS.

**Conclusions**

The prevalence of PCOS in Han women of reproductive age in Liaoning Province is 8.25%, with an infertility rate of 27.8%. The classical manifestation of PCOS among Han women in Liaoning Province is PCOs and abnormal menstruation, whereas obesity, acne, and hirsutism are minor indicators. In addition, androgen excess rate among PCOS patients is lower than that in other studies. FAI, HOMA-IR, increased serum testosterone and androstenedione, and decreased SHBG, long history of infertility, menstrual delay as menarche later than 16-years-old, and failure to have regular menstruation with two years after menarche are high-risk factors for PCOS.

**References**


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Adropin: a key component and potential gatekeeper of metabolic disturbances in polycystic ovarian syndrome

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Summary

Purpose: The aim of the current study was to evaluate potential relationships between serum adropin levels and metabolic parameters in polycystic ovary syndrome (PCOS). Materials and Methods: Twenty women with PCOS and 20 healthy, age and body mass index (BMI) matched controls were included in the study. All subjects underwent venous blood drawing on the early follicular phase after an overnight fasting. Serum adropin levels were measured with enzyme immunosorbent assay (EIA). The relationships between serum adropin levels and metabolic parameters were also assessed. Results: Serum adropin levels were found to be significantly lower in women with PCOS when compared to control group (p < 0.001). Serum adropin level was correlated negatively with fasting serum insulin levels, homeostasis model of assessment - insulin resistance (HOMA-IR) and serum lipid markers including cholesterol, very low-density lipoprotein, and triglycerides (TG) in PCOS patients (p < 0.05). Conclusion: The findings of current study suggest that women with PCOS have low serum adropin levels that may contribute to the underlying pathogenesis of metabolic disturbances in PCOS.

Key words: Adropin; PCOS; Insulin resistance; HOMA-IR.

Introduction

Adropin is a recently identified protein encoded by the energy homeostasis associated gene (Enho) that is expressed in the brain and the liver [1,2]. It has been demonstrated that the expression of adropin is regulated by energy status and dietary nutrient content, and altered with body fat composition [1,2]. In diet-induced obese (DIO) mice, systemic administration of adropin markedly attenuated insulin resistance and glucose intolerance [1-4]. Furthermore, the experimental studies have reported important findings that DIO mice are associated with diminished expression of adropin transcript in liver and low circulating adropin concentrations [1, 2]. Evidence from previous animal study showed that adropin knockout mice exhibit hepatic steatosis, insulin resistance, increased fasting triglycerides (TG), and propensity for impaired glucose tolerance [2].

PCOS affecting up to 20% of women of reproductive age is characterized by oligomenorrhea, hyperandrogenism, and a characteristic ovarian morphology on ultrasonographic examination [5]. The etiology of PCOS is complex and not fully known. Nevertheless, a significant proportion of women with PCOS suffer from insulin resistance (IR) and IR appears to be a central pathophysiological feature of PCOS. Despite its frequent association with obesity, the relation of IR in women with PCOS cannot be precisely explained solely due to obesity because data showing existence of higher IR in lean PCOS women than normal healthy controls is present [6].

Based on aforementioned observations, the authors hypothesized that adropin, an energy regulatory peptide, may be a factor in development of IR and dyslipidemia in women with PCOS. To date, adropin and its role in relation to IR, and lipid metabolism has not been studied in patients with PCOS. The aim of the current study was therefore to evaluate potential relationships between adropin levels and metabolic parameters, in women at reproductive age with PCOS.

Materials and Methods

This study was carried out in collaboration with Departments of Obstetrics and Gynecology in Pamukkale University in Denizli, in Inonu University Turgut Ozal Medical Centre, and Department of Medical Biochemistry, Firat University, Elazig in Turkey. The study protocol was approved by Instutional Ethics Committee. All subjects participating in the study were fully informed of the aim of the study, and informed consents were obtained.

This study enrolled 20 women with PCOS and 20 age- and body mass index (BMI)-matched healthy women as the control subjects. The subjects with PCOS were selected from a group of PCOS patients who were seeking treatment for menstrual irregularity, acne, hirsutism or infertility. The control group consisted of 20 age- and BMI-matched healthy volunteers who were menstruating regularly, normo-ovulatory, non-hirsute, and had normal biochemical and hormonal profiles, thereby excluding the diagnosis of PCOS in this group.

PCOS was defined when at least two of the following three features were present after the exclusion of other etiologies (Rotterdam criteria): (I) oligo/amenorrhea (fewer than six menstrual periods in the preceding year), (II) clinical and/or biochemical signs of hyperandrogenism, and (III) ultrasonographic finding [7]. The ultrasound criteria used for diagnosis of PCOS were: pres-
ence of 12 or more follicles in each ovary measuring two to nine mm in diameter, and/or increased ovarian volume (> ten ml). Clinical hyperandrogenism was quantified by the Ferriman-Gallwey scoring system [8] and the diagnosis was established when it was greater than 8. Patients taking antidiandrogen drugs, antidiabetics, lipid lowering medication, glucocorticoids or other hormonal drugs were excluded. Patients with anemia pregnancy, and adrenal disorders including congenital adrenal hyperplasia, diabetes mellitus, hypertension, myocardial infarction, stroke, and peripheral vascular disease were also eliminated.

For each subject, height, weight and BMI were evaluated by standard methods. BMI was measured as the ratio of the weight to the square of the height. All subjects underwent venous blood drawing for complete hormonal assays, lipids, adropin, fasting glucose, and insulin analysis. The blood of subjects was sampled in the morning following an overnight fast during early follicular phase (day 2–5) of spontaneous or progesterone induced withdrawal bleeding.

Biochemical analysis
The blood samples were centrifuged and then the plasma aliquots were frozen at -80°C until assayed. Serum adropin concentration was analyzed using an enzyme immunoassay kit (EIA) with a minimum detectable level less than 0.3 ng/ml. The intra- and interassay coefficient of variance ranged from 7.8 to 8.1 and from 9.2 to 11.3, respectively. Samples were processed by technicians that were blinded to the identity of samples.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, sex hormone-binding globulin (SHBG), insulin, and dehydroepiandrosterone sulfate (DHEAS) levels were measured by competitive chemiluminescent enzyme immunoassay method using the same trademark kits. Fasting glucose, TG, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic colorimetric assay methods using an autoanalyzer and commercially available kits. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using Friedewald formula [8]: LDL-C = TC - HDL-C – TG/5. Fasting insulin levels were measured in both PCOS and control subjects to estimate the insulin sensitivity. IR was calculated using the homeostasis model assessment IR index (HOMA-IR), according to the formula; HOMA-IR = fasting serum insulin (mU/ml) x fasting plasma glucose (mg/dl) /405[9].

Statistical analysis
The normality of distribution of variables was tested by using the Kolmogorov-Smirnov test. Categorical variables, presented as numbers and percentages, were compared using the Chi square test. Continuous variables with a skewed distribution were log-transformed. Continuous variables are presented as mean and standard deviation (SD). Comparison of continuous variables between the groups was assessed using Mann-Whitney U test. The correlation of variables was assessed by Pearson correlation test. For all comparisons, statistical significance was defined by p < 0.05. The data were assessed using the Statistical Package for Social Sciences software 19.0 for Windows package software.

Results
The demographic characteristics of study and control groups are compared in Table 1. Mean age and BMI were not different in the PCOS group compared to the controls (p = 0.14 and p = 0.58, respectively). All patients and subjects had normal renal, hepatic, and thyroid functions.

The mean LH, ratio of FSH to LH, HOMA-IR, fasting insulin, serum cholesterol, and TG levels were higher in PCOS group when compared those of the controls (p < 0.001, p < 0.001, p = 0.02, p = 0.03, p = 0.04, and p = 0.04, respectively); while there were no significant differences between the groups regarding mean serum estradiol, FSH, and thy-
Adropin: a key component and potential gatekeeper of metabolic disturbances in polycystic ovarian syndrome

Discussion

This study is the first, to the authors’ knowledge, study demonstrating the decrease of serum adropin levels in women with PCOS cases in whom each one of those patients had increased fasting serum insulin levels and HOMA-IR. Moreover, the current study has demonstrated significant negative correlation between serum adropin concentrations and fasting insulin, HOMA-IR, and lipid concentrations. These data has indicated an association between low serum adropin levels with IR and dyslipidemia in women with PCOS. In accordance with these findings, a previous study on humans has demonstrated that the low plasma adropin level is associated with an increased risk in metabolic factors including IR and dyslipidemia [10].

The mechanisms regulating the synthesis and secretion of adropin in PCOS women are not well known. However being a novel metabolic marker, its levels can be altered by any metabolic factor associated with PCOS. A recent study has demonstrated that overexpression of adropin in obese mice resulted in marked improvement in insulin sensitivity, a reduction in diabetes, and weight loss [1, 2]. A significant proportion of women with PCOS suffer from IR that appears to play a role in the pathophysiology of PCOS [11, 12]. However, there is no data whether the decrease in adropin levels seen in PCOS is mediated through IR or is a consequence of other metabolic factors.

Another possible explanation for the observed lower adropin levels in women with PCOS might be dyslipidemia. In the current study, the authors found a significant negative correlation between serum adropin and total cholesterol, TG, and VLDL-C levels. In addition, PCOS subjects with low serum adropin levels exhibited higher TG, HOMA-IR, fasting insulin, and VLDL-C levels compared to controls. Because most women with PCOS have IR [6, 11, 13, 14], we do not know whether decreased adropin level is secondary to IR or dyslipidemia contributes to IR and decreased adropin levels per se.

The current study has some disadvantages as involving a single measurement and a small population of women with PCOS. Second, the authors did not compare adropin levels in lean and obese PCOS patients. In spite of these limitations, this study was able to detect a difference between the serum adropin levels of the women with PCOS and those of controls.

Conclusion

In conclusion, the present results provide further indication of the significance of serum adropin in maintaining glucose homeostasis, lipid metabolism, and IR in PCOS subjects. Adropin deficiency associated with PCOS may attenuate the development of IR and dyslipidemia and might play a role in the pathophysiology of PCOS.

References


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Introduction

Bronchopulmonary dysplasia (BPD) is a common respiratory disease in premature infants, especially in very premature infants. It is a major reason for increasing incidence and mortality rates of premature infants. Approximately 30% of premature infants with birth weight <1,500 g suffer from BPD (most are those with birth weight between 501 g and 750 g). In recent years, the incidence of BPD shows an increasing tendency, which is generally presumed to reach 43% among the premature of <1500 g [1]. In addition, long-term BPD results in nervous system dysplasia and long-term pulmonary dysfunction [2, 3] seriously threaten premature infants’ life quality. In China, as neonatal intensive care techniques develop, more and more premature and very low birth weight infants have survived. Consequently, the incidence of BPD is also increasing, which seriously influences the survival rate and life quality of premature infants in this country. Therefore, early prevention and diagnosis of BDP play critical roles in improving premature infants’ survival rate and long-term prognosis.

In the past two decades, great achievements have been obtained from studies on the correlation between prenatal infection/inflammation and newborn respiratory prognosis [4]. Many clinical and animal models have proved that although maternal histological chorioamnionitis (HCA) can promote fetal lung maturity, it increases the risk of premature labor and influences the long-term prognosis of premature lung [5, 6]. Furthermore, another study has shown that changes in bioproteins are literally the form of fetus’ responses to intrauterine inflammation [7]. These changes play a part in the pathogenesis of BPD. Therefore, inflammatory mediators and some bioproteins serve as the best choices for the early prediction of premature BPD among various factors. Additionally, cord blood is the most easily obtainable blood sample and its obtaining does not cause harm to the premature.

Based on the aforementioned, cord blood samples were taken in the current study. Biological markers in the samples were determined to investigate their correlations with BPD and then to elucidate their feasibility being the predictive indices of BPD.

Materials and Methods

Subjects

A total of 134 very low birth weight infants born at Tianjin Central Hospital of Gynecology Obstetrics between January 1, 2010 and January 31, 2011 were recruited. Their birth weights were ≤ 1,500 g with gestational age (GA) ≤ 32 weeks. All GAs were estimated within 24 hours after birth using new Ballard scoring, and the estimated ages were taken as standard. Of the 134 infants, 35 diagnosed with BPD comprised the BPD group (one had severe BPD, two had moderate BPD, and the others had mild BPD), and the rest constituted the non-BPD (NBPD) group.

Cord blood collection

Three ml of placental umbilical cord umbilical vein blood was taken after neonatal birth. After clotting, the sample was centrifuged at 3000 r/min for 15 min. The supernatant was collected...
and immediately placed at -80 °C for detection. All samples were disposed in case that repeated freezing and thawing would influence experimental results.

**Placenta collection**
A total of 112 fetal placentas were selected and fixed in 10% formalin. They were instantly sent for pathological tests to determine whether HCA existed or not. HCA was defined by the existence of one to three polymorphonuclear leukocytes.

**Clinical data and diagnostic criteria**
Clinical data of GA, birth weight, sex, respiratory distress syndrome (RDS), ventilator treatment, and BPD were collected. BPD was defined according to the new definition of BPD given by the BPD reference co-held by the United States National Institute of Child Health and Human Development (NICHD), the United States National Heart, Lung, and Blood Institute, and the Rare Disease Committee; patients’ conditions were graded according to severity [8, 9]. The grading results are summarized in Table 1.

**Enzyme-linked immunosorbent assay (ELISA)**
ELISA was performed including IL-6, IL-6R, sgp130, and MMP-9 kits and an automatic analyzer.

**Statistical analysis**
Data were analyzed using the SPSS17.0 software. A normal distribution test was carried out for all variables. Data that did not satisfy normal distribution were evaluated using the rank test and presented as median + range. Multiple factor regression analysis was performed using Logistic.

### Table 1. *The diagnostic criteria and grading for BPD.*

<table>
<thead>
<tr>
<th>Diagnosis: Oxygen-inspiring time ≥ 28 d for premature infants</th>
<th>GA &lt; 32 weeks</th>
<th>GA ≥ 32 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading: Evaluation time</td>
<td>36 weeks of corrected GA</td>
<td>Postnatal 56 d</td>
</tr>
<tr>
<td>Mild BPD</td>
<td>Without oxygen use or discharged from hospital</td>
<td></td>
</tr>
<tr>
<td>Moderate BPD</td>
<td>FiO₂ &lt; 0.30 treatment</td>
<td></td>
</tr>
<tr>
<td>Severe BPD</td>
<td>FiO₂ ≥ 0.30 and/or continuous positive airway pressure or ventilator treatment</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. *Comparisons of the general data between groups.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>GA (weeks) (median; range)</th>
<th>Birth weight (g) (median; range)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>BPD</td>
<td>35</td>
<td>29 (26—29)</td>
<td>960 (600—1250)</td>
<td>22</td>
</tr>
<tr>
<td>NBPDG</td>
<td>99</td>
<td>30 (27—32)</td>
<td>1250 (940—1480)</td>
<td>43</td>
</tr>
<tr>
<td>Z or χ²</td>
<td>- 7.605</td>
<td>- 7.118</td>
<td>4,327</td>
<td>0.038</td>
</tr>
<tr>
<td>p value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.038</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. *Comparisons of the cytokines between groups.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>MMP-9 (ng/ml) (median; range)</th>
<th>IL-6 (pg/ml) (median; range)</th>
<th>IL-6R (pg/ml) (median; range)</th>
<th>Sgp130 (ng/ml) (median; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD</td>
<td>35</td>
<td>17.750 (10.250—283.000)</td>
<td>47.000 (0.700—179.750)</td>
<td>29.500 (13.250—191.750)</td>
<td>47.000 (13.750—227.500)</td>
</tr>
<tr>
<td>NBPD</td>
<td>99</td>
<td>12.250 (10.000—179.500)</td>
<td>1.500 (0.250—79.850)</td>
<td>14.500 (12.500—118.000)</td>
<td>17.000 (14.000—110.500)</td>
</tr>
<tr>
<td>Z</td>
<td>-1.569</td>
<td>-3.323</td>
<td>-3.536</td>
<td>-2.154</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.117</td>
<td>0.001</td>
<td>0.000</td>
<td>0.031</td>
<td></td>
</tr>
</tbody>
</table>

### Results

**Treatment comparison**
As shown in Table 2, the GAs and birth weights were not in normal distribution, according to normal distribution tests. Correspondingly, rank tests were performed to compare the GAs and birth weights between the groups. The results showed that the GA of the BPD group was significantly younger than that of the NBPD group (*p* < 0.05), and its birth weight was also noticeably lighter than that of the NBPD group (*p* < 0.05). As for gender, males were more than females in number, showing a significant difference (*p* < 0.05).

**Cytokine comparison**
The expression of IL-6, IL-6R, and sgp130 of the BPD group was significantly higher than those of the NBPD group (*p* < 0.05). Although MMP-9 expression of the BPD group was lower than that of the NBPD group, no significant difference was observed. The results are summarized in Table 3.

**The correlation between HCA and BPD**
As shown in Table 4, although the incidence rate of HCA in the BPD group was 60% which was higher than that in the NBPD group (54.5%), a Chi-square test did not show a significant difference (*p* > 0.05).
Biological markers in cord blood for prediction of bronchopulmonary dysplasia in premature infants

The correlations between bioactive markers and BPD

The Logistic regression analysis showed that GA, birth weight, sex, and IL-6 had influences on BPD: GA, birth weight, and IL-6R were negatively correlated with BPD (namely that a younger GA and a smaller birth weight indicate a higher risk of BPD; both \( p < 0.05 \)) (Table 5), whereas IL-6 was positively correlated with BPD (namely that higher IL-6 expression indicates a higher risk of BPD; \( p < 0.05 \)); males were more prone to BPD than females. Additionally, Sgp130 showed collinearity with IL-6 and therefore had a predictive value for BPD (Table 6).

Receiver operating characteristic (ROC) curves

Based on ROC curves, the following can be presumed: IL-6 at 46.125 pg/mL has the highest diagnostic value with sensitivity of 0.51 and specificity of 0.86 (Figure 1); Sgp130 at 47.125 ng/ml has the highest diagnostic value with sensitivity of 0.51 and specificity of 0.82 (Figure 2); and GA < 28.5 weeks and IL-6 > 46.125 pg/ml have sensitivity of 1, specificity of 0.59, and area under the curve of 0.849, which has an good predictive value for BPD (Figure 3).

Discussion

BPD is a common respiratory disease in premature infants, especially in very premature infants. It is a major cause for increases in disease incidence and mortality rates of premature infants. Numerous causes for BPD include external causes such as HCA and postnatal infection, iatrogenic causes such as mechanical ventilation-induced barotraumas and active oxygen free radical injury caused by long time oxygen and high-pressure oxygen, as well as internal host responses [10-12]. More than often, premature infants are exposed to adverse factors such as mechanical ventilation, high-pressure oxygen, barotraumas, and infection; these factors trigger the cascade reactions of inflammatory factors that further exacerbate airway, pneumoangiogram, and interstitial injuries to lead to pulmonary injury [13]. Therefore, inflammatory reaction is a key link in the development of BPD.

HCA is commonly used to describe an inflammatory status of intrauterine tissue, including fetal-maternal mixed tissue (the deciduous and chorial space) inflammation or fetal origin tissue (chorio-amnion, amniotic fluid, and the umbilical core) inflammation [14]. It is often accompanied with the evidence of the invasion of pathogenic bacteria into normal tissues [15, 16]. HCA reduces the incidence rate of RDS in infants with birth weight < 2,000 g but increases the incidence of chronic pulmonary disease [17]. This finding indicates that HCA promotes pulmonary maturation by stimulating adrenal function to promote cortisol secretion on the one hand [18]; on the other, intrauterine infection-induced fetal inflammation significantly disturbs the normal development of the pulmonary structure [19]. Despite these findings, the correlation of HCA with BPD remains controversial. In the present study, although the in-

| Table 4. — Correlation between maternal HCA and BPD. |
|---|---|---|---|
| Group | Cases (%) | χ² | p value |
| | HCA | Non-HCA |  | |
| BPD | 21 (60.000) | 14 (40.000) | 0.312 | 0.576 > 0.05 |
| NBPD | 54 (55.500) | 45 (45.500) |  | |

| Table 5. — Correlations of bioactive markers with BPD by multifactor logistic regression analysis. |
|---|---|---|---|
| Factor | OR | 95% CI | p value |
| GA | 0.135 | 0.042-0.436 | 0.001 |
| Birth weight | 0.992 | 0.987-0.997 | 0.002 |
| Gender | 7.206 | 1.380-37.636 | 0.019 |
| IL-6R | 0.947 | 0.912-0.983 | 0.005 |
| IL-6 | 1.056 | 1.019-1.096 | 0.003 |

| Table 6. — Correlation of the bioprotein Sgp130 with BPD using multifactor logistic regression analysis. |
|---|---|---|---|
| Factor | OR | 95% CI | p value |
| GA | 0.383 | 0.191—0.767 | 0.007 |
| Birth weight | 0.991 | 0.986—0.996 | 0.001 |
| Gender | 5.525 | 1.313—23.248 | 0.020 |
| MMP-9 | 0.990 | 0.980—1.000 | 0.057 |
| Sgp130 | 1.009 | 1.000—1.010 | 0.048 |

Figure 1. — The predictive values of IL-6 for BPD.
Cytokines are chemical materials secreted by various types of cells such as blood cells, endothelial and epithelial cells, fibroblasts, and type II alveolar cells. They mediate immune, inflammatory, and blood-producing responses caused by stimulation [10], thereby playing a part in the pathogenesis of BPD. In premature infants, stimulation can give rise to general and pulmonary inflammatory responses. Defects in tissue repair, differentiation, and growth can present changes in biomarker concentration as well as corresponding symptoms and signs in BPD infants [23, 24]. Conventionally, IL-6 is presumed to be a type of acute response and lymphocyte stimulating factor [25]. However, recent study has shown that IL-6 performs a series of actions including congenital immune reaction and immune response stimulation [26, 27]. The transformation between congenital and acquired immunity is the key point of inflammatory reaction; obstruction of the transformation can distort immune responses, which ultimately leads to the occurrence of chronic inflammation [28]. An experiment on in vitro fetal membranes has displayed that the levels of IL-6, IL-8, and TNF-α increase in HCA [8]. Another study indicates that premature infants exposed to pro-inflammatory cytokines or those with increased IL-6 in cord blood at the time of birth are more prone to chronic pulmonary disease and that prenatal fetal inflammatory reaction in infants with chronic pulmonary disease is a trigger factor of BPD [29]. The present study is further full proof that the correlation of increased IL-6 with the development of BPD does exist. Therefore, IL-6 level can serve as one of the predictive factor of BPD: IL-6 > 46.125 pg/ml signifies a high risk of BPD with an odds ratio of 1.056, a 95% confidence interval of 1.019 to 1.096, sensitivity of 0.51, and specificity of 0.86; under the condition of GA < 30 weeks, IL-6 > 46.125 pg/ml has a good predictive effect on BPD with sensitivity of 1 and specificity of 0.59. Although these results are basically consistent to those reported by others, differences in the predictive values of IL-6 do exit. Therefore, to further define the standard values of IL-6, larger samples are still necessary.

In addition, IL-6 can only perform its biologic activity by binding with its receptor. In view of the important role played by IL-6 in various diseases, regulating IL-6 expression by applying IL-6 receptor (IL-6R) has become a possible treatment method for associated diseases. IL-6 performs multiple functions in angiogenesis and vascular...
remodeling, and it participates in postnatal angiogenesis by circulating endothelial progenitor cells (EPCs) in blood. IL-6R expressed in EPCs (gp80) and the application of IL-6 can activate the gp80/gp130 pathway in EPCs including the phosphorylation of downstream extracellular signal-regulated kinase 1/2 and signal transduction and transcription activator-3 (STAT3) in EPCs, and anti-IL-6 antibodies or IL-6R can inhibit these influences [30]. In this study, because of the existence of the structural reconstruction of pulmonary alveoli in chronic pulmonary disease, IL-6R was detected to clarify whether it performs an effect on pulmonary disease in premature infants. The result shows that IL-6R is negatively correlated with BPD, namely that the lower an IL-6R level is, the more likely BPD is to occur. However, whether the incidence of BPD can be reduced by activating the activity of IL-6R remains to be explored. Meanwhile, the effect of IL-6 on BPD needs further studies to clarify considering that the action mechanism of the IL-6 and IL-6R signal transduction system underlying chronic inflammatory reaction remains unknown. Sgp130 is a cyclic IL-6 receptor subunit for the signal transduction of multiple kinds of cells and plays an important role in the IL-6 signal system. This study shows that in the premature with GA < 30 weeks, an increased Sgp130 level indicates a higher risk of BPD; Sgp130 at 47.125 ng/ml has the largest diagnostic value with sensitivity of 0.51 and specificity of 0.82. However, according to these results, Sgp130 has relatively low sensitivity. This entails large sample sized clinical experiments in the future.

In addition, to date, study has shown that deficiency of the matrix metalloproteinase MMP-9 aggravates pulmonary alveolus agenesia in genetically modified BPD; the activity of MMP-9 may be basically subject to defense mechanism, protecting lung tissues and inhibiting the damage of inflammation to the lungs [11]. In this study, although the MMP-9 content in the BPD group was lower than that in the NBPD group, no significant difference was observed. Therefore, the correlation between MMP-9 and BPD should be explored in the future.

This study shows that the combined consideration of birth weight, GA, IL-6, and Sgp130 for the prediction of BPD can reach accuracy as high as 88.1%. Furthermore, this study shows that males are more prone to BPD than females. However, due to social factors, some very premature female infants did not receive remedy and were excluded from this study. Therefore, whether there is a gender difference in BPD occurrence necessitates further exploration.

With the development of neonatal rescue techniques, the incidence of chronic pulmonary disease in the premature shows an increasing trend. How to predict the disease as early as possible for more effective prevention and treatment remains an urgent problem to solve. Recent years has witnessed bioactive markers become the hot topic of studies on pulmonary disease. Among various types of blood samples, cord blood is the most easily acquired sample whose acquisition does not harm premature infants, as well as the earliest channel for disease prediction. This study shows that IL-6 and Sgp130 have predictive values for BPD. However, their predictive values obtained in this study are not completely in line with those reported (the results reported by others in themselves are not in consistency with each other), and extensive clinical studies are still needed to define more scientific and accurate values. Nevertheless, the application of cytokines is still promising for predicting BPD.

References


Laparoendoscopic single-site surgery (LESS) for large benign adnexal tumors: one surgeon’s experience over one-year period

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Summary

Objective: To present the authors’ experience with laparoendoscopic single-site surgery (LESS) surgery for large benign adnexal tumors and to compare the removal time of resected specimen with that of conventional laparoscopy. Study Design: Ten consecutive patients underwent LESS for huge adnexal tumors at Myongji Hospital, Korea between March 2011 and July 2012. A modified open Hasson technique was used to gain access to the abdominal cavity. The single-port device was inserted trans-umbilically into the wound opening. After suction of large amount of fluid content, LESS salpingo-oophorectomy was performed. The resected adnexal specimen was placed into a LapBag for removal out of the abdominal cavity. The authors compared the removal time of resected specimen between LESS and previously performed conventional laparoscopy for large benign adnexal tumors. Results: The adnexal tumors in this study were all very large cystic tumors reaching near or over the umbilicus. It took less than ten minutes for the removal of the resected adnexal tumors in all LESS cases (three to ten minutes), much less time than that of the conventional laparoscopy (usually ten to 17 minutes). Conclusion: LESS for large benign adnexal tumors is feasible and removal of resected adnexal tumor is easier than conventional laparoscopic surgery.

Key words: LESS; Large benign adnexal tumor; Removal time.

Introduction

Benign ovarian cysts are now managed laparoscopically rather than by laparotomy. The advantages of laparoscopy over laparotomy are less post-operative pain, short hospital stay, earlier recovery, and improved quality of life in the post-operative period and cosmetic effect. However, the size of the ovarian cyst has been the major limitation of laparoscopic management due to the possibility of malignancy and inadvertent cyst rupture during surgery [1, 2].

Recently, there have been some reports on the feasibility of the laparoscopic removal of large benign ovary cyst [3, 4]. Wong et al. also described the technique for successful conventional laparoscopic removal of large ovarian cysts [5].

Since 2008, single-port laparoscopic surgery for benign gynecologic disease has become increasingly common [6]. In many reports, there was no difference in the median operation time between (laparoendoscopic single-site surgery) LESS and conventional laparoscopy [7-9]. However, it would take a long time to extract a large ovarian cyst through a conventional port due to small port size. On the contrary, removal of resected ovarian cyst through a larger umbilical incision would be easier and more rapid because of larger diameter of single umbilical incision. However, there has been no prior report on the comparison of specimen removal time between single port and conventional laparoscopy.

In this study, the authors attempted to show the feasibility of LESS for large adnexal tumors and easiness of removal of resected tumors compared with that of conventional laparoscopy.

Materials and Methods

Patients

The authors retrospectively reviewed the medical records of the ten consecutive patients who underwent LESS for large benign adnexal tumors at Myongji Hospital (Kyunggi-do, Korea) between March 2011 and July 2012.

Inclusion criteria for this study were sonographic and magnetic resonance imaging (MRI) features of the ovarian cysts consistent with benign disease, and the maximum diameter of the ovarian cyst > 15 cm on MRI imaging. Clinicopathologic data including age, body mass index, (BMI), surgical time, surgical results, and pathologic results were reviewed. This study was approved by the Institutional Review Board. The authors also reviewed the medical records of the eight patients who had conventional laparoscopic surgery for huge adnexal tumors by other surgeons before March 2011.

Surgical technique

The patient was placed in the dorsal lithotomy position. Regarding the LESS approach, a 2.5 to 3.0 cm vertical incision was made within the umbilicus using a modified open Hasson technique at the beginning of the surgery to gain access to the abdominal cavity. The single-port device was inserted trans-umbilically into the wound opening and suction irrigator...
was inserted into the cyst to drain the cyst. If the abdominal cavity was blocked by a large cyst wall, the authors placed a purse string suture in the cyst wall using 3-0 suture on a GI needle and penetrated the cyst using a suction irrigator and tied a single throw in the purse string to tighten the cyst wall around the suction irrigator and to prevent spillage. After the cyst fluid content was evacuated as completely as possible, the suction irrigator was removed and the purse string suture was tied up to minimize spillage. Then the single-port device was inserted trans-umbilically into the wound opening and the entire ovary cyst could be visualized.

The authors used a rigid 30-degree, five-mm laparoscope and conventional laparoscopic instruments for all LESS procedures. The infundibulopelvic vessels were sealed and ligated, and salpingo-oophorectomy was performed. The resected adnexal specimen was placed in a LapBag for removal from the abdominal cavity and to minimize intra-peritoneal leakage of the contents. They checked the time from the completion of resection of adnexal specimen to complete removal through the port site.

The peritoneum and fascia were approximated and closed layer by layer with 2-0 Vicryl suture. Skin adhesive material was applied for good cosmetic outcome and it was also convenient for the patient. All LESS procedures were performed by a single surgeon.

Statistical analysis was performed using SPSS version 10.0. Comparisons between groups were performed with Student t test and chi square test, including Fischer’s exact test. A p value < 0.05 was considered statistically significant.

Results

The adnexal tumors in this study were all very large cystic tumors ranging from 17 cm to 26 cm in the largest diameter. The pathologic diagnoses were confirmed as mucinous cystadenoma mixed with benign cystic teratoma (n = 2), endometriosis (n = 2), mucinous cystadenoma (n = 4), and serous cystadenoma (n = 2) (Table 1).

The median age of the patients was 56 years (range 31-70), and the median body mass index was 23.6 (range 19.5-27.1). The median surgical time was 52.5 min (range 45–70), and the median estimated blood loss during surgery was 50 ml (range 30–100). No patients received transfusions due to surgical blood loss. The median length of post-operative hospital stay was two days (range one to three days, Table 2).

Removal of the resected adnexal tumor through the large diameter (about 2.5 cm) umbilical single incision was much easier than through the small port diameter (about one cm) of conventional laparoscopy. It took less than ten minutes for the removal of the adnexal tumors in all LESS cases (three to ten minutes), much less time than that of the conventional laparoscopy (from ten to 17 minutes for huge adnexal cysts, Table 2).

All procedures were performed without complications. There were no perioperative port-related or surgical problems in all ten cases. One case needed one additional suprapubic port due to severe left pelvic wall adhesion arisen from the previous surgery. The other nine procedures were successfully performed without the use of additional ports.

<table>
<thead>
<tr>
<th>Table 1. — LESS patients’ characteristics.</th>
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<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Age (years, median)</td>
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<tr>
<td>Parity</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Previous surgery history (n)</td>
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<tr>
<td>Mucinous cystadenoma</td>
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<tr>
<td>Mucinous cystadenoma mixed with teratoma</td>
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<td>Serous cystadenoma</td>
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<td>Endometrioma</td>
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<table>
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<th>Table 2. — Surgical and pathological results.</th>
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<tr>
<td>Results</td>
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<tr>
<td>Maximal cyst diameter (cm)</td>
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<tr>
<td>Surgical time (min)</td>
</tr>
<tr>
<td>Tumor extraction time (min)</td>
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<tr>
<td>Estimated blood loss (ml)</td>
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<td>Post-op hospital stay (day)</td>
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The patients were followed-up at one and four weeks and at three months after discharge, and none showed early or late postoperative complications.

Discussion

Recently, LESS became more widespread than before owing to the improvement of flexible laparoscopic instruments and advances in technology. Several reports addressed the feasibility and safety of LESS for benign adnexal disease [10-12]. More recently, several case reports about LESS for large adnexal cysts also mentioned the feasibility, cosmetic aspect, and easiness of removal of large ovarian cyst [13-15].

In the current study, LESS surgery using the single-port device had a success rate of 90% with no conversion to laparotomy. The one failure case in which the authors used additional suprapubic port was due to severe pelvic wall adhesions originating from previous surgery.

Although the surgery period and operators were not the same between LESS and conventional laparoscopic adnexal surgery, this study shows that LESS for large benign adnexal cyst is feasible. There were no differences in patient’s age, BMI, post-operative hospital stay, total surgical time, and estimated blood loss between LESS and conventional laparoscopic groups. However the present results showed that the removal of resected adnexal mass out of abdomen in LESS was much easier and more rapid than that of conventional laparoscopy (six min vs 13 min, p-value < 0.01).

There are many reports comparing LESS adnexal surgery with conventional laparoscopic adnexal surgery [7, 9, 11]. These studies demonstrated that the surgical outcomes of LESS for adnexal lesions, such as total surgical time, blood
Laparoendoscopic single-site surgery (LESS) for large benign adnexal tumors: one surgeon’s experience over one-year period

loss, and operative complications did not differ from those of conventional laparoscopic surgery. However, no study compared the specimen removal time between the two types of surgery. They all compared total surgical time and speculated that specimen removal could be easier through a larger umbilical port. Accordingly, the authors analyzed the recorded film of previous conventional laparoscopy and indirectly compared the specimen removal time with that of LESS and discovered that removal of specimen was more rapid in LESS.

The removal of resected specimen consists of two parts: the first part consists of insertion of specimen into the laparoscopic pouch and the second part consists of morcellation of the adnexal specimen through port site. The first part usually takes only small portion of specimen removal time. The longest removal time in these LESS was ten minutes due to the longest first part time (4.5 minutes) and the longest second part time (5.5 minutes) owing to heavy cyst wall from the largest diameter (26 cm) with combined dermoid cyst content. (Figures 1, 2) In the remaining nine LESS cases, the first part did not take no more than two minutes and the second part no more than five minutes. From this experience, the authors can speculate that the second part of specimen removal comprises most of the specimen removal time in large adnexal cyst. Therefore removal through a larger umbilical incision site would be much easier and take less time than through a conventional smaller port site.

The authors generally used a rigid 30-degree, five-mm laparoscope with long shaft. Using this laparoscope, they could minimize the collision between the endoscope and surgical instruments compared with the endoscope and the surgical instruments compared with using 0-degree laparoscope. The relatively short surgical time in this study compared to others’ (ten minutes less time on average ) is assumed to be that the authors performed salpingo-oophorectomy in all ten cases instead of time-consuming cystectomy. In addition, they were already on the learning curve when they performed this surgery.

Laparoscopic drainage of large ovarian cysts followed by salpingo-oophorectomy may be considered to be a controversial approach to managing large ovarian cysts because of the possibility of occult malignancy. The risk of spillage during laparoscopic excision of large cyst still exists even after laparoscopic drainage of the ovarian cyst. Some authors concluded from their studies that there was no difference in the five-year survival rate between the patients who had intact removal of the cyst compared with those with intraoperative cyst rupture in Stage I epithelial ovarian cancer patients [16, 17]. However, the recent reports advocate the impact of perioperative capsule rupture on survival. Improvement was observed in the five-year disease-free survival for Stage I epithelial ovarian cancer patients without intraoperative tumor rupture compared with those with tumor rupture [18]. Although the significance of this spillage in cases of malignant cysts is controversial, the importance of patient selection and capability of the surgeon to managing this situation should be stressed before applying the current result. Based on the previous reports and on the present study, however, the authors believe many patients with large benign adnexal tumors could share the benefits of minimally invasive surgery with surgeons’ careful surgical evaluation and careful patient’s selection on the basis of no evidence of gynecologic malignancy on imag-

Figure 1. — Magnetic resonance imaging of large ovary cyst measuring 26 cm (T2-sagital).
Figure 2. — Pictures illustrating LESS. A) Purse-string sure to the cyst wall; B) Evacuation of the cyst content; C) Placing of resected specimen into the Lapbag; D) Morcellation of the specimen.
ing studies such as computed tomography (CT), magnetic resonance imaging (MRI), and/or positron emission tomography CT (PET-CT).

There are many limitations in this study due to the small number of patients enrolled, its retrospective nature, and different surgeons and varying surgery periods between LESS and conventional laparoscopy. Although the surgical skill is different between surgeons, the authors do not believe that simple removal of specimen through port site would employ experienced skills.

Despite shortness of retrospective and indirect comparison of this study, this study adds to accumulating evidence supporting the feasibility and safety of LESS for large benign ovarian cysts and shows the removal of resected specimen is easier than the conventional laparoscopy. Considering the above, the authors believe LESS may be the first choice when it comes to large benign cystic adnexal tumors.

References


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Total pelvic floor reconstruction versus transvaginal hysterectomy for pelvic organ prolapse: a retrospective cohort

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Summary

Aims: To evaluate the surgical outcomes following total pelvic floor reconstruction (TPFR) and transvaginal hysterectomy (TVH).

Materials and Methods: This was a retrospective cohort study of all patients who underwent TPFR or TVH repair for pelvic organ prolapse (POP) between January 2005 and January 2011. A total of 251 consecutive women were evaluated prior to, and at two, six, and 12 months after surgery. Anatomical, symptoms, and quality of life were measured using the Pelvic Organ Prolapse Quantification system (POP-Q) and pelvic floor distress inventory (PFDI). The surgical outcomes were compared between groups using Student’s t-test and ANCOVA tests (p < 0.05). Results: Of the 251 patients, 129 had a total pelvic floor reconstruction (TPFR group), and concomitant modified transobturator inside-out tension-free urethral suspension (TVT-O) was used in pelvic floor dysfunction patients with stress urinary incontinence. The patients that underwent vaginal hysterectomy surgery (TVH group) were 122. At two, six, and 12 months, respectively, 12.40% (TPFR group) and 18.85% (TVH group) of the patients were lost to follow-up. There were no significant differences between TPFR group and TVH group for all preoperative variables (p > 0.05). The TPFR patients had significantly lower operation time, blood loss, anus exhaust time, remaining catheter time, and the length of stay in hospital (p < 0.05). Postoperatively, the recurrence rate in TVH group was higher than that of TPFR group after surgery at six and 12 months (p < 0.05). The PFDI score was significantly different between the groups. Conclusions: The short-term clinical results suggest that the two surgeries are safe and effective in treating female POP. The patients’ quality of life was improved, but TPFR technique was more conspicuous for treating POP.

Key words: Total pelvic floor reconstruction; Transvaginal hysterectomy; Quality of life; Prolapse.

Introduction

Pelvic organ prolapse (POP) is a relatively common condition, and around 50% of parous women will have prolapse with symptoms. Lousquy et al. have estimated that 30.8% women at 70 years of age will have pelvic problems and approximately 10% women will need a POP repair in their lifetime [1]. A recently updated Cochrane review on surgery for POP showed that total pelvic floor reconstruction with its excellent success rates of 84–99% was associated with a lower vault prolapse and dyspareunea recurrence rate than vaginal sacrospinous colpopexy [2]. Abdominal sacrocolpopexy, which is considered the gold standard for apical prolapse repair, has a higher success rate but at the cost of including higher morbidity and longer operative and recovery time vaginal procedures. The difference in success is attributed to the use of synthetic material. The polypropylene gynecological mesh has been used as fascial strengthening, with tension-free technique, reducing the possibility of relapse, with attempts to merge the benefits of both approaches for prolapse repair [3]. Current opinion suggests that transobturator vaginal tape from inside to outside or tension-free vaginal tape obturator from inside to outside (TVT-O) was described for the first time in 2003 [4].

Apical support of the vagina is the hallmark of pelvic floor surgery and is required to prevent recurrence in other compartments. Furthermore, vaginal hysterectomy at the time of POP surgery has been traditionally recommended, although it remains unclear as to whether this is required or prevents recurrence [5]. Several studies investigating the polypropylene system have reported high short-term success rates, with few intra- and postoperative complications [6]. Because total pelvic floor reconstruction (TPFR) and transvaginal hysterectomy (TVH) have not been directly compared in the literature and it is currently unclear as to the appropriate management of a patient scheduled for total pelvic floor with a uterus in situ. The aim of this retrospective cohort study was to determine whether a difference in peri- and postoperative outcomes existed between patients that underwent TPFR and TVH.

Materials and Methods

This was a retrospective cohort study comparing outcomes for patients that had undergone either TPFR or TVH between January 2005 and January 2011 at the Second People’s Hospital of Changzhou, Nanjing Medical University, Changzhou, Jiangsu Province, China, with 251 women aged from 48 to 82 years. Inclusion criteria were as follows: women with stress urinary incontinence lasting for at least two years as diagnosed by clinical evaluation and urodynamics and age > 40 years. Exclusion criteria were as follows: overactive bladder and mental disease, previous surgical and/or pharmacological treatment of POP, re-dominant or isolated urge incontinence, and serious contraindications to surgical procedures. Patients were excluded from this retrospective analysis if only the anterior or posterior portion of the polypropylene kit was placed, rather than the total vaginal...
Patients were also excluded if follow-up after surgery was less than 12 months. Estrogen cream was administered for one or two weeks preoperatively for patients with thin vaginal wall. They agreed to buy a single set for operation, and met the inclusion criteria. All patients were operated on at the Department of Obstetrics and Gynecology, Hospital of Second People’s Hospital of Changzhou, Nanjing Medical University (by the same surgeon).

The preoperative assessment consisted of an in-depth interview covering anthropometric data, medical, surgical and obstetric history, symptoms of prolapse, pelvic pain, voiding and defecatory dysfunction, and the impact of the prolapse on daily life (especially sex life). Prolapse was assessed using the International Continence Society (ICS) Pelvic Organ Prolapse Quantification (POP-Q) system. Additional studies included a full urodynamic work-up, a standing stress test, uroflow, and postvoid residual. All patients who were consulting for symptomatic genital pelvic organ prolapse (Grade II and above), who had been informed of their inclusion in the registry and of the technique used, and who had agreed to regular monitoring for 12 months, were included.

Surgical technique. The patient was placed in the lithotomy position and her thighs flexed approximately at 90 degrees. Anesthesia was either epidural or at times general. Antibiotics were administered preoperatively and postoperatively for 24 hours (one gram of cefalozine). A bladder catheter and a vaginal pack were left in place for 24 hours. Post-void residual (PVR) was measured after catheter removal. The technique involves: implantation of a large sheet of polypropylene mesh (10.0 x 3.5 cm) (Figure 1) between the urinary bladder and the vagina in case of cystocele (Figure 2), or a mesh (10.0 x 4.5 cm) was placed between the vagina and the rectum in case of rectocele (Figure 3). The surgical technique has been described elsewhere. In some patients, a modified transobturator inside-out tension-free urethral suspension (TVT-O) procedure was performed through a separate incision at the mid urethra after the mesh procedure for proper positioning and to avoid displacement. The TVT-O procedure was performed according to De Leval [7]. TVH was also described by Ethicon Women’s Health and Urology [8].

Patients were scheduled for their postoperative visit at two, six, and 12 months after surgery. Data were entered for the follow-up preoperative basic characteristics: age, body mass index (BMI), number of vaginal deliveries, history of prior incontinence surgery, history of prior prolapse surgery, sexual life, POP quantification (POP-Q) vaginal measurements and stage of vaginal prolapse [9]. The following operative assessments all included: operative time (from beginning to end of anesthesia), estimated blood loss, length of stay in hospital recorded in days, and intraoperative complications. The follow-up postoperative information consisted of: POP-Q vaginal measurements and pelvic floor distress inventory short form 20 (PFDI-20) score. These assessors were not blinded to the type of surgery performed. The aforementioned information was compiled into a single de-identified spreadsheet. A second individual performed a quality assessment of the data collected from the charts of every tenth patient.

Results

A total of 251 charts were identified from January 2005 to January 2011 at the Second People’s Hospital of Changzhou, Nanjing Medical University, Changzhou,
Jiangsu Province, China. Figure 4 depicts patient distribution for each surgical group. All patients attended the month 12 postoperative appointment but 12.40% (TPFR) and 18.85% (TVH) of the patients were lost to follow-up. (Figure 4). The final analyses included 212 patients, with 113 in the TPFR group and 99 in the TVH group (Figure 4). The baseline characteristics of the patients’ are shown in Table 1.

### Characteristics of the operation

Characteristics of the operation and complications were compared between the two surgical groups and are summarised in Table 2. There were significant differences between the two groups at the time of surgery, blood loss, anus exhaust time, remain catheter time, and length of stay in hospital. Immediately postoperatively, the following complications were observed: In the TPFR group 3 patients had a hematoma and ten patients operated for prolapse developed acute urine retention that resolved after three weeks of intermittent self-catheterization. Two patients presented with urinary tract infection after a total reconstruction repair. Three patients required wide mesh excision for reoperation. Patients undergoing vaginal hysterectomy had slightly more complications (Table 2).

### Postoperative outcomes

Postoperative data analyses comparing postoperative POP-Q measurements and stage of prolapse between the TPFR and TVH groups are summarized in Table 3, respectively. TPFR patients were found to have a significantly higher curative rate than patients that underwent TVH. There were also significant differences for other POP-Q measurements and grades after the surgery at six and 12 months follow-up.
months. However there were no significant differences between the surgical groups for POP-Q stage of prolapse at two months after surgery. Secondary outcome analyses also showed significant differences between the two groups in relation to the PFDI scores (Table 3).

Discussion

Symptomatic pelvic organ prolapse has long been considered an indication for hysterectomy. Transvaginal hysterectomy has been the traditional surgical treatment of uterovaginal prolapse for many years. However, vaginal hysterectomy alone often fails to address the underlying deficiencies in pelvic support that cause uterovaginal prolapse. Indeed, Maher had reported that there were up to 40% of women undergoing hysterectomy subsequently present with vaginal vault prolapse [10]. An increasing number of women desire uterine preservation and alternatives to hysterectomy. As the main reasons for opting to preserve their uterus, women most often cite the feeling that the uterus is an integral part of them, provides a sensation of wholeness, and serves a reproductive function, the uterus and cervix may have an important role in sexual function and wellbeing. Management of uterine prolapse in women who do not wish to undergo hysterectomy remains a challenge. In this report we compare the two surgical techniques. The advantages of this approach include better haemostasis, decreased hospital stay, reduced blood loss, less anus exhaust time, less more rapid recovery and smaller incisions.

Over the last 70 years, there were several surgical procedures for uterine preservation have been developed, including Manchester repair, sacral hysteropexy, sacrospinous hysteropexy and various laparoscopic uterine suspension techniques [11]. In 2008, Jia et al systematically reviewed that any type mesh significantly reduced objective prolapse recurrence rates compared with no mesh [12]. Fatton et al. reported the results of 110 women who underwent a new tension-free vaginal mesh repair of genital prolapse using polypropylene mesh. In that retrospective multicentric study, the authors reported one bladder injury, two hematomas, five mesh exposures and a failure rate of 4.7% with minimum follow-up of three months [13]. However, most of the published reports are small retrospective studies and the success rates reported vary widely. These studies show overall success rates of 77–97% [14–17], with slight variations when individual compartments are separately reported. From a surgical technique standpoint, it is becoming more evident that mesh implantation can be done safely if the surgical technique is standardized.

This study demonstrated that there were significant differences in operative variables and postoperative outcomes between TPFR and TVH groups. Follow-up beyond 12 months could therefore reveal more significant differences between the surgical groups for POP-Q stage of prolapse at two months after surgery. Secondary outcome analyses also showed significant differences between the two groups in relation to the PFDI scores (Table 3).

Table 2. — Characteristics of the operation and complications compared between the two surgical groups.

<table>
<thead>
<tr>
<th></th>
<th>TPFR* (n=113)</th>
<th>TVH* (n=99)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Operative time (minutes)</td>
<td>52.3±15.3</td>
<td>115.3±11.3</td>
<td>p &lt; 0.000</td>
</tr>
<tr>
<td>Anesthesia:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Epidural anesthesia</td>
<td>17 (15%)</td>
<td>12 (12%)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>General anesthesia</td>
<td>96 (85%)</td>
<td>87 (88%)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>147.8±30.5</td>
<td>212.1±43.5</td>
<td>p &gt; 0.001</td>
</tr>
<tr>
<td>Anus exhaust time(min)</td>
<td>26.2±4.7</td>
<td>39.3±9.8</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Remain catheter time(min)</td>
<td>80.3±12.7</td>
<td>120±18.1</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Mean hospital stay (days)</td>
<td>5.5±0.9</td>
<td>8.1±1.2</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Number of complications:</td>
<td></td>
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</tr>
<tr>
<td>Bleeding (&gt;300ml)</td>
<td>0</td>
<td>6</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Bladder or rectum damage</td>
<td>2</td>
<td>6</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Hematoma, ecchymosis</td>
<td>3</td>
<td>4</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>5</td>
<td>10</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Acute urine retention</td>
<td>10</td>
<td>12</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Reoperation</td>
<td>3</td>
<td>2</td>
<td>P&gt;0.05</td>
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</tbody>
</table>

Table 3. — Postoperative data analyses comparing postoperative POP-Q measurements and stage of prolapse between the TPFR and TVH groups.

<table>
<thead>
<tr>
<th>Post-operative Grade n.:</th>
<th>TPFR* (n=113)</th>
<th>TVH* (n=99)</th>
<th>p-value</th>
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<tr>
<td>Month 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>113</td>
<td>98</td>
<td></td>
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<tr>
<td>I</td>
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<tr>
<td>II</td>
<td>0</td>
<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Month 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>109</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
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</tr>
<tr>
<td>II</td>
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<td>3</td>
<td>p &gt; 0.05</td>
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<tr>
<td>III</td>
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<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Month 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>105</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>13</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>4</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>IV</td>
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<td>0</td>
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<tr>
<td>SUIa</td>
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<td>Month 2</td>
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<td></td>
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<tr>
<td>Cure</td>
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<td>59</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Recurrence</td>
<td>3</td>
<td>5</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Month 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure</td>
<td>76</td>
<td>51</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Recurrence</td>
<td>3</td>
<td>13</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Month 12</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cure</td>
<td>74</td>
<td>46</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Recurrence</td>
<td>5</td>
<td>18</td>
<td>p &gt; 0.05</td>
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<td>PFDIb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8.6±2.1</td>
<td>12.7±4.3</td>
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<tr>
<td>Month 6</td>
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<td></td>
<td></td>
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<tr>
<td>9.7±3.0</td>
<td>14.4±5.5</td>
<td>p &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Month 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0±2.3</td>
<td>13.2±4.4</td>
<td>p &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

a Stress urinary incontinence. b Pelvic floor distress inventory.
between the two procedures for both PFDI score and apical stage of prolapse. The follow-up period here was only 12 months and that longer-term assessment is warranted. The findings of this study may have been limited by several factors. As this study involved the retrospective collection of data, the two surgical groups were heterogeneous and the comparative analyses may also have been limited by the sample size. Given the observed variability and number of patients in each group, the sample collected had sufficient power to detect difference in the outcome measure. Indeed, with regards to this comparison, some significant differences were found between the two surgical groups, but a limited sample size may have impacted the ability to demonstrate significant differences for other surgical outcomes. There may be several explanations for such a lower complications in the TPFR group. First of all, the dissection of the vaginal wall was performed not too thin. Second, positioning the mesh without tension, displacement, and overlap. In addition the total pelvic floor reconstruction is a technically challenging procedure that requires specific operative high skills and extensive experience form the surgeon.

Conclusion
The results of 12-month follow-up showed that the two surgeries are safe and effective surgical outcomes for treating female POP. The total pelvic floor reconstruction has more advantages in terms of safety, feasibility, and functional outcomes. The TPFR group showed less incidences of postoperative complications than the TVH group.

References
Study of an antiangiogenesis gene therapy with endostatin on endometriosis in the nude mouse model

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Summary

Objective: This work aims to investigate the treatment effect of endostatin (ES) in the nude mouse model with endometriosis (EMs). Materials and Methods: Recombinant adenovirus Ad-ES carrying ES gene was constructed. Apoptosis of ECV-304 cells induced by Ad-ES was observed. The nude mouse model with EMs was established by subcutaneous implantation. After the local focus was injected with the Ad-ES, the Ad-Track or the physiologic saline, respectively, the morphological features of ectopic focuses were observed under microscopy. The microvessel densities (MVD) and the apoptosis were detected. Results: The recombinant Ad-ES was successfully constructed. Apoptosis of ECV 304 cells could be induced by Ad-ES. The nude mouse model with EMs was successfully established by subcutaneous implantation. There were statistical differences in the volumes of endometriotic lesions and MVD after treatment by Ad-ES compared with those in the other two control groups \((p < 0.05)\). Apoptosis of the cells were significantly increased in the group of treatment by Ad-ES compared with those of the two control groups. Conclusion: ES could induce ECV 304 cells to apoptosis and inhibit the growth of endometrium in the nude mouse model. The findings suggest that antiangiopoeisis may be used as a promising therapy for the treatment of EMs.

Key words: Endostatin; Endometriosis; Antiangiogenesis; Nude mouse; Apoptosis.

Introduction

Endometriosis (EMs) is a common disease in women of childbearing age and has showed an increasing trend of the incidence rate. The main clinical symptoms include dysmenorrheal, chronic pelvic pain, and infertility. Although it is a benign disease, EMs has some similar tumor malignant features such as local invasion, distant organ involvement, and multiple lesions. At present, as the etiology and pathogenesis of EMs is unclear and lack effective method based on cause of disease as target, treatment of EMs by drugs or surgical therapies has limited effect and is easy to relapse \([1-7]\). Therefore, tracking the latest research progress of the EMs pathogenesis and finding a new therapeutic approach have become an urgent need for the current study on EMs. Previous studies suggested that biological behavior of ectopic endometrial tissue (transferring, planting, growing) similar to tumor metastasis may be critical for the pathogenesis of EMs \([8-14]\). As the formation and growth of endometriosis focus must rely on the formation of new blood vessels, angiogenesis mechanisms has been recognized as an important pathogenesis of EMs. Some scholars attempted research of antiangiogenic therapy on EMs using angiogenesis inhibitor and good results were preliminarily showed. Therefore, antiangiogenesis would be an effective way for the treatment of EMs. Endostatin (ES) could specifically induce apoptosis of vascular.

Therefore, ES is a potent inhibitor for endothelial cells which only specifically inhibited the neovascular endothelial cells but no significant inhibitory effect for the non-endothelial origin cells and the normal mature vascular endothelial cells. ES antiangiogenic therapy has entered into Phase II in clinical studies, and studies about ES protein or gene antiangiogenic therapy on animals has been performed. However, ES mainly used in cancer treatment, there have been no reports about treating EMs by ES. With characteristics of unstable and difficult to prepare and apply, ES proteins need to inhibit angiogenesis with the help of gene therapy \([15-20]\). In the present study, recombinant adenovirus with ES gene was constructed, apoptosis of the vascular endothelial cells was induced in vitro, and the inhibitory effect of ES to endometriosis lesions in the nude mouse models was further studied. The authors attempted to explore the feasibility and security of antiangiogenic gene treating EMs by ES, and find a convenient and economical new way with small side-effect to treat EMs.

Materials and Methods

Female BALB/c nude mice six to eight weeks old and weight of 18-20 g, five for one cage, were reared under class specific pathogen free (SPF) conditions. The mice were given sterile water and food, nutrient food like sunflower seeds and egg yolk were added per week. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Zhujiang Hospital of Southern Medical University.

The patients with uterine resection for benign disease between 41 and 45 years old were selected. The surgery was performed on the 20th to 22nd day of the menstrual cycle. Hormone therapy had not been given six months prior to the surgery, and no other complications and comorbidities were found. This study was con-
Infection efficiency

agarose gel after PCR amplification. ES DNA as a template, the primers and reaction conditions were and centrifuged at 8,000 r/min for ten minutes to obtain the Ad-

Ad-ES identification

The designed primers were listed as follows: the upstream primer P1 (5’-GGGTACCATGGCTCGTACCATG-3’), the downstream primer P2 (5’-GCTAGAATTCTTGGAGGCAGT-3’). Primers P1 and P2 were randomly designed according to ES sequence. pShuttle-ES was taken as a template, ES fragment (about 650 bp) was isolated by PCR amplification, double digestion Kpn I and Xba I were connected with pAdTrack-CMV to obtain the recombination shuttle plasmid pAdTrack-ES. Recombinant adenoviral plasmid pAd-ES was selected by homologous recombining pAdEasy-1 and pAdTrack-ES linearized by Pme I in competence cells BJ5183. PAd-Track without exogenous gene was prepared by the same method as a negative control.

The pAd-ES linearized by Pac I transfected 85-90% confluent AAV293 cells. The cells were collected when they became round, suspended, and green fluorescent protein (GFP) expression was strong (approximately on the 14th day). After freezing (-80°C) and thawing (37°C) repeatedly, the supernatant (recombinant adenovirus Ad-ES) was collected to purify by cesium chloride density gradient centrifugation. The end point dilution method was adopted to determine the virus titer (Titer), which was calculated according to the Spearman-Karber method: 

\[
T (\text{pfu} / \text{ml}) = 10 (x + 0.8),
\]

X indicated the sum of lesions rate for each dilution.

Ad-ES was sent for DNA sequencing. Ad-ES was added to proteinase K at 60°C for two hours, boiled at 100°C for ten minutes, and centrifuged at 8,000 r/min for ten minutes to obtain the Ad-ES DNA as a template, the primers and reaction conditions were the same as 1.2.1. Electrophoretic detection was performed on 1% agarose gel after PCR amplification.

Infection efficiency

ECV-304 was inoculated in 6-hole plates by 1×10⁶ holes. Virus solutions with 1, 10 and 100 multiplicity of infection (MOI) were respectively used to infect. The percentage of GFP-positive cells was discarded. The nuclear staining and apoptotic bodies were observed by fluorescence microscope.

Preparation of the nude mouse models

A dose of 0.1 ml/10 g of chloral hydrate was used to anesthetize the nude mice. After fixation, the abdominal skin of the nude mice was cut to a 0.5 cm incision, endometrial debris were implanted subcutaneously, sutured by No. 1 thread, and covered by sterile tape after disinfection. Nutrition was attended after the operation, and wounds and the living conditions of the nude mice were observed daily. The growth of the animals was inspected three times a week.

The morphological observation of the ectopic lesions by light microscope

Mice were sacrificed two weeks after injection. The subcutaneous lesions were removed and stained by hematoxylin and eosin (HE). The morphology of the ectopic lesions was observation under light microscope.

Growing suppression of the endometrial lesions by Ad-ES in nude mouse

Four weeks after the model preparation, the 30 nude mice implanted subcutaneously were randomly divided into the treatment group (Ad-ES group), the negative control group (Ad-Track group), and the control group (the normal saline group), the injection dose was 75μl for each mouse. From the second day after injection, the maximum diameter (a) and the maximum diameter (b) of the subcutaneous lesions were measured once every three days.

With regards to tumor volume, the lesion volume could be calculated by the formula of 

\[
V = \frac{1}{2}a \times b^2.
\]

The calculation of the growth inhibition for lesions could be referred to the tumor inhibition rate (the tumor inhibition rate = (1 - the volume change of the experimental group / the volume change of the control group) × 100%).

Mice were sacrificed two weeks after injection and the lesions were removed. Some lesions were stained by HE and histological forms were observed under light microscope. The other lesions were stained by TUNEL and immunohistochemical CD34.

The detection of apoptosis were performed according to the TUNEL kit instructions. Results were determined by the standard classification of brown particles found in the cytoplasm. Negative (-) indicated the cells without coloring or positive cells < 5%, weakly positive (+) indicated that the cells were pale yellow color, and the percentage of the positive cells was 5-25%, positive (+ +) indicated that the cells were dark yellow and the percentage of positive cells was 25-50%, strong positive (+ + +) indicated that the cells were brownish yellow, and the number of positive cells > 50%. The procedure was carried out by two pathologists under a blinded condition.

A microvesSEL could be clustered by CD34 immunohistochemical stained pathological slicing and the brown endothelial cells or endothelial cell with clear boundaries in the adjacent capillaries, smooth muscle or other interstitial connective tissue components. The manner in which microvesSEL is quantified is called microvesSEL density counting method. Lumens were not used as indicators of judging microvascular structure. The red blood cells were regarded as a microvesSEL not as vascular lumen because of their branched and non-complete independent structure. The procedure was carried out by two pathologists under a blinded condition.

Statistical methods

SPSS 12.0 software was used to analyze the results, the data were indicated by ± S, one-way analysis of variance was used to
compare the average of the groups, repeated measures analysis of variance was used to compare the measurement data of each group before and after the experiment, and a $p < 0.05$ indicated that the variance was significant.

Results

The construction and packaging of adenovirus

The expected ES fragment (about 650 bp) was amplified by PCR using Psuttle-ES plasmid as template and P1 and P2 as primers (Figure 1). Sixteen hours after the recombinant adenovirus plasmid PAD-E transfecting AAV293 cells, the fluorescence was increasing and brightening gradually when the expression of GFP could be seen under a fluorescence microscope. Five to nine days after transfection, the cells swelled and rounded, the antennae gradually disappeared, the fluorescence non longer increased, and partial cells suspended and changed to beaded form. These morphological aspects indicated the formation and amplification of the virus. About 14 days after the transfection, a majority of AAV 293 cell bodies shrank and suspended (Figure 2). The titers of Ad-ES and Ad-Track were determined as $2.06 \times 10^{10}$ pfu/ml and $1.73 \times 10^{10}$ pfu/ml.

Ad-ES identification

The sequenced and spliced results of the Ad-ES corresponded to the Endostatin gene of human beings published in the GeneBank, without sequence mutations.

The Ad-ES DNA was identified by PCR amplifying the expected bands (about 650 bp), while there was no amplification products of the Ad-Track appeared, indicating that Ad-ES was constructed successfully (Figure 3).

Figure 1. — Amplification of gene encoding ES. 1: ES; 2: Water (Control; M: DL2000 DNA Marker).

Figure 2. — Linear pAd-ES was transfected into AAV293 cells to package adenovirus. A: three days after transfection; B: six days after transfection; C: eight days after transfection; D: 14 days after transfection.
**Apoptosis**

Detected by the flow cytometry, apoptotic characteristic sub-diploid peak appeared 48 hours after Ad-ES infecting the ECV-304 cells, while there was no such phenomenon in the negative control group of Ad-Track infecting the ECV-304 cells (Figure 4A, B), suggesting that ES ECV-304 cells could be induced apoptosis by ES in vitro.

Stained by Hochest 33258, the apparent generation of the apoptotic bodies could not be seen 24 hours after AD-ES infecting the ECV-304 cells, and most of the cells were still in the state of chromosome condensation. Forty-eight hours after infection, a large number of intracellular apoptotic bodies appeared (white arrow, Figure 4C, D), while there was no such phenomenon in the
negative control group. These results implied that the ECV-304 could induce apoptosis as Ad-ES continued acting.

**Preparation of the nude mouse models**

The nude mice healed three to five days after the abdominal incision, the lesions were implanted, then the edema faded and narrowed gradually. Nodular protruding structure with size of $2.5 \times 2.5 \times 2.5$ mm formed on the 10th to 12th day and small blood vessels confirmed pathological endometrial tissue growth on the surface (Figure 5).

**The morphological observation of the ectopic lesions by light microscope**

After stained by HE, the growth of the endometrial stromal cells and glandular epithelial were observed under light microscope. The edges of the ectopic lesion tissues were continued by the muscle cell layer of mice. The phenomenon of mesenchymal cells invading the muscle layer was seen in some sections. Oval or round glands lumens were surrounded with flat or cuboidal epithelial cells, infiltrated by inflammatory cells, and interstitial cells in surroundings. The formation of new blood capillaries, proliferation of connective tissue, and degeneration of hyaline were seen around the lesions, which were similar to the performance of endometrial hyperplasia.

**Growing suppression of the endometrial lesions by Ad-ES in nude mouse**

The nude mice were sacrificed two weeks after injection, and the ectopic lesion volume was no different among the nude mice in each group before injection. The lesions of the Ad-Track negative control group and the normal saline control group continued to grow after the injection, but the growth rate was slower than that of the first four weeks after the model preparation. The growth of lesion volume in the Ad-ES therapy group was slow, and the lesion volume gradually reduced five days after injection (Figure 6).

The volume of ectopic lesions in nude mice was measured respectively two weeks before and after the treatment. According to the repeated measures analysis of variance, the lesion volume of the Ad-ES treatment group was lower than that of the Ad-Track negative control group and the normal saline control group, indicating significant differences ($F = 817.754, p = 0.000$). There was treatment interaction between the volume and the therapy methods ($F = 753.460, p = 0.000$), suggesting that the volume trend was different before and after treatment in different groups.

The solo effects were further analyzed, the results showed that the lesion volume change of the Ad-ES treatment group was significant ($p = 0.000$). The Ad-Track-negative control group and the normal saline control group

---

**Table 1.** — Comparison of volumes before and after injection between different groups (±S) (mm$^3$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Case (n)</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Total</th>
<th>$t/F$ value</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>10</td>
<td>30.0620 ± 1.62353</td>
<td>0.2230 ± 0.35299</td>
<td>15.1425 ± 15.34974</td>
<td>57.005</td>
<td>0.000</td>
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<tr>
<td>Negative control</td>
<td>10</td>
<td>29.4540 ± 1.76043</td>
<td>29.8280 ± 1.56288</td>
<td>29.6410 ± 1.63151</td>
<td>0.527</td>
<td>0.611</td>
</tr>
<tr>
<td>Blank control</td>
<td>10</td>
<td>30.0540 ± 1.47299</td>
<td>28.8380 ± 1.43321</td>
<td>29.4460 ± 1.54592</td>
<td>1.990</td>
<td>0.078</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>29.8567 ± 1.59281</td>
<td>19.6297 ± 14.01449</td>
<td>24.7432 ± 11.15243</td>
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<td>$F$ value</td>
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<td>0.462</td>
<td>1835.264</td>
<td>612.717</td>
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<tr>
<td>$p$ value</td>
<td></td>
<td>0.635</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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Table 2. — Comparison of TUNEL and MVD between different groups (± S).

<table>
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<th>Group</th>
<th>n</th>
<th>TUNEL</th>
<th>MVD</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>10</td>
<td>0.1500 ± 0.00667</td>
<td>3.1680 ± 0.53987</td>
</tr>
<tr>
<td>Negative control</td>
<td>10</td>
<td>0.1420 ± 0.00493*</td>
<td>5.1940 ± 0.61278*</td>
</tr>
<tr>
<td>Blank control</td>
<td>10</td>
<td>0.1440 ± 0.00516*</td>
<td>5.1780 ± 0.61226*</td>
</tr>
</tbody>
</table>

*p value = 0.000, Table 2.*

had no significant changes (p > 0.05). The lesion volume difference among groups before the treatment was not significant (p > 0.05, Table 1).

After treating for two weeks, the results of one-way analysis of variance showed that the apoptosis of the Ad-ES treatment group was higher than that of the AD-Track negative control and the normal saline control group (p = 0.000), the difference of the apoptosis in the Ad-Track negative control and the normal saline control group was not significant (p = 0.120). The microvessel density of the Ad-ES treatment group was lower than that of the AD-Track negative control and the normal saline control group (p = 0.000); there was no significant difference between physiological microvascular density of the Ad-Track negative control group, and the normal saline control group (p = 0.952, Table 2).

Discussion

Angiogenic mechanism has been recognized as an important pathogenesis for EMs. As ES is a potent antiangiogenic substances with unstable protein structure, gene therapy has been used to improve action [21-23]. One of the key aspects of gene therapy is to choose a safe and efficient gene vector. The adenovirus AdEasy-1 system in this study is a safe and efficient gene vector. With security, low genotoxicity, high infection rate, easy to breed, wide host, and E3 gene (coding the toxic products), the system would not be integrated into the chromosome of the host cell and no self-replicating owing to the knocking out of the E1 gene (controlling the transcription replication units) and E3 gene (coding the toxic products), the system would not lead to serious side-effects [24-33].

Nude mice were animals with functional deficit of T lymphocytes and two primary immunodeficiency characterization (no thymus and dysplasia of body hair). Their cell immunity was low, while the function of the B cells was normal and the activity of the NK cells was high. The serum immunoglobulin levels of the nude mice were low. The organization, genetics, and biochemical properties of the foreign tissue implanted in nude mice could be generally kept, and the sensitivity of the transplanted tissues to various drugs was not changed. Therefore, the nude mice were more ideal models animals to research EMs of human beings than other animals [34-40].

In this study, recombinant adenovirus Ad-ES with ES gene was successfully constructed, and nude mouse model of EMs was established by subcutaneous implanting human endometrium. The recombinant adenovirus Ad-ES successfully induced the apoptosis of vascular endothelial cells ECV-304 in vitro. The volume of the endometriosis lesions significantly reduced after local injecting recombinant adenovirus Ad-ES into the endometriosis lesions of the nude mice. Dyed by HE and observed under light microscope, glandular atrophy, partial uncompleted structure, and varying degrees of interstitial necrosis were seen. The apoptosis and microvascular density were further detected in the endometriosis lesions. The results showed that significant apoptosis occurred in the endometriosis lesions treated by recombinant adenovirus Ad-ES, and microvascular density in lesions dropped. Therefore, the morphological changes indicated that the apoptosis of vascular endothelial cells and inhibition of angiogenesis could be induced by ES to inhibit the growth of ectopic endometrial lesions in the nude mouse models. The anti-angiogenesis might be a new way to treat EMs.

References


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Introduction

Uterine myomas (UM) are the most common benign tumors of the female genital tract, affecting 20% to 30% of women during reproductive age and approximately 70% of women after 50 years [1]. Despite multiple risk factors (including genetic and hormonal mechanisms), related to the fibroids development and growth, their exact etiopathology remains unclear [2].

UM can be associated with abnormal uterine bleeding (AUB), pelvic pain or infertility because of submucous uterine myoma. The authors considered eligible: single G1 or G2 submucous uterine myoma, at least 0.5 cm ultrasound ‘myometrial-free-margin’ and two months GnRH pre-surgical treatment (myoma > three cm). Group A patients were treated by bipolar resectoscope and Group B by monopolar resectoscope. Primary endpoint was to compare the groups in term of complete or incomplete myomas resection (“second-step-procedure” rate). Secondary endpoint was to compare two treatments in term of surgical time and intraoperative complications rate.

Results: Group A (60 patients) and Group B (216 patients) were homogeneous for general features and myomas location but they differed for G2 type prevalence (73.3% vs 50.5%), mean myomas diameter (33.17 ± 11.93 vs 29.45 ± 9.63), and surgical time (29.43 ± 12.6 vs 23.2 ± 8.2 minutes). In Group A patients both G1 and G2 myomas were completely removed in single step without intraoperative/ postoperative complications; in Group B surgical outcomes of G1 myomas were similar to those of Group A, while G2 myomas required procedure termination in 12% of cases because of light electrolyte disturbance (22 cases) and severe iponatremia in four cases. All intraoperative complications occurred when procedure time exceeded 30 minutes and when myomas diameter was greater than 37.5 millimeters. Conclusion: in the era of mini-invasive surgery, hysteroscopic approach by bipolar device should be considered as a useful, safe, and large scale feasible procedure for submucosal myoma treatment, particularly when G2.

Key words: Uterine submucosal myoma; Hysteroscopic myomectomy; Monopolar energy; Bipolar device; Surgical outcome.
Material and Methods

In the period from January 2011 to December 2013, a multicenter observational case-controlled study was conducted on premenopausal women affected by submucous myoma. All patients underwent operative hysteroscopy because of menorrhagia, pelvic pain or infertility. In order to confirm the previous ultrasound suspicious for sub-mucous myoma, all patients received preoperative outpatient hysteroscopy to define its number, site, size and type.

The authors considered eligible for the study patients with: single grade G1 or G2 submucous myoma, estimated ultrasound ‘myometrial free margin’ at least 0.5 cm and two-months- GnRH pre-surgical treatment when myoma was greater than three cm in diameter.

Group A (case group) included patients sent to Endoscopic Unit of University of Cagliari and treated by bipolar resectoscope and Group B (control group) patients sent to Endoscopic Unit of University of Padua and treated by monopolar resectoscope.

In Group A, all procedures were performed using bipolar resectoscope and isotonic electrolyte-containing solutions as distension medium, while in Group B, surgical procedures were performed by monopolar resectoscope and hypotonic distention medium (1% glycine, 1% mannitol in 1,000 ml solution). All cases were performed in operative setting using unconscious sedation for G1 and subarachnoidal anesthesia in G2 type myoma.

For all cases, the authors collected data regarding general features (age, BMI, and parity), preoperative characteristics (surgical prescription, hysteroscopic myomas’ grade, diameter, site), intraoperative outcomes, and complications (surgical time, fluid input, and output balance in monopolar procedures, complete or incomplete myomas resection, uterine perforation, excessive bleeding, fluid overload, and postoperative outcomes (hospital recovery length and necessity of second step procedure).

In both groups, all procedures were performed by same skilled Surgeons (S.A. in Group A and P.L. in Group B) using a slicing technique in case of G1 myoma and a “enucleation in toto” technique in case of G2 myoma.

Primary endpoint of the study was the comparison between two groups in term of complete or incomplete myomas resection and “second step procedure” rate. Secondary endpoint was the evaluation of surgical time and intraoperative complications rate during monopolar versus bipolar procedure.

Statistical analysis was performed by SPSS software for Windows version 19, using parametric and non-parametric tests, when appropriate. Continuous data were tested with the t test, when appropriate. The results obtained from the data collection were expressed in absolute numbers, percentages for discrete variables, and in means ± standard deviations for continuous variables. The statistical significance was defined as p < 0.05.

Results

In considered interval time, the authors collected data regarding 276 eligible patients: mean age was 41.4 ± 6.38 years and mean BMI was 24.33 ± 4. Among them, 108 were nulliparous (39.13%) and 168 were primiparous or multiparous (60.87%). Surgical myoma removal was required because of menorrhagia in 74.6% of cases (206 patients), pelvic pain in 13.4% (37 cases), and infertility in 12% (33 cases).

In preoperative work-up, 44.6% of myomas resulted grade 1 type (123 cases) while 55.4% were G2 (153 cases).

Myomas were sited in anterior uterine wall in 29.3% (81 cases), in posterior one in 36.2% (100 cases), in lateral one in 12% (33 cases), in uterine fundus in 14.9% (41 cases), in cornua in 5.1% (14 cases), and in uterine isthmus in 2.5% (seven cases). Mean diameter of removed myomas was 30.26±10.2 mm.

Group A included 60 patients while Group B 216 patients. The two groups were homogeneous for general features. Between the groups, significant statistical differences were found in Group A compared to Group B in terms of prevalence of G2 type myoma (73.3% vs 50.5%) (p < 0.001), mean myomas diameter (33.17±11.93 vs 29.45±9.63) (p < 0.01), but no differences were found in relation to myomas location.

In Group A patients mean surgical time was 29.43 ± 12.6 minutes compared to the 23.2 ± 8.2 in Group B (p < 0.01).

In Group A both G1 and G2 myomas were completely removed in single step and without intraoperative and postoperative complications: all patients were discharged six hours after procedure.

Similarly to Group A, in Group B G1 myomas removal was not burdened by intraoperative and postoperative adverse outcomes (p: n.s.) while G2 myomas excision required procedure interruption in 12% of cases (26 patients): unbalanced fluid input/output >1,500 ml caused light electrolyte disturbance in 22 case and severe hyponatremia in four cases (three cases without neurological symptoms and one case with suspicet of cerebral oedema, requiring recovery in intensive Care Unit) (p < 0.05).

A surgical second step was scheduled for these 26 patients in aim to complete surgical procedure. No other intraoperative and postoperative complications occurred and all patients were discharged six hours after treatment.

In Group B patients with G2 myomas, data stratification showed that all intraoperative complication occurred when procedure time exceeded 30 minutes and when myomas’ diameter was greater than 37.5 millimeters (Figures 1 and 2). Nevertheless, the complications rate was too small to be meaningfully compared with myomas location.

Discussion

Uterine fibroids were detected in 25–40% of women presenting AUB and concomitant pelvic pain and infertility [15]. Although a direct cause–effect relationship has not been completely established, observational studies suggest that shrinkage or removal of any identified uterine fibroids is effective in alleviating menstrual bleeding abnormalities, while data about pelvic pain resolution and fertility restore are still inconclusive [16-19].

Hysteroscopic myomectomy may be sometimes a highly complex procedure and its real feasibility must be thoroughly preoperatively evaluated [9].

Despite that office investigative hysteroscopy represent the gold standard in the myoma assessment (evaluating the percentage of intracavity protusion, its localization and
Monopolar versus bipolar device: safety, feasibility, limits and perioperative complications in performing hysteroscopic myomectomy

From the first introduction of monopolar hysteroscopic myomectomy, in the last two decades this procedure has been affirmed as a gold standard technique in mini-invasive conservative surgical treatment of submucosal myoma. Monopolar energy requiring non-electrolytic solutions represent the “Achilles’ heel” of this ideal endoscopic surgery which is potentially fatal in case of massive overload may lead to hyponatremic encephalopathy and brain edema: even a minimal glycine absorption could cause a borderline electrolytes disturbance responsible of transient blood oxygen desaturation, hypercapnia, coagulopathy, as well as postoperative hyperammonemia from oxidative deamination of the amino acid glycine [20].

These unpleasant intraoperative complications are responsible of most procedure interruption, incomplete myoma removal, and consequently a second surgical step scheduling [9]. The aforementioned adverse effects and the risk of tissue damage (due to thermal energy spread particularly in case of G2 myomas with low myometrial free margin), may influence surgeon’s performance, inducing him or her to reduce the surgical time as soon as possible and to opt for complete removal in a following step. In fact in the present study, the mean surgical time results significantly lower in monopolar compared to bipolar procedures. Despite surgical time does not represent an exclusive risk factor for distension fluid adsorption, the present data showed that fluid overload always occurred when procedure time exceeded 30 minutes.

Certainly, myoma size and myometral depth (usually associated with low myometrial free margin) represent a known risk factor: their role in increasing both surgical time and intraoperative complications (such as early procedure interruption and thermal damage due to monopolar energy spread) must not be underestimated.

In agreement with previous evidences, the present data showed that 12% of G2 myomas larger than 37.5 mm and treated by monopolar electrode were postponed to a following step to avoid severe intraoperative complications. On the contrary, all myomas were enucleated in toto by bipolar device in a single step procedure without intraoperative and postoperative complications.

Existing evidences regarding bipolar surgical devices demonstrated a lower peripheral thermal tissue damage: this advantageous property results in a higher surgical usefulness and safety (particularly in cases with minimal tissue thickness and neighboring damageable organs) compared to monopolar energy equipment [9, 20-22].

In addition, the use of isotonic saline solution gives to the surgeon an additional operating time, which is useful in a larger fluid deficit, and higher surgical difficulty cases.

In the era of mini-invasive surgery, hysteroscopic bipolar approach for submucosal myoma treatment should be considered as a useful, safe, and large-scale feasible procedure, particularly when G2. Innovative non-surgical procedures (such as magnetic resonance-guided focused ultrasound myomectomy) applied with interesting outcome have been proposed to further reduce invasiveness, but they are still considered experimental and not feasible in a large scale population [23, 24].
The strengths of the present study were: surgical procedures performed by same skilled surgeons in both groups, inclusion of “a very low myometrial free margin” cases (five mm), preoperative performance of both ultrasound and hysteroscopic investigation, a very low severe intraoperative complication rate, performance of spinal anesthesia during all G2 surgical procedure [25].

As limitations the authors include: case group comprising a little cohort of patients, only single submucosal myoma cases, a non-comparable distribution of myomas type between case and control groups, lack of correlation between myoma location, and complication rate.

References


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Successful completion of the first trimester despite the inappropriate rate of rise of the serum beta human chorionic gonadotropin levels

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Summary

Purpose: To report an exception to the rule that once slow rising serum beta human chorionic gonadotropin (hCG) levels are observed, a live fetus after 12 weeks is not possible even if fetal viability was detected earlier. Materials and Methods: The fetus of a woman with a slow rising serum beta-hCG levels which even plateaued was evaluated by serial pelvic sonography. Results: The fetal pole grew appropriately but at one point the sac size was a week behind. Nevertheless the woman completed the first trimester with a viable fetus and a normal fetal heart rate. Conclusions: Though rare, a live fetus at the end of the first trimester is possible if even if there is a slow rise of sera hCG levels where there is at least one instance when the hCG levels do not double in two days.

Key words: Beta-hCG level; Rate of rise; Live fetus; Sac size.

Introduction

The authors previously published a manuscript showing that in 16 women who showed a positive heart beat despite at least one serum beta human chorionic gonadotropin (hCG) level that did not double appropriately demonstrating no viable pregnancies at 12 weeks [1]. Since this publication in 2003 they have been seeking one case that would be an exception to the rule that if the beta-hCG level fails to double appropriately, even once, there will be an inevitable fetal loss by 12 weeks even if viability is found earlier in the first trimester. This case report identifies the first exception to the rule.

Materials and Methods

A 35-year-old woman with primary infertility was treated with clomiphene citrate with luteal phase progesterone support because she was anovulatory. She conceived and her first beta-hCG level was obtained 15 days from conception and was 134 mIU/ml. Table 1 lists the serum beta-hCG levels according to days post-conception.

Her first ultrasound was performed on day 23 from conception. An eight-mm gestational sac was found consistent with 5.3 weeks. One week later a five-mm crown-rump length was found but the sac only grew to ten mm, consistent with 5.6 weeks.

One week later (37 days from conception) the sac grew to 16 mm, consistent with 6.38 weeks whereas the fetal pole grew to 11 mm, consistent with 7.2 weeks. Three weeks later (day 57) with a drop in the beta-hCG level from a week earlier from 48,813 to 40,363, the crown-rump length (CRL) measured 33 mm, consistent with an appropriate ten-week size. However, the sac was only 35 mm, consistent with 8.4 weeks.

Up to this point there had been a subchorionic hematoma. This disappeared the following week with the ultrasound showing a CRL of 46 mm, consistent with 10.26 weeks. Her last ultrasound was performed 77 days from conception; everything was perfect on ultrasound with a heartbeat of 153 beats per minute.

Discussion

Not only did this woman show an inadequate rise in the beta hCG level from 21 to 23 days from conception, there was also a very inappropriate rise the following week. Finally the ultimate plateauing of the serum beta-hCG levels strongly suggested that the fetus would die; but it did not happen. Also, against a normal outcome was that when the CRL measured eight weeks, the sac was one week behind at seven weeks [2].

Though the present authors published the manuscript showing that slow rising beta-hCG predict fetal demise by the end of the first trimester, even if viability was seen earlier, the data used was from a different study involving pregnancies from 12/95 to 8/00. These data found 16 women out of 158 consecutive pregnancies that had a slow rise of the serum beta-hCG levels with viability at eight weeks but with a miscarriage by 12 weeks [1]. Since that time the present authors decided to evaluate a larger series to see what is the true chance of a live fetus under these circumstances. After failing to find even one exception to the
rule after evaluating 1,000 consecutive pregnancies, the authors abandoned the prospective study and just alerted their staff to be on the lookout for even one case report.

As an infertility practice the present authors carefully follow 95% of their pregnancies through the first trimester. Despite thousands of pregnancies over nine years since their previous publication showing an incidence of slow rising beta-hCG levels with early viability at eight weeks to be about 10%, this is the first one completing the first trimester and actually did deliver a live healthy fetus (actually she did deliver a healthy full-term girl).

The knowledge that at least it is possible to have a live baby with a slow rising serum beta-hCG is important since with no precedent some women will discontinue therapy aimed at reducing miscarriage risk, e.g., progesterone supplementation. This case report may encourage some women to continue aggressive therapy aimed to prevent a pregnancy loss.

References


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Table 1. — Serum beta-hCG levels according to days post-conception.

<table>
<thead>
<tr>
<th>Days post-conception</th>
<th>Serum beta-hCG level (mIU/ml)</th>
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<tbody>
<tr>
<td>15</td>
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<td>17</td>
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<tr>
<td>21</td>
<td>2,161</td>
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<td>64</td>
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<td>41,221</td>
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<td>77</td>
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Pregnancy in a patient with premature ovarian failure

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Summary

Background: Premature ovarian failure (POF) occurs in about one in 100 women under 40 years of age. The authors report a case of a POF patient who conceived during hormonal replacement therapy (HRT). Case: A 24-year-old woman with confirmed POF conceived spontaneously during HRT. Conclusion: Pregnancy is possible in women with POF.

Key words: Premature ovarian failure, Hormonal replacement therapy; Pregnancy.

Introduction

Premature ovarian failure (POF) is defined as secondary amenorrhea, hypoestrogenism, and elevated levels of gonadotropins occurring in women before the age of 40 years [1]. The estimated incidence is one in 100 women under the age of 40, one in 1,000 women under the age of 30, and one in 10,000 women under the age of 20 [2, 3]. The etiology of most POF cases remains unknown (idiopathic POF). Known factors leading to this condition include X-chromosome anomalies, oocyte-specific gene mutations, autoimmune disorders, hypothryoidism, radiation, chemotherapy or surgery [4, 5]. Cessation of ovarian function in POF is not necessarily permanent. Young women with POF can experience intermittent and unpredictable resumption of ovarian activity and spontaneous pregnancies have been reported in about five to ten percent of cases subsequent to the diagnosis [6].

Case Report

A 21-year-old woman was referred to the present department because of secondary amenorrhea lasting for three years, irregular menses since the age of 16, acne, hot flashes, headaches, and moodiness. She had menarche at the age of 13 and reported regular menses, lasting four to five days, until the age of 16. After that her menses became irregular, the average cycle length was between 14 days and three months. She was treated with oral contraceptives since the age of 18. She had no history of chronic diseases and previous operations. The results of hormonal tests performed six months previously were: follicle stimulating hormone (FSH) 80.7 IU/l; luteinizing hormone (LH) 25.3 IU/l; estradiol (E2) 10.5 pg/ml. Her physical examination demonstrated a seemingly healthy woman with body mass index (BMI) of 23, Tanner stage V, small uterus during pelvic exam, including a hypoestrogenized vaginal epithelium. Transvaginal ultrasound revealed small uterus (2.9 cm length) with thin endometrium (four mm) and small ovaries (right ovary 4.9cm³, left ovary 2.9cm³) with a few follicles in the right ovary. Hormonal tests revealed FSH 90.9 IU/l; LH 35.9 IU/l; E2 8.5 pg/ml. Serum prolactin, thyroid and adrenal function tests were within the normal ranges. Karyotype was 46 XX. POF was diagnosed after clinical and ultrasonographic examinations and hormonal replacement therapy (HRT) with sequential two mg 17β-estradiol and ten mg dydrogesterone was proposed. HRT was continued for six years until secondary amenorrhea occurred during therapy. Ultrasongraphy performed two weeks later revealed the presence of gestational sac inside the uterine cavity with visualization of the embryonal tissue. Hormonal supplementation (ten mg dydrogesterone orally, twice a day) was administered for the next three months. Down syndrome and Trisomy 18 screening using biochemical and ultrasound markers performed at 13 gestational weeks were below the risk cut-off for these diseases. Pregnancy was uneventful aside from cholestasis of pregnancy, which was an indication for labor induction at 37 weeks of gestation. The patient delivered a healthy boy and her puerperium was uneventful.

Discussion

The possibility of spontaneous pregnancy in women with POF has been reported in about five to ten percent of cases [6], especially in case of fluctuating FSH levels, the possibility to identify ovaries on ultrasound, autoimmune POF or caused by chemotherapy [7]. Primary amenorrhea seems to be the worst predictive factor of intermittent ovarian function [8].

More than one-third of women with normal karyotype and idiopathic POF demonstrate appearances of ovarian follicles on pelvic ultrasound. Around 20% of these patients ovulate spontaneously during four months of observation, and nearly 50% of those affected have intermittent ovarian follicle function [9]. Pregnancy may occur after the diagnosis of POF, even in women with no follicles observed on ovarian biopsy [10].

The mechanism of possible pregnancy might involve the possibility of elevated gonadotropin causing downregulation of gonadotropin receptors and restoration of the sensitivity of the few remaining ovarian follicles by lowering of serum gonadotropin levels with estrogen therapy. Estrogen may increase the number and sensitivity of FSH receptors in granulosa cells and start the recruitment of follicles. Besides, gene coding for the FSH receptor or its regulatory enzymes may be augmented through activation of the hormone-sensitive adenylate cyclase and consequent elevation.

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of the intracellular cAMP [11], what might explain the pregnancy in the presented case.

It is recommended that patients with POF are treated with HRT and are closely monitored for ovulation before resorting to oocyte donation. The combination of corticosteroids with pituitary suppression, followed by ovarian stimulation with gonadotropin, was shown to be beneficial in restoring ovarian function in patients with idiopathic POF. In patients with POF due to autoimmune disease, immunosuppressive treatments such as corticosteroids could improve ovarian function [8, 12]. Furthermore, bone marrow transplantation (BMT) was demonstrated to improve fertility in a portion of patients and in mice, but the mechanism by which BMT improves fertility remains unknown [13].

References


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Introduction

The syndrome of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a progressive neurodegenerative disorder that is most commonly the result of an A-to-G transition mutation at position 3243 of the mitochondrial genome [1]. It is a multisystem disorder first affecting tissues with high energy consumption, such as brain and skeletal muscle, but then proceeds to cause auditory, visual, psychiatric, renal, gastrointestinal, and dermatological symptoms as well [1-4]. MELAS remains one of the most common mitochondrial disorders, with an incidence as high as 12.48 per 100,000 in one study [4]. However, like all mitochondrial disorders, the concept of hertoplasmy – the presence of two or more different genomes within one cell – makes the disease prevalence and inheritance pattern difficult to appreciate [5, 6].

Since it was first reported by Pavlakis et al. in 1984 [7], an abundance of research has been dedicated to MELAS but no clear pathophysiological mechanism has been linked to the implicated point mutations in the mitochondrial genome. One theory posits mitochondrial neuropathy as the underlying cause of migraines, seizures, and stroke-like symptoms [8]. Iizuka et al. report that, despite the name, stroke-like episodes are more likely non-ischemic events. They found that these episodes were characterized by increased capillary permeability, hyperperfusion, neuronal vulnerability, and neuronal hyperexcitability. In subsequent neuroimaging studies, they found that the initial change during acute stroke-like episodes appeared as a focal brain lesion along the cerebral cortex, up to half of which progressively spread to adjoining cortex of the brain beyond the major vascular territories with various degree of vasogenic edema [9]. It is possible that this edema secondary to increased capillary permeability is the direct cause of the characteristic neurologic findings in MELAS patients.

Summary

Purpose: To evaluate the efficacy of sympathomimetic amine therapy on a mitochondrial abnormality known as the mitochondrial encephalopathy lactic acidosis and stroke-like symptoms syndrome (MELAS syndrome). Materials and Methods: Dextroamphetamine sulfate 15 mg extended release capsule was prescribed to a woman with a 25 year history of MELAS syndrome refractory to most other therapies. Results: Within one month of therapy the woman noticed considerable improvement in her chronic fatigue, pain, and edema. Conclusions: The MELAS syndrome is thus another condition to add to the list of various chronic refractory disorders that improve considerably after dextroamphetamine therapy. This is the first mitochondrial disorder shown to improve with sympathomimetic amines which could suggest that dextroamphetamine could prove useful in decreasing the risk of aneuploidy in women of advanced reproductive age.

Key words: Mitochondria; Chronic fatigue; Encephalopathy; Sympathetic hypofunction; Sympathomimetic amines.

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Improvemnet in symptoms of the syndrome of mitochondrial encephalopathy, lactic acidosis, and stroke-like symptoms (MELAS) following treatment with sympathomimetic amines – possible implications for improving fecundity in women of advanced reproductive age

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would reason that sympathomimetic amine therapy would be effective for patients with MELAS as well as idiopathic orthostatic cyclic edema.

The current study demonstrates the efficacy of sympathomimetic amine therapy for treatment of migraines, seizures, stroke-like episodes, and fatigue associated with MELAS.

Materials and Methods

At the age of 27, shortly after the birth of her second child, this patient began to experience consistent throbbing headache associated with slurred speech, drooping eyelids, and muscle pain. She was admitted to the hospital more than 15 times in order to evaluate these symptoms. Based on her clinical picture of facial muscle weakness, she was diagnosed with myasthenia gravis.

That same year, the patient started to experience stroke-like episodes. Her legs became paralyzed for several days at a time and then spontaneously returned to full strength. She also developed seizures, and began topiramate therapy. She also began to experience narcolepsy, falling asleep in public places such as a restaurant and family gatherings.

Six years after the onset of symptoms, the patient was referred to a molecular medicine specialist. At that time, she was found to have variable lactate and pyruvate levels. Muscle biopsy showed diffuse mitochondrial proliferation. Skeletal muscle oxidative phosphorylation enzymology showed a decrease in the Complex I Assay (CoQ1) (31 nmol/minute/mg mitochondrial protein, normal values 93-325). This combination of this clinical syndrome, muscle biopsy, and enzyme assay led to the diagnosis of MELAS.

As of November 2010, the patient still experienced headaches, stroke-like illness, and extreme fatigue despite treatment with coenzyme Q-10, lidocaine injections, carnitine, and even ketamine therapy. She reported that ketamine therapy helped her extreme fatigue, headaches, and stroke-like symptoms for about four or five weeks after treatment. Directly after her ketamine therapy, she rated her pain as 5 out of 10 in severity; but reports that the pain slowly escalated over the next five weeks and returned to a baseline 10 out of 10.

The woman presented to the present authors at age 52, aware that the group treats chronic fatigue syndrome and was hoping to gain some relief from at least this aspect of her disorder. A trial of dextroamphetamine was started.

Results

The present patient reported improvement in her edema symptoms after the first month of treatment, during which she took 15 mg of dextroamphetamine per day. She also experienced decreased fatigue symptoms and reported decreased pain and increased quality of life. A month after initiating sympathetic amine therapy, she described moderate improvement in her subjective level of pain with no fluctuation.

Encouraged by the initial results, she agreed to increase her dose of dextroamphetamine to 30 mg per day. With the increase, she notes significant improvement still with no fluctuation.

Discussion

Sympathomimetic amines have been found to markedly improve symptoms of chronic neuromuscular disorders that were refractory to standard therapy. Some of these neuromuscular disorders include chronic fatigue which was one of the main debilitating problems of the subject of this case report [16]. Dextroamphetamine sulfate has proven very effective for migraine headaches that were recalcitrant to other therapies [17-19]. One case that was associated with weight gain and edema showed marked improvement in the migraine without improving the edema. This suggests that at least in some cases the headaches may be related to the absorption of chemicals and toxins into brain tissue rather than the edema per se. On the other hand, the present authors recently treated a woman with severe headaches and papilledema diagnosed with pseudotumor cerebri who did not respond to acetazolamide whose severe headaches not only completely disappeared following treatment with dextroamphetamine sulfate, but two months later fundoscopic evaluation showed no signs of papilledema.

The link between these disorders involving a multitude of organ systems seems to be a hypofunction of the sympathetic nervous system [13]. Some other local environmental, genetic or infectious factors may involve certain organ systems over the other. The MELAS syndrome may be another manifestation of this disorder of the sympathetic nervous system, all linked by their positive response to sympathomimetic amine therapy.

Similar to the disorders described above, it is not clear whether the prime reason for the symptoms of MELAS is capillary permeability in cerebral and neurological tissue or cellular permeability and absorption of chemicals and toxins into these tissues or both. Regardless, dextroamphetamine sulfate may prove to be the most effective therapy with the least side effects for MELAS.

Advanced reproductive age is associated with embryos that have a high frequency of aneuploidy related to meiosis I and meiosis II errors, leading to low pregnancy rates and high miscarriage rates [20]. Errors of nondisjunction of chromosomes during meiosis I and meiosis II may be related to mitochondrial errors related to aging of mitochondria, as polymerization of spindle microtubules appears dependant on ATP [21, 22]. With the considerable improvement of this serious mitochondrial disorder with the sympathomimetic amine dextroamphetamine sulfate gives food for thought as to whether this therapy could help reduce meiosis errors in women of advanced reproductive age and possibly improve fecundity and reduce miscarriage rates.

References


Improvement in symptoms of the syndrome of mitochondrial encephalopathy, lactic acidosis, and stroke-like symptoms (MELAS) etc.


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Celiac disease and endometriosis: an insidious and worrisome association hard to diagnose: a case report

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Summary

Background: Primary infertility is an unusual presentation of celiac disease (CD). When non-classical symptoms are present, the diagnosis is not easy and it becomes even more difficult when CD is associated with endometriosis, representing a diagnostic challenge for medical practitioners and gynecologists. Case Report: A 34-year-old patient presented to the authors’ observation with primary infertility. Formerly she was treated for endometriosis and the diagnosis of CD was delayed. A favorable clinical and serological response following a gluten-free-diet (GFD) was achieved and a successful pregnancy was obtained. Discussion: This case report emphasizes the role of the CD in women’s infertility and the possible association between CD and endometriosis. Even if the relationship between these two diseases is still unclear and further studies to address this issue are required, more attention from gynecologists is needed, considering that the later this association is diagnosed, the greater the probability of adverse outcomes of health developing.

Key words: Celiac disease; Endometriosis; Infertility; Pregnancy; Gluten-free-diet.

Introduction

Celiac disease (CD) is an immune-mediated enteropathy [1, 2] caused by the ingestion of foods containing gluten such as wheat, barley, and rye [1]. It is characterized by a damage of the small intestine with consequent micronutrient malabsorption and a wide clinical variability [1, 2]. The prevalence is estimated to be one percent in the general population [3] and it is often found in over two percent in women undergoing investigation for infertility [4], whereas other studies found that CD is about as prevalent in infertile women as in the general population [5, 6]. The disease is classically though to affect children under two years of age, but the epidemiology of CD has shifted over the years, and now the majority of patients are adults (40-50 years), with a wide array of symptoms, accounting for the high rate of missed diagnoses [7]. Besides the classical symptoms (diarrhea, abdominal pain, bloating, and weight loss) [8, 9], there are many non-specific, extra-gastrointestinal manifestations associated with CD, which do not promptly suggest to test for this disease [8], such as osteoporosis, malignancy, autoimmune disorders, infertility, recurrent abortions, and pregnancy complications (intrauterine growth retardation, low birth weight, and preterm birth) [10]. Another common cause of infertility is endometriosis, a chronic disease of the reproductive age characterized by the presence of active endometrial tissue outside the uterus and associated with various symptoms such as dysmenorrhea, dyspareunia, chronic pelvic pain, irregular bleedings, and infertility [11]. Endometriosis is found in 20% to 50% of all women with infertility and is associated with a lower pregnancy rate [12]. CD and endometriosis, when associated, may lead to difficult diagnosis due to the overlap of symptoms (abdominal pain, bowel changes, menstrual irregularities, spontaneous abortion, and infertility). To date, there are only two studies on the association between endometriosis and CD [2, 13], and further evidence is needed to definitively assess there shared pathogenesis and their possible association.

Case Report

A 34-year-old Caucasian woman presented to the authors’ observation in 2010 with main complaints of primary infertility. Her clinical history revealed a normal physical and psychic development and lactose, yeast, and egg intolerance. She denied a history of smoking. She had menarche at the age of 13, her menstrual cycles were irregular since then, and she has always been suffering from dysmenorrhea, dyschezia, chronic pelvic pain, and menometrorrhagia. In order to reduce the above-mentioned symptoms, she was treated with combined oral contraceptives (discontinuously administered) from 16 to 29 years of age. At the age of 29, after many years of bothersome symptoms such as constipation, bowel changes, abdominal pain, and dyschezia, which have always been attributed to irritable bowel syndrome, an ovarian cyst was detected in the left ovary with a transvaginal ultrasound during a medical examination. The ultrasound pattern of the cyst suggested endometriosis, thus Ca 125 was measured and resulted positive (83 U/ml) (normal value: < 35 U/ml). The cyst was laparoscopically removed and the histological examination of her specimen was reported as endometriosis, when associated, may lead to difficult diagnosis due to the overlap of symptoms (abdominal pain, bowel changes, menstrual irregularities, spontaneous abortion, and infertility). To date, there are only two studies on the association between endometriosis and CD [2, 13], and further evidence is needed to definitively assess their shared pathogenesis and their possible association.

A 34-year-old patient presented to the authors’ observation with primary infertility. Formerly she was treated for endometriosis and the diagnosis of CD was delayed. A favorable clinical and serological response following a gluten-free-diet (GFD) was achieved and a successful pregnancy was obtained.

Case Report: A 34-year-old patient presented to the authors’ observation with primary infertility. Formerly she was treated for endometriosis and the diagnosis of CD was delayed. A favorable clinical and serological response following a gluten-free-diet (GFD) was achieved and a successful pregnancy was obtained.

Discussion: This case report emphasizes the role of the CD in women’s infertility and the possible association between CD and endometriosis. Even if the relationship between these two diseases is still unclear and further studies to address this issue are required, more attention from gynecologists is needed, considering that the later this association is diagnosed, the greater the probability of adverse outcomes of health developing.

Key words: Celiac disease; Endometriosis; Infertility; Pregnancy; Gluten-free-diet.
she had been attempting to conceive for two years without success, and in 2010, when she came to the authors’ observation, three cycles of ovarian stimulation with clomiphene and gonadotropins were performed, but the first intrauterine insemination (IUI) resulted in a spontaneous abortion. As a consequence of the failure of the assisted reproductive techniques (ART), the authors decided to perform another ovarian stimulation, but after further investigations her hemoglobin was 9.1 g/dl and the peripheral blood examination was suggestive of microcytic hypochromic anemia. In view of a history of bowel symptoms, menstrual irregularities, infertility, and the recent anemia, the authors decided to investigate the patient for CD. Her IgA tissue transglutaminase (tTG) antibodies were positive (84.57 U/ml) (normal value: < 4.0 U/ml), thus duodenal biopsies were performed and the histological examination revealed Marsh III atrophic mucosa, confirming the presence of CD. Following the diagnosis, the patient was placed on a gluten-free diet (GFD). Three months later she was evaluated again and at that time, her hemoglobin was 12.3 g/dl and her IgA tTG levels decreased to 28.42 U/ml. The diet also led to an improvement in her celiac-related symptoms and within six months she spontaneously conceived. She had no complications during pregnancy and she delivered a healthy baby. To date, she is still on a GFD and she is continuing a careful follow-up.

Discussion

Menstrual and reproductive disorders are now known to be among the atypical symptoms of CD. These disorders include late menarche, early menopause, secondary amenorrhea [1, 9], menstrual irregularities [10], infertility, recurrent miscarriages, intrauterine growth restriction [1, 9, 10], lower birth weight of celiac women babies, and preterm birth [1, 10]. Many times patients with CD are completely asymptomatic, aside from infertility or they may have none of the symptoms classically attributed to CD. For this reason, the diagnosis on average is delayed up to ten years and many women are only diagnosed in their adulthood [10]. CD can induce malabsorption and deficiencies of micronutrients such as iron, folic acid, vitamin K, zinc, and selenium, which are essential for organogenesis and ovarian function and their deficiency may lead to celiac-associated infertility [14, 15]. The rate of menstrual and reproductive disorders is reduced with early diagnosis and treatment with a GFD [9, 10]. However, studies on the association between CD and infertility are often inconclusive and contradictory. The prevalence of CD in women undergoing investigation for infertility was often found in over two percent [4], whereas other studies found that CD was about as prevalent in infertile women as in the general population [5, 6]. Overall, it is not possible to draw any definitive conclusion on this issue and there are no guidelines for CD testing in patients with infertility or in women with a history of adverse pregnancy outcomes [10]. Nevertheless, given the likelihood that the GFD improves pregnancy and fertility outcomes and the low cost of serological screening compared with the great medical expense associated with infertility and complications of pregnancy, CD testing should be strongly considered [10], especially among women with unexplained infertility. As it is commonly known, endometriosis is associated with infertility, however, the etiology of this association is unclear, thus complicating management. Several mechanisms have been proposed to explain the endometriosis-related infertility, such as [16-18]: distorted pelvic anatomy, altered peritoneal function, altered hormonal and cell-mediated function, endocrine and ovulatory abnormalities, impaired implantation, oocyte and embryo quality, and abnormal utero-tubal transport. Some women with endometriosis will conceive without difficulties, however others may encounter a substantially longer time to conception. Several controlled trials have suggested reduced fecundity in women with endometriosis ranging from two to ten percent [19]. The impact of endometriosis on oocyte quality has been suggested by studies evaluating the donor oocytes of patients with and without endometriosis and implantation rates in recipients. Specifically, in a retrospective analysis women who received embryos from endometriotic ovaries had significantly reduced implantation rates [17]. To the authors’ knowledge, there are only two studies addressing the association between endometriosis and CD. In one study, Aguiar et al. screened 120 women with endometriosis and 1,500 controls with CD serology followed by a small intestinal biopsy where indicated. CD was confirmed in 3/120 (2.5%) cases with endometriosis and in 10/1500 (0.66%) controls, but the difference was not statistically significant [2]. In the other study, Stephansson et al. found similar results and endometriosis seemed to be associated with prior CD [13]. Shared etiological factors are possible explanations for the positive association between the two diseases. Endometriosis is associated with chronic inflammation as well as CD [20] and it is possible that the two diseases share certain pathways of inflammation. Indeed, major increases in interferon-γ and interleukin-6 are seen in both CD [21] and endometriosis [22]. Moreover, CD and endometriosis are both considered immunologically mediated diseases [2]. In addition, it has been demonstrated that HLA-DQ7 is twice as common in patients with endometriosis [23], and it may also influence the risk of future CD [24]. As an alternative, assuming that there is a casual link between CD and endometriosis, a common genetic/immunologic link is more likely than a possible nutritional deficit. The association of CD and endometriosis is still unclear and represents a challenge for medical practitioners and gynecologists due to the overlap of symptoms, such as infertility, abdominal pain, constipation, diarrhea, menstrual irregularities, amenorrhea, and multiple spontaneous abortions. Furthermore, some of these conditions may be mistaken for irritable bowel syndrome, remaining misdiagnosed for years.
Celiac disease and endometriosis: an insidious and worrisome association hard to diagnose: a case report

Conclusion

In the past, CD was considered as a solely gastrointestinal disease of infancy, whereas nowadays many patients present with the disease in adulthood and experience a wide range of non-classical symptoms, some of which are specifically relevant to women’s health, such as infertility and unfavourable pregnancy outcomes. It is recommended, according to the authors’ experience and on the available literature on the subject, that all women with unexplained infertility and a history of adverse pregnancy outcomes should be tested for CD. The diagnosis may be very difficult when CD is associated with endometriosis and it represents a diagnostic challenge for physicians. The presented case report sheds additional light on the role of the CD in women’s infertility and on the possible association between CD and endometriosis. Even if the relationship between these two diseases is still unclear and further studies to address this issue are required, more attention from gynecologists is needed, considering that the later this association is diagnosed, the greater the probability is of adverse health outcomes to develop.

References

Rare extrapelvic endometriosis on iliac vein wall – diagnosis and treatment

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Summary

Although endometriosis is a relatively common illness in women during the reproductive period, extrapelvic localization of endometriosis is a relatively rare finding which can pose a differential diagnostic problem. Diagnosis and surgical treatment of a patient with endometriotic tumor located on the outer wall of the left common iliac vein are discussed. Patient underwent surgery because of circulatory disorders and left leg edema. Histopathological findings verified endometriosis on the outer wall of the common left iliac vein without other localizations.

Key words: Endometriosis; Iliac vein compression.

Introduction

Endometriosis is defined as the occurrence outside the uterine cavity of areas of glandular structures surrounded by specific stroma, histologically and functionally resembling uterine endometrium. Although they may be found in any organ in the body, the lesions predominantly occur in the pelvic region, involving the ovaries, broad ligaments, rectovaginal septum, uterosacral ligaments, uterovesical pouch, the surface of uterus, and oviducts.

Localization of endometriosis can be divided into two broad anatomical categories – pelvic and extrapelvic. Pelvic lesions may be found within the ovaries, most frequently in fallopian tubes or pelvic peritoneum. Extrapelvic lesions may be localized to the genitalia, perineum, urinary tract system, gastrointestinal tract, intrathoracic structures, musculoskeletal tissue, abdominal wall or nervous system [1].

Endometriosis is an estrogen-dependent inflammatory process affecting 10% to 15% of fertile women, and as many as 50% of infertile women [1, 2]. As the disease is often asymptomatic and remains undiagnosed, its true incidence is not known. The diagnosis of extrapelvic endometriosis is difficult, usually dictated by clinical suspicion, followed by imaging studies (ultrasound, CT and magnetic resonance imaging), laparoscopy, tissue biopsy and immunohistochemistry [3, 4].

Due to high frequency of occurrence of endometriosis in women in reproductive period, especially in cases followed by cyclical repetition of discomforts, extrapelvic endometriosis should be taken into consideration regardless of numerous differential and diagnostic dilemmas. The aim is to achieve better preoperative preparation and to conduct operative procedure with minimum intraoperative and postoperative complications leading to better treatment prognosis.

Case Report

Case is presented of a patient age 31 who was surgically treated for clinically diagnosed compressive tumor of left common iliac vein. After such treatment, extrapelvic endometriosis, i.e., endometriotic tumor on the outer wall of the left common iliac vein was histopathologically verified.

Discomforts which caused patient to report for checkup started one year before that. Swelling of left foot started first. Over time, the edema kept spreading upwards so after 6 months the entire left leg was swollen. That is when clinical suspicion arose differentially diagnostically either about left paracolic lymphadenopathy or deep vein thrombosis in iliacophemoral region. Color Doppler ultrasonographic examination was performed and clinically suspicion set about external compression of left common iliac vein. An MNR scan of the pelvis was performed because of suspected lymphadenopathy and it was ruled out. MNR scan showed that uterus and both ovaries were normal in size and shape which, together with previously performed detailed gynecological exam, confirmed absence of gynecological condition. Patient underwent surgery. Intraoperatively, a tumor lesion was found on the left common iliac vein which was the cause of compression; it was removed completely and sent to histopathological exam. Iliac vein was reconstructed by ringed tubular graft and an artificial arteriovenous fistula was made. Postoperative course was complicated again at first by fever and 5 days later by repeated occurrence of edema of the left leg. Checkup phebography then showed vein graft thrombosis while blood flow in AV fistula was normal. Cavography showed normal flow through inferior vena cava, with no external compression. Anticoagulant, antibiotic and physical therapies were prescribed. Patient reacted well to administered therapy. After all discomforts and clinical symptoms of infection disappeared, and normal vein flow through the reconstructed iliac vein was confirmed, AV fistula ligation was performed and patient was released from hospital in good general state. Histopathological exam of the tumor surgically removed from the wall of the left common iliac vein verified endometriosis. Regardless of completely normal gynecological findings, i.e. no signs of any gynecological condition, regular checkups with vascular surgeon...
and with gynecologist were continued. All findings were normal. Patient was discomfort-free. The only problem that appeared later was sterility issue. In spite of completely normal gynecological finding, ovulatory cycles, normal hormonal status and husband’s normal spermogram finding, patient was not able to conceive naturally through spontaneous cycle nor through stimulated cycle, or by any in vitro fertilization (IVF) method.

Discussion

According to references, extrapelvic endometriosis is rare. The most common localizations of extrapelvic endometriosis are scars remaining after surgical procedures (episiotomy, Cesarian section) [2, 5], followed by gastrointestinal tract. The gastrointestinal tract is the most common site of extrapelvic endometriosis, affecting 5% - 15% of women with pelvic endometriosis. Among women with intestinal endometriosis rectum and sigmoid colon are the most commonly involved areas [6]. Inguinal endometriosis is rare and accounts for 0.3-0.6% of patients affected by endometriosis [7]. An isolated case of endometriosis of obturator nerve was reported in 1990 [8]. A unique case of retroperitoneal endometriosis causing deep vein thrombosis from extrinsic compression of the right iliac vein is described [1] as well as endometriosis causing cyclic compression of the right external iliac vein with cyclic edema of the right leg and thigh [9].

Also, there are two case reports of endometriosis occurring around large veins. The first case reported endometriosis involving the left femoral vein [10]. The patient presented with a painful groin mass that showed characteristics of endometriosis in the adventitial layer of the vein. The second case was due to endometriosis encircling the right external iliac vein causing edema of the right leg and thigh. Successful surgical resection of the endometriosis was performed in both cases [9].

Management of endometriosis is individualized and driven by lesion localization and symptoms presentation [2, 11]. Considering that endometriosis relapse is a frequent finding, especially in cases of pelvic localization, and that remission depends on adequate and complete surgical removal of endometriotic tumor; adequate surgical intervention is necessary in order to prevent relapses especially in cases of extrapelvic localization. However, rare extrapelvic localization of endometriosis and varying symptomatology is often the cause of erroneous preoperative diagnosis.

Sterility poses as a particular problem and occurs in about 50% of patients suffering from extrapelvic endometriosis even after it is successfully surgically removed as in this case.

The role of endometriosis in development of infertility (except in cases of mechanical obstruction to conception) is still unknown. Considering the fact that some patients with this condition conceive without any difficulties, while other are infertile, it is clear that there is no unique explanation for all infertility cases.

Question whether completely removed extrapelvic endometriosis should be postoperatively treated by medicaments, which medicaments should be used and for how long, i.e., whether such a treatment of adequately surgically removed extrapelvic endometriosis can later affect reproductive ability i.e. fertility, still remains open.

References


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Postpartum posterior reversible encephalopathy syndrome (PRES) in a twin pregnancy complicated by preeclampsia-eclampsia: case report

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Summary
This is the second case in literature of posterior reversible encephalopathy syndrome (PRES) in a twin pregnancy complicated by preeclampsia-eclampsia. A 27-year-old primigravida with dichorionic diamniotic twin pregnancy was admitted at 36 weeks of gestation for induction of labour due to preeclampsia. On the second day postpartum, the patient developed severe hypertension, visual symptoms, confusion, headache, and eclamptic fits. Head computed tomography (CT) showed hypodense basal ganglia lesions. The patient was treated in the intensive treatment unit with hydralazine and labetalol infusions and anticonvulsants. Five days later, there was complete clinical improvement and follow-up magnetic resonance imaging (MRI) was normal. The patient was discharged 11 days post-delivery. Diagnosis of PRES is based on the presence of clinical features of acute neurologic compromise, abnormal neuroimaging findings, and complete reversibility of findings after prompt treatment. Early recognition and proper treatment result in complete reversibility of this condition.

Key words: PRES; Preeclampsia; Eclampsia; Twin pregnancy.

Introduction
Posterior reversible encephalopathy syndrome (PRES) is a clinical entity that presents in a wide variety of conditions. It was first described by Hinchey et al. in 1996 [1] but the currently used term “PRES” was proposed by Casey et al. in 2000 [2]. Diagnosis of PRES is based on the simultaneous presence of clinical features of acute neurologic compromise, brain neuroimaging findings, and clinical/radiologic proof of reversibility [3]. Despite the increasing number of reports indicating awareness of PRES among clinicians [4], its prevalence is still unknown [5,6].

The authors present the case of a primigravid woman with a twin pregnancy who was induced at 36 weeks of gestation due to preeclampsia and was diagnosed postpartum with PRES. To the best of the authors’ knowledge, this is the second case report on the association between twin pregnancy and PRES.

Case Report
A 27-year-old primigravida, who conceived via assisted conception treatment was diagnosed with a dichorionic diamiotic twin pregnancy at her dating scan. Her antenatal course was unremarkable with normal growth of both fetuses until she was admitted at 36 weeks of gestation with a diagnosis of preeclampsia. On admission, the patient complained of visual disturbances and headache and had increased blood pressure of 150/90 mmHg with 2+ proteinuria. Induction of labour was planned with dinoprostone. While in labour the suspicion of chooroamnionitis was raised due to a maternal temperature of 38.1°C and fetal tachycardia and the patient received intrapartum antibiotics. Pain relief was provided with epidural analgesia. Several hours later, two healthy babies were born both by ventouse delivery because of delayed second stage. The patient was subsequently taken to the postnatal ward.

On second day postpartum, the patient developed a high blood pressure (210/120 mmHg), with severe photophobia, confusion, and headache. Hypertension was treated with hydralazine and magnesium sulphate was given for seizure prevention. Once stabilised, a head computed tomography (CT) was performed that showed hypodense basal ganglia lesions. The patient was treated in the intensive treatment unit with hydralazine and labetalol infusions and anticonvulsants. Five days later, there was complete clinical improvement and follow-up magnetic resonance imaging (MRI) was normal. The patient was discharged 11 days post-delivery.

Discussion
PRES syndrome has been associated with a variety of conditions. The main obstetric precipitating factor is preeclampsia-eclampsia [7] with cases of PRES having been observed both postpartum and antepartum [4, 5, 8, 9]. Other associa-
tions highlighted in literature include retained placental fragments leading to delayed eclampsia [10], dural puncture/regional anesthesia [11], infection/sepsis with gram-positive organisms [12], molar pregnancy [9,13] and only one case of twin pregnancy [14]. Even though features of PRES are almost universally present in eclamptic patients [15], it is yet unclear what proportion of eclamptic patients have PRES [6]. Non-obstetric conditions include abrupt hypertension, post-transplantation, autoimmune diseases, acute renal failure, blood transfusion, and immunodepressant drugs [7,16,17]. In a large case-series of \( n = 113 \) patients [3], etiologies included hypertension (61%), cytotoxic medication (19%), sepsis (7%) and preeclampsia-eclampsia (6%).

Clinical features of PRES include acute onset headache, visual symptoms, altered mental state, focal neurological deficits, and seizures [3,7,8]. In the largest case-series study of \( n = 27 \) women with preeclampsia-eclampsia and PRES [8], symptoms included seizures (62.5%), headache (58%), disturbed vision (50%), altered mental state (12.5%) and focal neurological deficits (4.1%). Laboratory findings indicative of endothelial injury are often seen such as thrombocytopenia and increased lactate dehydrogenase [7].

The “posterior” description in PRES has been suggested because of the involvement of the parietal-occipital lobes (83%-94%) [1-3,16]. Other brain regions include the frontal lobes (54.2%-77%) [3, 16], temporal lobes (64%) [3], cortex (8.3% - 90%) [7, 16], cerebellum (53%) [3], brainstem (27%) [3], and basal ganglia (4.2% - 34%) [3, 6-8, 16]. In preeclampsia-eclampsia, there is a trend for increased basal ganglia involvement [3,8], less severe oedematous lesions, and less occurrence of brain hemorrhage in comparison to other predisposing factors [8].

In the acute setting, diagnosis of PRES is more likely to be made by correlation of head CT findings and clinical features of the patient. Nevertheless, MRI imaging has the ability to detect subtle parenchymal changes [7] and distinguish vasogenic edema from cytotoxic edema that is associated with early onset infarcts [16]. To date, there is no clear correlation between clinical characteristics and severity/location of neuroimaging abnormalities in PRES [9].

The pathophysiology of PRES still remains unclear. An early theory suggests that there is vasoconstriction leading to brain hypoperfusion, subsequent ischemia, and cytotoxic oedema [3,18]. The current theory proposes that severe hypertension leads to a breakdown in cerebral autoregulation and leakage of serum into the cerebral interstitium producing focal vasogenic edema [3, 18, 19]. This vasogenic edema is seen as hypodense CT/MRI lesions [5] particularly in the posterior regions because of relatively less sympathetic innervation in the posterior circulation resulting in less protection against abrupt hypertension [3,9]. A third mechanism suggested is that of endothelial dysfunction in cases of preeclampsia-eclampsia and sepsis, leading to vascular instability, vasoconstriction and hypoperfusion [18].

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**Figure 1.** — Transverse head CT scan obtained without intravenous contrast medium on day two postpartum. There are areas of low attenuation (hypodense) in the left ganglionic region and to a lesser extent to the right (white arrows). There is no intracranial hemorrhage. Differential diagnosis includes an embolic event and dural venous sinus thrombosis.

**Figure 2.** — T2-weighted MRI of the brain from the same patient five days after the head CT scan. There are no parenchymal abnormalities noted and no evidence of infarction or bleeding. The basal ganglia are normal (white arrows). The patient at this stage has clinically improved.
Early recognition of PRES is vital since reversibility of PRES is not spontaneous but depends on prompt management of hypertension and withdrawal of precipitating factors [4,7,16]. If treated adequately, symptoms typically resolve within seven days [5]. In preeclampsia-eclampsia, there is less severe disease course with a shorter hospital stay and a better outcome in comparison to other etiologies [6].

Recurrent PRES has an incidence of 3.8% [20] and is associated with non-obstetric causes [6,7,20]. Even though recurrent eclampsia has an incidence of two percent [21], no recurrent PRES has yet to be reported in a subsequent pregnancy [6].

This is the second case report on the association between twin pregnancy and PRES. In the presented patient, precipitating factors such as preeclampsia, infection during labour and use of epidural anesthesia were present. Diagnosis was based on clinical and radiological findings and the complete recovery of the patient. Prompt recognition and aggressive treatment led to complete reversibility of this condition.

References


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Early diagnosis of limb body wall complex: a case report

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Summary

Limb body wall complex (LBWC) is a rare polymalformative syndrome. The majority of cases presented in the literature have been diagnosed in the second or third trimester of pregnancy. The authors present a case of LBWC diagnosed in the first trimester of pregnancy. The combination of two- and three-dimensional ultrasounds proved to be useful for establishing the diagnosis and differentiating from other abdominal wall defects. The diagnosis was followed by therapeutic abortion at 14 weeks’ gestation, and the pathological report confirmed the diagnosis.

Key words: Limb body wall complex; Umbilical cord syndrome; Body stalk anomaly; Two-dimensional ultrasound; Three-dimensional ultrasound.

Introduction

The limb body wall complex (LBWC), a rare polymalformative syndrome, first described by Van Allen is diagnosed based on the presence of at least two of the three following characteristics: 1) exencephaly/encepalocele and facial clefts; 2) thoraco- and/or abdominoschisis, and 3) limb defects (1,2). The authors present a case of LBWC diagnosed early during pregnancy using two-dimensional (2D) three-dimensional (3D) ultrasonography.

Case Report

A 24-year-old woman, gravida 1, para 0, and abortus 0 presented for ultrasound for nuchal translucency scanning at 12 weeks and five days of amenorrhea. The woman had a normal history, and neither her family nor the baby’s father’s family had a history of genetic anomalies or malformations. The ultrasound revealed a large defect in the anterior abdominal wall (Figure 1). The fetal head showed exencephaly with evidence of scoliosis. The umbilical cord was very short. Both the spinal and abdominal wall defects were clearly visible with 3D ultrasound (Figure 2).

Based on the ultrasound features, a diagnosis of LBWC was formulated. The patient was informed of the poor prognosis and counseled about the condition. The 3D ultrasound images proved useful in helping the patient understand the syndrome. Because of the gestational age, after counseling, the patient decided to have therapeutic abortion at 14 weeks’ gestation.

The diagnosis was confirmed by anatomopathologic examination (Figures 3, 4). The liver and intestines were outside the body. Fetal head showed exencephaly and cleft lip. The superior right arm was shortened and had an aberrant site of implantation. There was no right forearm and the right hand had only three fingers. Usually limb anomalies described in the literature are more frequent in the inferior arms. The umbilical cord was short, straight, incompletely covered by amnion and adherent to the placental membranes, as well as eviscerated mass. After the abortion the parents were counseled that there was no risk of recurrence for the subsequent pregnancy. The woman gave birth after one year to a healthy newborn (female, 3,200 g, at 38 weeks gestation).

Discussion

LBWC, a rare polymalformative fetal syndrome anomaly of the anterior wall with a very poor prognosis, has an incidence of 0.21 to 0.31 per 10,000 live births (3). Diagnosis is based on the presence of at least two of three essential features: exencephaly/encepalocele and facial clefts, thoraco- and/or abdominoschisis, and limb defects (1,2). A constant finding is coelosomia, which can be variably associated with encephalic, vertebral, visceral or limb anomalies. The anomaly has also been referred to in the literature as a short umbilical cord syndrome or body stalk anomaly.

Some authors assert that LBWC simply represents a severe form of amniotic band syndrome. Indeed, in almost 40% of cases, amniotic bands are present and the defect of the limbs could appear to be secondary to the amniotic bands. The pathogenesis of LBWC is unclear, and different pathogenic mechanisms have been suggested. Several theories have attempted to explain the syndrome: germ disc defect with early embryonic maldevelopment (4,5), early amnion rupture theory (6,7), vascular disruption theory (8,9), and the disturbance of the embryonic folding process (4). Hunter et al. claimed a new theory in which association of malformations originates as early as the embryonic disc stage and that some of the associated anomalies are secondary complications of the primary disturbance in embryogenesis (10). LBWC will always appear as a combination of a large abdominal wall defect with protrusion of the viscera, a severe spinal scoliosis and a continuous juxtaposition of the fetus to the placenta. The eviscerated organs form a complex mass entangled with membranes. The defect comprises most often both the ab-
demen and the thorax or rarely could be limited only to the abdomen or thorax. Associated malformations include central nervous system lesions, facial abnormalities, cardiac malformations, urogenital anomalies, limb defects, amniotic bands, and placental abnormalities.

The limb defect can manifest as clubfoot, oligodactyly, arthrogryposis, and absent limbs or digits. Severe scoliosis develops as a consequence of the irregular attachment of the fetus to the placenta. Russo et al. identified two distinct LBWC phenotypes: placento-cranial and placento-abdominal types (11). The placento-cranial type is characterized by craniofacial defects, facial clefts, and amniotic adhesions. The placento-abdominal type has no cranial defects, rather, more frequently has been associated with coelosomia lumbosacral meningomyelocele and kyphoscoliosis, as well as urogenital anomalies. The present case has the characteristic of the former type of LBWC. Usually, LBWC is diagnosed during the second trimester of pregnancy by ultrasound. Although the syndrome can also be diagnosed in the first trimester of pregnancy, very few cases of diagnosis in early gestation have been reported (12-14). Nonetheless, the diagnosis cannot be formulated before ten weeks' gestation because of the physiological herniation characteristic of this period. In the present case, the diagnosis was established at the end of the first trimester of pregnancy. The presentation of this case clearly showed the importance and the benefit of a correct prenatal diagnosis. Because the ultrasound examination was correctly performed, this condition was diagnosed early and a therapeutic abortion was performed.
It is important to distinguish LBWC from other anterior wall defects to determine the prognosis and management options (15-17). The site of the defect should be considered when differentiating LBWC from other abdominal wall anomalies. In ectopia cordis, the defect is situated at the abdominal wall and anterior aspect of the thorax, but also involves the heart, while cloacal extrophy involves the lower abdominal wall (14). Also, it is important to discern LBWC from isolated gastroschisis, which has a much better prognosis (15).

The prognosis for LBWC is very poor and because it is considered incompatible with life, pregnancy should be terminated after a correct sonographic diagnosis. It is also important to explain to the families affected that there is no risk of recurrence of this anomaly.

Conclusion

The present case demonstrated the importance of performing a thorough morphologic survey at the time of nuchal translucency screening, and the value of 3D sonography in the delineation of first-trimester anomalies. Also, 3D ultrasound is a valuable tool for counseling parents to provide a better understanding of the problems that could appear.

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Recovery from endometrial thinning and successful pregnancy following vitamin E and C supplementation in infertile woman undergoing myomectomy for diffuse leiomyomatosis of the uterus: a case report

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Summary
The authors here report a case of an infertile woman with diffuse leiomyomatosis of the uterus, which is a rare benign pathological condition in which the myometrium is occupied by innumerable small fibroid nodules. Due to the progressive abdominal distension of the patient and the desire for pregnancy of the couple, myomectomy was performed as a primary treatment. Urgent relaparotomy was required for hematoma debridement on the following day. Despite the evidence of the follicular growth and cyclic ovarian steroid secretion, the patient had postoperative endometrial thinning that was unresponsive to hormone replacement therapy. Supplementation of oral tocopherol nicotinate/vitamin E and ascorbic acid/vitamin C was effective for immediate recovery of withdrawal bleeding and gradual gain of endometrial thickness. The patient had a successful pregnancy in an in vitro fertilization-embryo transfer program and gave a birth to a healthy baby.

Key words: Ascorbic acid; Diffuse leiomyomatosis; Endometrial thinning; Tocopherol nicotinate.

Introduction
Diffuse leiomyomatosis of the uterus (DLU) is a rare benign pathological condition in which the myometrium is occupied by innumerable small fibroid nodules, which is distinct from common uterine leiomyoma [1]. The cause and etiology of DLU remain yet undetermined, although the clonality analysis for random X chromosome inactivation patterns suggests the polyclonal origins of the leiomyoma cells [2].

DLU often involves young women of reproductive ages in menorrhagia, menorrhagia, and infertility. Early reports reviewed the cases that were found patho-anatomically in the hysterectomyed uterus, but recent advances in medical imaging techniques such as ultrasound and magnetic resonance imaging facilitated its non-invasive diagnosis. Literature introduced a variety of fertility-sparing strategy including surgery, pharmacotherapy, and conservative follow-up for women with DLU and desire for pregnancy [3-5]. However, there is no consensus or guideline on the clinical management for fertility preservation in DLU.

The authors here report a case of an infertile woman with DLU. Due to progressive abdominal distension and wish for a baby, myomectomy was chosen as a primary treatment. On the following day, urgent relaparotomy was required for hematoma debridement. Despite the evidence of the follicular growth and cyclic ovarian steroid secretion, the patient had postoperative endometrial thinning that was unresponsive to hormone replacement therapy using estrogens and progestogens. Supplementation of tocopherol nicotinate/vitamin E and ascorbic acid/vitamin C was effective for immediate recovery of withdrawal bleeding and gradual gain of endometrial thickness. The patient was able to conceive in an in vitro fertilization-embryo transfer program and gave a birth to a healthy baby.

Case Report
A 29-year-old nulliparous infertile woman suffering from abdominal distension and menorrhagia presented to the outpatient clinic. Physical examination revealed a bulging abdominal wall with a palpable, solid, fixed mass. Ultrasound tomography demonstrated the presence of innumerable hypoechoic nodular lesions throughout the myometrium. The maximal diameters of the nodules ranged from five to 80 mm, which were confirmed by magnetic resonance imaging and thus diagnosed with DLU. The patient had iron-deficiency anemia (hemoglobin concentration 7.8 g/dl, serum iron concentration < 20 mg/dl), which was treated with sodium ferrous citrate. Due to narrowness and sharp bend of cervical canal, the authors were unable to evaluate the degree of the uterine cavity deformation with office hysteroscopy or hysterosalpingography. They opted for myomectomy under a written informed consent, as the patient had progressive abdominal distension and desire for pregnancy. Prior to the operation, a total of 1,200 g venous blood were collected for autologous blood transfusion.

Under general anesthesia, cervical canal was dilated using Hegar dilators and a double lumen catheter was installed in the uterine cavity. Following the peritoneal incision, indigo carmine dye injection through the catheter revealed bilateral tubal occlusion...
sion. Twenty IU arginine vasopressin (diluted in 50 ml saline) was injected into the fibroid nodules prior to incision. More than 80 subserosal and intramural nodules were surgically removed with an estimated blood loss of 3,300 g (Figure 1). On the following day, the patient complained of uncontrolled abdominal pain. Venous blood hemoglobin concentration measured low (8.2 g/dl) despite full transfusion of autologous blood and additional homologous red blood cells (2,000 g) and frozen fresh plasma (six units). A hyperechoic lesion was detected in the mid-to-low section of the uterine corpus on ultrasound, implicating hematoma formation. Urgent relaparotomy was performed under general anesthesia. Hematoma debridement and surgical suture were added to the bleeding points. Histopathologic diagnosis was leiomyoma except the largest nodule with atypical leiomyoma.

Residual fibroid nodules were treated with intramuscular injection of gonadotropin releasing hormone agonist leuprolide acetate depot (3.75 mg, every four weeks) for three times. Over the next three months, the patient had a secondary amenorrhea with endometrial thinning measured between 3.2 mm and 4.6 mm thickness, despite the evidence of the follicular growth and fluctuating serum estradiol (61 – 312 pg/ml) and progesterone (0.8 – 11 ng/ml) concentration. Withdrawal bleeding did not occur even following three cycles of hormone replacement therapy with conjugated equine estrogens (0.625 mg, twice a day, for 21 days) and progestogens (two mg, twice a day, for ten days). Vaginal bleeding was first noted following oral supplementation of tocopherol nicotinate (600 mg/day) and ascorbic acid (2,000 mg/day) along with hormone replacement therapy. After four years of continuous medication, the endometrial thickness gradually increased to seven mm or more. During these periods, the size of two major fibroid nodules (the maximal diameter 22 mm and 18 mm) remained unchanged on ultrasound. The patient underwent conventional in vitro fertilization, conceived in the first fresh embryo transfer cycle, and delivered a healthy baby weighing 1,720 g at 34 weeks of gestation by elective cesarean section in a maternal care unit.

**Discussion**

It is difficult to determine the best therapeutic path to preserve fertility for infertile women with DLU. Previous reports presented the successful cases that were managed with myomectomy, gonadotropin releasing hormone agonist, or a combination of these treatments [3-5]. Considering the progressive abdominal distension of the patient and the desire for pregnancy of the couple, the authors chose myomectomy as a primary treatment. They removed as many nodules as possible, but had to perform urgent relaparotomy for hematoma debridement.

Following two consecutive surgeries and three months of gonadotropin releasing hormone agonist treatment, the patient had a secondary amenorrhea for a total of six months. Despite the evidence of the follicular growth and cyclic estradiol and progesterone secretion, endometrial thickness
Recovery from endometrial thinning and successful pregnancy following vitamin E and C supplementation in infertile women etc.

was always less than five mm. Hormone replacement therapy was not effective for induction of withdrawal bleeding, suggesting postoperative endometrial thinning.

Vitamin E is capable of improving microvascular blood flow by inhibiting lipid peroxidation in erythrocyte membranes [6] or protecting endothelial cells from oxidative stress [7]. Vitamin E was demonstrated to increase the endometrial thickness and the clinical pregnancy rates in infertile women with thin endometrium [8]. In addition, vitamin C was shown to stimulate in vitro proliferation of human endometrial epithelial cells [9]. The authors thus attempted tocopherol nicotinate and ascorbic acid, along with conventional hormone replacement. Menstruation immediately recovered following the supplementation of these agents, while the endometrium remained unresponsive and thin.

It is generally accepted that endometrial thickness with less than six to seven mm in the proliferative phase is closely associated with low pregnancy rate in the cycle [10]. Following four years of continuous medication, the patient was able to gain the endometrial thickness and eventually had a successful pregnancy in an in vitro fertilization-fresh embryo transfer program. Recently, infertile women with endometrial thinning were found to have high blood flow impedance of uterine radial arteries and low endometrial vascular endothelial growth factor expression, which was improved by vitamin E administration [11]. Vitamin C was also shown to support endometrial epithelial cell proliferation via nitric oxide-mediated signalling [9]. Thus, vitamin E and C are likely to contribute to endometrial growth via the pathways distinct from estrogens.

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Conclusion

The authors presented a case report of endometrial thinning following myomectomy for DLU and its recovery with a long-term combination therapy of tocopherol nicotinate, ascorbic acid, and hormone replacement. Continuous vitamin E and C administration may be a therapeutic option for women with postoperative endometrial thinning.
Abdominal wall endometriosis after a caesarian section - an interesting case report

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Summary

Background: Endometriotic foci can be rarely found on the surgical incision following caesarean delivery and on perineotomy site following vaginal delivery. Case: A 33-year-old woman with a history of caesarian section five years prior was admitted to the present clinic due to right groin pain with increasing intensity during menstruation. Ultrasound revealed an endometrioma-like subcutaneous mass directly under the right edge of the Pfannenstiel scar. The mass (3.5 x 2.4 x 2 cm) was removed en bloc with ultrascissor. Conclusion: The prevailing argument supports that it is a complication caused by the iatrogenic dispersal of endometrial material. Symptoms onset vary from one to five years postoperatively and mainly include pain and enlargement of the mass during menstruation. Diagnosis may be demanding due to the atypical presentation of the disease. Symptoms exacerbate during menstruation in only 20% of all cases. Abdominal ultrasound is extremely useful for diagnosis. The treatment of choice is surgical excision.

Key words: Endometriosis; Caesarian section; Infertility.

Introduction

Endometriosis is an estrogen-dependent, benign gynecologic disorder, characterized by the ectopic presence of endometrial tissue that is morphologically and biologically similar to eutopic endometrium. It mainly affects women of reproductive age, regardless of race or social status [1, 2].

Endometriosis is most commonly found on the ovaries, fallopian tubes, uterine ligaments, and pelvic peritoneum. Less common sites include the bladder, bowel, cervix, rectum, vagina, and vulva. Rarely, endometriosis can be found in distant organs such as the lungs, brain, diaphragm, kidneys, spleen, gallbladder, nasal mucosa, spinal canal, stomach, breast, and skin. Endometriotic foci have also been found on the surgical incision following caesarean delivery and on perineotomy site following vaginal delivery. These cases are known as “scar endometriosis” (incidence 0.03% - 1.5%) [3-5].

The authors present an interesting case report of endometriosis diagnosed on caesarian section scar of a woman who delivered five years prior. They also present a brief review of the literature regarding this rare manifestation of the disease.

Case Report

A 33-year-old woman attended the gynecology outpatient clinic complaining of right groin pain, which initiated one year prior and exacerbated during the last three months. She had two vaginal deliveries and a caesarean section performed five years prior.

On clinical examination, a painful, hard, irregular 2 x 2 cm mass directly under the right edge of the Pfannenstiel incision was easily palpated. Routine blood and biochemical tests and CA-125 were normal. Pain intensity and mass size was increasing during menstruation. Ultrasound revealed a compact, round, hypoechoic subcutaneous mass (18.8 x 14.9 mm), above the fascia of the rectus abdominis muscle, with endometrioma-like imaging (Figure 1A). There were no other pathological findings. Further investigations with magnetic resonance imaging (MRI) or computed tomography (CT) were not considered necessary.

A small incision was performed at the right sight of the Pfannenstiel scar. A subcutaneous mass (3.5 x 2.4 x 2 cm) of hard texture and irregular shape, firmly attached to the surrounding fatty tissue was removed en bloc with ultrascissor (Figure 1B). Histology came back as endometriosis. Postoperative course and hospitalisation were uneventful. The patient was discharged the following day with a prescription of six months with oral dienogest. On her follow up appointments at six months postoperatively, there were no clinical signs of any further disease.

Discussion

Post-surgery scar endometriosis is a very rare condition. Its incidence after a caesarian section is 0.03% - 1.5% [5, 6]. The prevailing argument supports that it is a complication caused by the iatrogenic dispersal of endometrial material at the incision site [7]. Similarly, the disease can be rarely found at the perineum following vaginal delivery. Symptoms onset vary from one to five years postoperatively and mainly include pain and enlargement of the mass during menstruation.

Diagnosis may be quite demanding due to the atypical nature and presentation of the disease. Symptoms are exacerbated during the menstrual cycle in only 20% of all cases [5, 7, 8]. Differential diagnosis needs to exclude subcutaneous abscess collection, lipoma, inguinal hernia, femoral hernia, and primary/metastatic cancer. Abdominal ultrasound is extremely useful for di-
agnostic purposes. Ultrasound image reveals a round, hypoechoic, area with fibrous elements surrounded by an inflammatory, echogenic ring [3, 9]. Usually, there is no need for further CT or MRI investigation. Final diagnosis is always established by histological confirmation.

The incidence rate of malignant transformation following endometriosis is 0.6% - 1.0% and it usually concerns the ovarian localization of the disease (80% of cases). As for other localizations, about four percent were found on surgical incision. The most frequent types of ovarian malignancies are endometrioid adenocarcinoma (69%) and vitreous cancer (14%). Extra-ovarian transformation of endometriosis usually results in vitreous cancer and adenosarcoma [4, 10]. The treatment of choice for subcutaneous endometriosis is extended surgical excision of the mass. Progestogen, contraceptive or danazol treatment offer temporary relief from symptoms before treatment, and perhaps small reduction of the endometriotic mass. Post-surgery management with pharmaceutical agents is not compulsory and its significance for these kinds of complications is not actually established.

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Isolated torsion of fallopian tube complicating pregnancy: case report

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Summary

Objective: Isolated fallopian tube torsion is a very uncommon condition in pregnancy. Most of the cases presented were in the third trimester. Only one case was reported in labor. The authors report the second case of the isolated tubal torsion during labor. Case report: A 18-year-old primigravid woman at 37 weeks of gestation was admitted to labor room with painful uterine contraction. Cervix was one-cm dilated and 70% effaced. Her sonographic and laboratory findings were unremarkable. Approximately four hours later the patient reported sudden pain at lower abdomen. The fetal heart rate tracing showed late deceleration. Preoperative diagnosis was considered as ablatio placentae. Isolated torsion of the right fallopian tube was revealed in cesarean delivery. Healthy infant was delivered and right salpingectomy was performed. Postoperative course was uncomplicated. Conclusion: In case of pain unrelated to uterine contraction during labor may be a sign of fallopian tube torsion which is an uncommon condition complicating pregnancy. In such condition, fallopian tube torsion should be kept in mind since early diagnosis may help to preserve the affected tube during labor.

Key words: Tubal torsion; Term pregnancy; Labor.

Introduction

Isolated torsion of the fallopian tube is a rare condition during pregnancy. The most important sign is lower abdominal pain [1]. It might be diagnosed in nonpregnant women with doppler ultrasonography [1, 2]. However it is very difficult to diagnose preoperatively in labor. In this report, a pregnant woman with isolated tubal torsion complicating pregnancy is reported.

Case Report

A 18-year-old primigravid woman at 37 weeks of gestation was admitted to labor room with the complain of painful uterine contraction every five minute. Her medical history and prenatal course were unremarkable. She reported no pain between contractions. She was afebrile with a blood pressure of 125/72 mm/Hg and heart rate of 92 beats per minute. Physical examination was normal. Cervix was one-cm dilated and 70% effaced. The fetal heart rate was approximately 130 beats per minute. Her hematocrit level, white blood cell, and platelet counts were within normal range for pregnancy. In ultrasonographic evaluation, fetal biometric measurements was appropriate with her last menstrual period. Patient received no analgesia and medication. Four hours later, the patient reported sudden pain at lower abdomen with normal vital signs. Physical examination revealed strong uterine contraction and tenderness. Fetal heart rate tracing showed late deceleration. Low transverse cesarean section for fetal distress was performed. Ablatio placenta was not detected. Cesarean delivery of a viable, 2,700 gr infant with Apgar scores of 6 at one minute and 8 at five minutes. Pelvic exploration showed isolated torsion of the distal half of the right fallopian tube. The right tube was twisted three times around itself and its size was 5 x 3 x 2 cm (Figure 1). The right ovary was not involved in torsion. Appendix and contralateral adnexa were normal. Postoperative course was uncomplicated. Histopathologic examination of the specimen showed necrosis and hemorrhage.

Discussion

Fallopian tube torsion was first reported in 1890 [3]. The annual incidence of isolated torsion of the fallopian tube is 1/1,500,000 in non-pregnant women [4]. Approximately 80% of tubal torsions are observed in reproductive period, while 12% of them during pregnancy [5]. Most of them is in the third trimester [6]. The diagnosis of fallopian tube torsion was made in labor in the present case.

Torsion usually occurs in abnormal fallopian tubes. Etiologic factors of the tubal torsion is classified in two groups [7]. Intrinsic causes of the tubal torsion are hydrosalpinx, haematosalpinx, tubal neoplasms, and previous tubal ligation or surgery. Ovarian and paratubal cysts, trauma, pelvic congestion, pregnancy, and sudden body position changes are the extrinsic causes [1]. In the present case, the authors thought that the torsion occurred in the result of pregnancy and uterine contractions. Some authors claimed that the gravid uterus exerts a rotational force on the adnexa as the fundal height rises during pregnancy [8]. The force of contractions in labor may contribute to the pathogenesis.

Tubal torsion is seen three times more frequently in the right fallopian tube as in the present case [5]. It is thought to be due to prevention of torsion by the sigmoid colon on the left side or to slow venous flow on the right side which may result in congestion [5].

The most important symptom of tubal torsion is lower abdominal pain which may be accompanied by nausea and vomiting [1]. In advanced cases, abdominal tenderness may
be obtained. Body temperature and white blood cell count maybe slightly elevated or within normal ranges. Ovarian torsion, acute appendicitis, ruptured ovarian cyst, ablatio placenta, and pelvic inflammatory disease can mimic the symptoms and findings of tubal torsion [9]. Preoperative diagnosis is very difficult and it is always identified during the operations [10, 11]. The present ultrasonographic study showed no abnormal finding. The authors performed cesarean section for fetal distress secondary to the possibility of ablatio placenta. The right fallopian tube torsion was diagnosed incidentally in laparotomy.

Fallopian tube is often edematous when it diagnosed. Detorsion might be attempted in a fallopian tube which has no sign of infarction with early diagnosis [12]. However salpingectomy is performed in most cases [13]. Ovaries should always be preserved unless severely deteriorated or necrosis develops [14]. Tubal detorsion may help preserving fertility but it may also increase ectopic pregnancy risk [14]. The authors performed right salpingectomy because of its gross necrotic appearance and observation of no blood flow return following detorsion procedure.

In case of pain unrelated to uterine contraction during labor may be a sign of fallopian tube torsion which is an uncommon condition complicated pregnancy. In such condition, fallopian tube torsion should be kept in mind since early diagnosis may help to preserve the affected tube during labor.

References


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