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Figure 2. — NT distribution of pregnancy with NT and CHD (yes: increased NT > 3 mm; no: NT < 3 mm.

Figure 3. — Percentage of pregnancies with CHD and chromosomal abnormalities with elevated NT (yes: NT > 3 mm; no: NT < 3 mm.

Figure 4. — Percentages of elevated NT (above 3 mm) measurements in fetuses with CHD and with fetuses with chromosomal anomalies.

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Editorial Article

An Editor’s opinion of the recent committee opinion of the American Society for Reproductive Medicine that the luteal phase deficiency as a clinical entity causing infertility has not been proven

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Summary

Purpose: To present an opposing view to the recent conclusions reached by the Practice Committee of the American Society for Reproductive Medicine and a recent review of the role of progesterone in subfertility by Sonntag and Ludwig that there is no evidence to support using progesterone in the luteal phase as exclusive therapy. Materials and Methods: A large quasi randomized study not mentioned by either review is presented. Results: In this study published in 1989 when women with luteal phase deficiencies and subfertility were evaluated for follicular maturation, the majority seemed to form mature follicles. This majority group found far better with pregnancy outcome by taking exclusive progesterone in the luteal phase than follicle maturing drugs. A recent prospective series confirmed its beneficial effect. Conclusions: Physicians should not empirically treat with follicle maturing drugs but should use progesterone in the luteal phase, preferably in those women who seemingly create a mature follicle.

Key words: Luteal phase deficiencies; Progesterone; Follicle maturing drugs; Follicular maturation; Immunosuppression.

Introduction

As an Editor of this Journal, I was asked to write one editorial with each issue, especially, but not limited to my field of expertise and that is reproductive endocrinology and infertility. One editorial that I wrote was about the nature of the editorials that I would be writing entitled “The diagnosis and treatment of infertility – one person’s philosophic approach” [1]. I reviewed in this editorial the difficulties encountered by the physician treating infertility in making therapeutic decision.

One physician recently was facetiously stated that it makes no sense to go to medical school because the insurance companies tell him what diagnostic tests he should perform, the drug representatives school him on what drugs he should use, and the patients have already self-diagnosed themselves by simply “going on line”. The treating physician if not conducting there own review learn from lectures in their own hospitals or at national or international meetings by “experts” in the field and some predominantly base their treatment methods from didactic teaching and advice. Others will read the literature and will assume that the latest article from a reputable journal is the treatment philosophy to follow. Others rely heavily on committee practice opinions from the top experts in their field. The expectation is that the committee is composed of well-respected scientists in their field with both clinical and research experience. Ideally, each member should have both clinical and research experience, but a mixture of a team with clinical or research expertise would be acceptable. The committee chairman should do due diligence in selecting the appropriate committee members.

A recent practice committee of the prestigious American Society of Reproductive Medicine, published their opinion of the clinical relevance of luteal phase deficiency in the prestigious Journal Fertility and Sterility, in November 2012 [2]. The committee was composed of 19 well-known and well-published reproductive endocrinologists. I suspect, but am not sure that Samantha Pfeifer, M.D., was the committee chairman since she was listed first in the recognition of participants. The manuscript was received June 25, 2012 and was published online July 20, 2012. Thus it was not subject to peer review.
There were five key points in their summary, but I will just quote two of them which is pertinent to my opinion about the committee views of use of luteal phase support with progesterone as a sole treatment entity for infertility. “No diagnostic test for luteal phase insufficiency has been proven reliable in a clinical setting. The roles of BBT, luteal progesterone levels, endometrial biopsy and other diagnostic studies have never been established and performance of these tests cannot be commended”. I actually do not disagree with this statement and I have provided a detailed summary of what information has been accrued by these aforementioned studies and their limitations as a definitive test of luteal phase deficiency. Furthermore I mention the studies on putative molecular markers of endometrial development and their failure as a method to diagnose luteal phase deficiency [3]. In this article I mention that the hope will be in finding a correlation with luteal phase deficiency and insufficient generation of the immunosuppressive protein, the progesterone induced blocking factor (PIBF). Along with our fellow Rachael Cohen, I will be publishing another editorial in the near future concerning new and exciting findings concerning PIBF.

The second statement from the ASRM practice committee that I want to summarize is the following: “No treatment for luteal phase insufficiency has been shown to improve outcomes in natural, unstimulated cycles”. There was no mention of any studies published by the members of this committee that showed that exclusive use of progesterone in the luteal phase was ineffective as a treatment modality for infertility. The committee states that “to date all attempts to link poor fertility endpoints have been unsuccessful” and they refer to four references [4-7]. However in my opinion, one cannot make the jump that because so far no good documentation of luteal phase deficiency exists that therefore treatment with progesterone is doomed for failure.

A lack of progesterone as a cause of infertility was first published in 1949 by Georgianna Jones who coined the term luteal phase deficiency [8]. In 1962 she published an uncontrolled series of 555 private patients and found that the use of progesterone in the luteal phase was associated with achieving pregnancies [9]. It is clear that once a pregnancy is established, surgically removing the ovary with the corpus luteum or the use of a progesterone receptor antagonist, e.g., mifepristone in the early first trimester will abrogate a pregnancy [10, 11]. However, another progesterone receptor antagonist, Ulipristal, which similar in structure and function to mifepristone, prevents binding of circulating progesterone to the progesterone receptor and inhibiting transcription and translation, documentation has been approved for emerging post-coital contraception [12]. The approval was granted upon the documentation that similar to other emerging contraceptives, e.g., levonorgestrel and combination estrogen-progesterone regimens, Ulipristal acetate also inhibits ovulation [13]. Ulipristal is more effective than levonorgestrel and other oral contraceptives emerging contraception and it is likely that the improved efficacy is related to inhibiting embryos from implanting if failure to prevent ovulation occurred [14]. There was no evidence of delayed endometrial maturation of the endometrium by a single dose of Ulipristal. However, there is a strong likelihood that this one time dosage, which has a half-life of 32 hours and can be detected seven days later, may have suppressed PIBF and thus allowed immune rejection of the conceptus [14-18]. Thus, the studies with Ulipristal in this manner suggest that inadequate progesterone effect, whether due to a small decrease in the integrated secretion of progesterone or relative resistance of the progesterone receptor to progesterone, may be present and may respond to therapy with extra progesterone and thus correct some infertility problem.

An integrated view on the diagnosis and treatment of luteal phase deficiency as a cause of subfertility was also recently published [19]. They wrote “Despite the existing recommendation for rational work-up in subfertility, luteal phase evaluation and progesterone therapy alone is still common in daily practice”. I take this to mean that there is no rational reason for using exclusive luteal phase support with progesterone as a treatment for subfertility. Yet, in their review there is no mention of any studies refuting the use of exclusive progesterone therapy. The practice committee of the ASRM seem to favor the use of follicle maturing drugs in that they state that the “use of agents that induce ovulation may improve the fertility of subfertile women”. Similarly, Sonntag and Ludwig state “as luteal deficiency is not an entity on its own but a consequence of defective follicular maturation and growth, it should be diagnosed and treated as such [19]. The use of follicle maturing drugs to correct luteal phase deficiency became popular in the late 1970’s to early 1980’s [20-22]. Sonntag and Ludwig implied that the empirical use of progesterone in the luteal phase may still be popular in Germany. However, in the United States, from evaluating thousands of subfertile couples, I would state that empirical usage of ovulation inducing drugs is the standard not only for reproductive endocrinologists, but OB/GYN generalists whereas exclusive luteal phase progesterone is uncommon.

Do I disagree that using follicle maturing drugs is never the right answer? No, sometimes they are appropriate. Do I agree that all cases of luteal phase deficiency should be treated with follicle maturing drugs? Absolutely not. Do I think that there is no use for exclusive use of progesterone in the luteal phase? No – I totally disagree and, in fact, would argue that more women should be treated with the exclusive use of progesterone than with drugs that induce ovulation. I will also state that I do not think that those women requiring follicle maturing drugs should be treated with them exclusively but in fact should also be supplemented with progesterone in the luteal phase.
To this point I have provided no better argument than the ASRM practice committee or Sonntag and Ludwig to favor my position but merely conjecture. However, in contrast to those opposed to progesterone, I have evaluated the efficacy of progesterone in the luteal phase as exclusive therapy. The study I refer to was published in a peer review journal in 1988 [23]. The study was a quasi-prospective randomized trial in that the randomization was by last digit of social security number rather than present day techniques as random numbers table and there was no placebo control [23]. The study randomly compared the use of clomiphene citrate (or human menopausal gonadotropins if the post-coital test was poor related to the anti-estrogen effect of clomiphene citrate) versus the exclusive use of progesterone vaginal suppositories in the luteal phase. The study group consisted of women with a minimum of one year of infertility with a male partner with normal semen parameters, bilateral tubal patency, and a normal post-coital test. After enrollment, if they showed evidence of an unruptured follicle in their initial evaluation of follicular maturation, they were excluded. Only women with endometrial biopsies performed in the late luteal phase which dated two or more days out-of-phase were included. One hundred consecutive women were randomly stratified into two groups based on their initial observation cycle - those who seemed to make a mature dominant follicle (using the aforementioned definition described earlier in this section), and those who showed follicle collapse and secretion of progesterone in the luteal phase, but did not seem to attain a mature follicle based on serial transvaginal sonography and serum estradiol levels. The 58 women making a mature follicle were randomized into treatment with clomiphene citrate or low dosage human menopausal gonadotropins in those with poor post-coital tests on clomiphene (n=27) or just with vaginal progesterone (n=31). The requirement was six cycles of good post-coital tests so the group receiving clomiphene had even extra cycles if in the first or subsequent cycles the post-coital test was poor when they would be switched to hMG. Only three of 27 (11.1%) conceived with follicle maturing drugs and two of three miscarried during the first six months of therapy. Thus the live delivery rate was only 3.7%. In contrast, 24 of 31 (77.4%) women conceived with luteal phase progesterone supplementation with only one miscarriage. The live delivery rate was 74.2% [24]. Interestingly, 25 women who failed to conceive during the six-month study with follicle maturing drugs during the first six months were switched to just progesterone in the luteal phase, and 16 of 25 (64.0%) conceived within six months with only one miscarriage [24]. In contrast, with a three-way randomization in the 42 women who did not attain a mature follicle, seven of ten conceived with follicle maturing drugs, but there were four miscarriages. Combining follicle maturing drugs in the follicular phase and progesterone in the luteal phase, the same percent achieved a pregnancy (14 of 20, 70%) but there was only one miscarriage. There were only three of 12 conceiving with just progesterone supplementation alone but no miscarriages [24].

If one did not separate the group according to follicle maturation, overall 43.8% achieved a clinical pregnancy with follicle maturing drugs vs. 60.4% with exclusive use of progesterone. The author is not aware of any subsequent study that refutes these data. Nevertheless, even to the present day, the authors having evaluated thousands of infertility couples and find a high percentage had been previously treated empirically with follicle maturing drugs by other infertility specialists or gynecologists successfully conceiving with progesterone.

As previously mentioned, the endometrial biopsy as performed in the aforementioned study has been criticized as to its accuracy in diagnosing luteal phase deficiency. This has led to a treatment philosophy in our infertility practice to empirically treat women with infertility with regular menstrual cycles who seem to make mature follicles, have made partners with normal semen parameters, normal post-coital tests, and bilateral tubal patency with progesterone in the luteal phase. This is especially important in women aged 30 or above or even younger women with symptoms or signs of endometriosis. Though, as mentioned, there have been no studies refuting the aforementioned study published about 30 years ago, there have been no studies corroborating it either [24].

We decided to attempt to corroborate our previous study. However, with no remuneration, it would be difficult to convince women to be treated with a placebo for a period of time or give women follicle maturing drugs considering our previous negative data when using these drugs in the presence of mature follicles. Thus we decided to perform a prospective observational series of exclusive use of progesterone in the luteal phase without the use of an endometrial biopsy in women with a minimum of one year of infertility [3, 25].

For 32 women aged ≤ 39 with an average length of infertility of 2.3 years, (71.7%) achieved a live pregnancy past the first trimester within six months of progesterone therapy [3]. Also, of great importance, 26 of the 32 women had failed to have a successful pregnancy despite being previously treated for at least three cycles with follicle stimulation drugs prescribed by other previous physicians. Clomiphene citrate and/or letrozole may cause vasomotor side effects, depression, thin endometria, ovarian cysts, hostile cervical mucus, and multiple follicles and thus multiple births. Gonadotropins, though not causing vasomotor symptoms, hostile mucus, or thin endometria, have an even greater likelihood of causing multiple births or persistent ovarian cysts (from unruptured follicles), but worst of all, they are extremely expensive. Based on these data, I would recommend empirical luteal phase progesterone therapy for infertility in women with “unexplained infertility” rather
than empirical use of follicle stimulating drugs, or worse, going to the most expensive of all therapies, in vitro fertilization. These data suggest that luteal phase deficiency is common but there is no good method at present to detect it. If the diagnosis is wrong, the treatment with progesterone is without risk and relatively inexpensive.

Perhaps computer searches from the ASRM Committee and Sonntag and Ludwig did not go back far enough to find this aforementioned 1988 study. However, these data were re-presented in the editorial I wrote in this journal in 2002 entitled “Progesterone therapy versus follicle maturing drugs – possible opposite effects on embryo implantation” [25]. They were also summarized in my editorial “Ovulation defects despite regular menses” [26]. These titles should not have been missed in any reasonable computer search, so my assumption is that these data were purposely left out of the article by the Practice Committee and Sonntag and Ludwig’s article because the data conflicted with their personal opinions even though these opinions were unsubstantiated by any studies performed by these authors or others [2, 19].

Luigi Mastroianni, the head of reproductive endocrinology and infertility at the University of Pennsylvania School of Medicine was a pioneer in the field of infertility and he made a great number of discoveries that helped to allow the field of infertility to progress to its present state. I knew him well but he did not agree with my pro-progesterone attitude which was the Thomas Jefferson School of Medicine and later Robert Wood Johnson School of Medicine (Camden Division) philosophy. His students followed his doctrine and supplemental progesterone has not been a normal treatment modality at the University of Pennsylvania. I am assuming that the committee chair person would be the first member of the committee mentioned and Dr. Samantha Pfeifer is at this institution. The committee chair person enlists other members to form the committee and there are three additional members of the committee who were also once at the University of Pennsylvania. With 76 references in their article, I have to assume that the omission of our study was not fortuitous. Our data were not even mentioned to be criticized!

Prior to this editorial I wrote another editorial entitled “Infertility for the OB/GYN generalist” [27]. I mention our data in this article and I take the position that the generalist should consider empirical progesterone therapy themselves for women over 30 or those with pelvic pain for infertile women before referring to a reproductive endocrinologist because the “standard” of care by these specialist seem to be three cycles of intrauterine insemination with follicle maturing drugs (and frequently with no luteal phase support) then encouraging them to proceed to IVF. I emphasize in that editorial that the generalist can easily distinguish those with a potential luteal phase defect with immature follicles vs. mature follicles by evaluating serum estradiol at mid-cycle and looking for a level >200 pg/ml. This group will do very well with just progesterone support without risk or cost to the patient. This group is more common than those with follicle maturation defects. I can assure the reader that after 40 years of using luteal phase progesterone exclusively for selected cases I have not soured on this therapy one iota. I can also assure the reader that we have achieved many successful pregnancies by simply using progesterone support in the luteal phase in patients who have failed with ovulation drugs or even in vitro fertilization in patients who were previously treated by infertility specialists.

Thus, I return to my editorial which explained the type of editorials I will write for this Journal and re-emphasize the difficulty we all have in developing treatment paradigms for our patients [1]. The information leading practicing physicians to establish treatment protocols will be based on textbooks, publications in journals, lectures, review courses, and personal experience. Very rarely is there uniformity of opinions and thus the treating physician must decide what makes sense to them and base treatment on what they think is both logical and feasible [1]. There will be always biases. Though I have no commercial interests, my Ph.D. thesis was “The role of progesterone in promoting fertility and preventing miscarriage may be through the stimulation of immunomodulatory proteins”. My basic science work coupled with clinical experience and publications could give me an edge against the members of the committee or Sonntag and Ludwg. However it could be considered that I am biased and will continue to try to put the square peg in a round hole.

I strongly suggest that whatever treatment course you choose based on what approach seems most logical and practical, you should keep statistics of your own patients so you can decide if this therapy seems efficacious or is it time to try an alternative therapy.

References

An Editor’s opinion of the recent committee opinion of the American Society for Reproductive Medicine that the luteal phase deficiency etc.


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Original Articles

Reproductive Biology Section

Very unusual symptoms consistent with a possible migraine immediately following the injection of recombinant follitropin beta

J.H. Check1,2

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Summary

Purpose: To present a new side effect of follitropin beta not shared with highly purified urinary follicle stimulating hormone (FSH).

Materials and Methods: Follitropin beta was administered for preparation of in vitro fertilization-embryo transfer (IVF-ET).

Results: Within a few minutes of injection of subcutaneously of follitropin beta, a 27-year-old woman developed a feeling of fever, headache, nausea, vomiting dizziness, and a visual aura consistent with migraine syndrome. These side effects did not occur when switched to highly purified urinary FSH.

Conclusions: Since this reaction did not occur when injected with FSH not made with recombinant DNA technology, it is concluded that recombinant FSH (at least with follitropin beta) can produce an immediate migraine-like syndrome.

Key words: Recombinant FSH; Follitropin beta; Migraine headaches; High purified urinary FSH.

Introduction

It is important to make the public and medical field aware of side effects from medications even if extremely rare. It is even more important if alternative equally effective therapy can be recommended to avoid these side effects. A case is reported of an unusual constellation of symptoms that immediately followed the injection of recombinant follicle stimulating hormone (FSH) (follitropin beta). However, these side effects were not seen following injection of highly purified urinary FSH.

Case Report

A 27-year-old woman was treated with recombinant FSH (follitropin beta). Immediately after her first injection she felt feverish although her temperature was only 37.2°C. This was associated with visual hallucinations in the form of colored zigzag lines on the wall that looked like neon lights. She also experienced headaches, dizziness and nausea. The symptoms dissipated spontaneously two hours after the subcutaneous injection of 150 IU. She thought that these symptoms were coincidental, so she took a second injection the next day. Though the same symptoms occurred, they disappeared again about an hour later, she tried it one more time the next day. However, the same symptomology occurred and she consulted our office. The present authors advised her to seek the opinion of a neurologist before proceeding with any more gonadotropin injections.

The neurologist ascertained that she had no history of migraine headaches or hypertension. Her only reaction to medication was to oxycodone and other analgesics which caused severe nausea and vomiting. Occasionally wine would give her a headache.

Her complete neurological examination was negative. The woman was further evaluated by electroencephalogram (EEG) and an electrocardiogram (EKG). Both the EEG and EKG were normal. She asked the neurologist if she could recommend some type of treatment that could counteract the adverse reaction to FSH injections. The neurologist had no suggestions nor any explanations for these symptoms other than somehow the injection of follitropin beta may have cause a migraine headache with visual aura.

Since the patient wanted to proceed with in vitro fertilization (IVF) because of unexplained infertility, having failed to conceive despite luteal phase support with progesterone and intrauterine insemination. She consented to try FSH injections again. However, this time she was switched to highly purified urinary FSH. She has had no reaction at all to highly purified urinary FSH despite generation of supra-normal sera estradiol levels.
Discussion

Something in the manufacturing process for recombinant FSH (at least follitropin beta) can induce a very unusual migraine-like syndrome. The reaction is too quick to consider it was related to a sudden increase in estradiol. Most likely it is an allergic reaction to some ingredient needed to form recombinant FSH. It could be specific to the manufacturing of just follitropin beta. However, follitropin alpha was never tried.

The fact that there was no reaction to high purified urinary FSH suggests that the reaction was specific to some chemical used to prepare FSH using recombinant DNA technology rather than what is used for highly purified urinary FSH.

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Effect of different luteal support schemes on clinical outcome in frozen-thawed embryos transfer cycles

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Summary

Objective: To evaluate the clinical outcome of frozen-thawed embryo transfer (FET) when using different luteal support schemes.

Study Design: Retrospective analysis of FET cycles was performed from June 2013 and December 2013. Infertile women, who underwent FET cycles utilizing embryos cryopreserved on day 3 post-insemination following an initial fresh IVF cycle. Patients were divided into three groups according to the luteal support scheme. Grade A (oral administration of progesterone, n=156), Group B (vaginal administration of progesterone, n=345), Group C (dissolved progesterone in oil with intramuscular injection, n=885), and group C was divided into two subgroups according to with (subgroup C1, n=521) or without (subgroup C2, n=364) human chorionic gonadotrophin (hCG) injected intramuscularly. The authors compared patients’ characteristics and the pregnancy outcomes of each group. Results: There was no difference in the patient characteristics of each group. There was no difference in the implantation rate or clinical and ongoing pregnancy rate among oral, vaginal, and intramuscular progesterone groups. The abortion and ectopic pregnancy rates were not significantly different among the three groups. Conclusion: Oral progesterone in the FET cycles is convenient and has similar pregnancy outcomes compared with intramuscular or vaginal administration.

Key words: Frozen-thawed embryo transfer; Luteal support scheme; Pregnancy outcome.

Introduction

The first successful pregnancy undergoing frozen-thawed embryo transfer (FET) was reported in 1983 and this strategy has been progressively used in assisted reproductive technology (ART) [1]. It is well known that FET has become a routine treatment of infertility. Surplus embryos in fresh cycles were cryopreserved for subsequent FET cycles. FET was often a better choice when mild stimulation or luteal phase ovarian stimulation was used in fresh cycles [2].

FET can provide several benefits in ART such as decreasing risk of multiple pregnancy and ovarian hyperstimulation syndrome, increasing cumulative pregnancy rate [3, 4]. FET perhaps avoids embryo-endometrium asynchrony which is a major cause of impaired endometrial receptivity after ovarian stimulation [5]. FET is safe and has similar neonatal outcome in terms of prematurity, low birth weight, stillbirth, neonatal death, and major malformation compared with fresh ET [6].

Due to the important role of corpus luteum, various luteal phase support schemes are applied, aiming to improve the FET success rate. Several medicinal products containing progesterone are in widespread use orally, intramuscular injection or vaginally for support of luteal function during assisted reproduction. In fresh IVF cycles, both intramuscular and vaginal progesterone have become the standard of care for luteal phase support. However, the dosage, type, route of administration, and the length of treatment for luteal phase support in FET cycles still remains conflicting. Therefore, the present study was conducted to evaluate clinical outcomes of FET with different luteal support schemes.

Materials and Methods

Retrospective analysis was carried out with FET cycles between June 2013 and December 2013 in Children’s Hospital of Shanxi & Women Health Center of Shanxi. A total of 1,386 cases with FET cycles were enrolled. In order to avoid potential bias, only the first two FET cycles of each patient was included. The cycles with preimplantation genetic diagnosis and cancelled cycles were excluded.

All FET cycles were classified as Grade A to Grade C, according to luteal support scheme. These main outcomes were analyzed including the implantation rate, clinical pregnancy rate, ongoing pregnancy rate, abortion rate and ectopic pregnancy rate. Other parameters, such as age, duration of infertility, and endometrium thickness on the day of ovulation or day 3 before FET in HRT cycles were also analyzed.

FET protocol

A transvaginal sonography was performed in all patients on day 2 or 3 of the cycle for exclusion of ovarian cysts. Women with a spontaneous cycle did not have any medication during their follicular phase, and second transvaginal sonography was performed.
Group A: Oral administration of progesterone. The luteal phase was supported with oral micronized progesterone tablets (200 mg, three times daily) or vaginal progesterone gel (90 mg/d) n = 345.

Group B: Vaginal administration of progesterone. The luteal phase was supported with vaginal micronized progesterone tablets (200 mg, three times daily) or vaginal progesterone gel (90 mg/d) n = 345.

Group C: Progesterone in oil injected intramuscularly (IMP). Progesterone injection (80 mg, im, qd), n = 885. Subgroup C1: IMP with human chorionic gonadotrophin (hCG). Beside IMP, the luteal phase was supported with hCG for Injection (2,000 I.U., im.QoD±3). n=521. Subgroup C2: IMP only, n=364. The luteal phase support continued until the 10th gestational week.

Diagnosis of pregnancy and follow-up

In the present study, biochemical pregnancy was defined as a serum hCG >35 IU/L two weeks after transfer but then declined to negative. If fetus with fetal heart activity in the seventh gestational week was visualized by ultrasonography, it was considered clinical pregnancy. Ongoing pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound at the fifth to seventh weeks of gestation and the existence of a fetal heartbeat at approximately 12 weeks gestation. Spontaneous abortion: loss of fetus with gestational age before 20 weeks. Ectopic pregnancy: the diagnosis of extra uterine pregnancy confirmed by laparoscopy or ultrasound. The authors compared patients’ characteristics and the pregnancy outcomes of each group.

Statistical analysis

Statistical analysis was performed using SPSS 13.0. Continuous characteristic values were compared using t-test. Ordinary were analyzed by the χ²-test. A p-value < 0.05 was reported as statistically significant.

Results

The results showed there was no significant differences in the patient characteristics such as age, duration of infertility, and endometrium thickness on day 3 before the transfer was noted among groups (Table 1).

Data on early pregnancy outcome in terms of implantation rate, biochemical pregnancy, ongoing pregnancy rate, spontaneous abortion, and ectopic pregnancy are detailed in Tables 2 and 3. There were no losses to follow-up pregnancies in this study.

Table 1. — Patient characteristics of FETs (x ± sd).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.19 ± 4.59</td>
<td>30.58 ± 4.31</td>
<td>30.42 ± 4.54</td>
<td>30.20 ± 4.49</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>4.11 ± 0.42</td>
<td>4.03 ± 0.36</td>
<td>3.91 ± 0.38</td>
<td>4.04 ± 0.40</td>
</tr>
<tr>
<td>*EM thickness (mm)</td>
<td>10.45 ± 1.75</td>
<td>10.22 ± 1.88</td>
<td>10.08 ± 1.63</td>
<td>10.23 ± 1.78</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. FET: frozen-thawed embryo transfer; NS: not significant; EM: endometrium.

* presents the value of the day of ovulation or day 3 before FET in HRT cycles.

Table 2. — Pregnancy outcomes of FETs.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate (%)</td>
<td>18.47</td>
<td>18.50</td>
<td>18.32</td>
<td>0.992</td>
</tr>
<tr>
<td>Biochemical pregnancy rate (%)</td>
<td>39.10</td>
<td>40.00</td>
<td>36.61</td>
<td>0.508</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>36.54</td>
<td>37.10</td>
<td>22.99</td>
<td>0.332</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>30.13</td>
<td>29.86</td>
<td>23.95</td>
<td>0.051</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>17.54</td>
<td>16.41</td>
<td>22.95</td>
<td>0.261</td>
</tr>
<tr>
<td>Ectopic pregnancy rate (%)</td>
<td>0.00</td>
<td>3.13</td>
<td>2.05</td>
<td>0.420</td>
</tr>
</tbody>
</table>

Table 3. — Pregnancy outcomes of subgroups C1 and C2.

<table>
<thead>
<tr>
<th></th>
<th>Subgroup C1</th>
<th>Subgroup C2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate (%)</td>
<td>17.54</td>
<td>18.86</td>
<td>0.415</td>
</tr>
<tr>
<td>Biochemical pregnancy rate (%)</td>
<td>35.99</td>
<td>37.04</td>
<td>0.748</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>32.42</td>
<td>33.40</td>
<td>0.760</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>25.27</td>
<td>23.03</td>
<td>0.051</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>19.49</td>
<td>25.29</td>
<td>0.248</td>
</tr>
<tr>
<td>Ectopic pregnancy rate (%)</td>
<td>2.54</td>
<td>1.72</td>
<td>0.689</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using SPSS 13.0. Continuous characteristic values were compared using t-test. Ordinary were analyzed by the χ²-test. A p-value < 0.05 was reported as statistically significant.
Effect of different luteal support schemes on clinical outcome in frozen-thawed embryos transfer cycles

Discussion

It is well known that luteal support with progesterone is necessary for successful implantation of the embryo following egg collection and embryo transfer in IVF cycle. Early studies showed the effect of different luteal support scheme on clinical outcome in fresh embryos transfer cycles.

Progesterone can be administered by several routes. The oral, intramuscular (IM), and vaginal routes have been chosen frequently in the past. There are disadvantages to each method. The oral route is ineffective, since progesterone has a liver first-pass effect, and is associated with a high rate of metabolites which may result in side effects such as a somnolent effect [7]. Friedler et al. and Licciardi et al. showed oral progesterone to be associated with significantly lower implantation and pregnancy rates, and higher miscarriage rates, or both, compared with IM or vaginal administration [8,9].

Progesterone in oil injections serve as an effective route for without first passing through the liver. It results in a depot effect and continuous release of progesterone over time, with a long elimination half-life, allowing once-daily injections, but in some cases intramuscular progesterone injections may be painful, especially if prolonged for up to ten weeks.

Vaginal progesterone may be inconvenient, though progesterone reaches the uterus directly and endometrial progesterone concentration reaches a steady state within five hours, the short half-life of natural progesterone and intermittent peaks of absorption with vaginal administration, it is generally required to be inserted multiple times daily and may cause vaginal irritation in some women. Uterine contractility and endometrial wave activity are higher after vaginal administration compared with intramuscular administration, which might adversely affect the implantation of the embryo [10]. However, the effect of different luteal support scheme for FET cycles still remains conflicting. Smitz et al. showed that vaginal administration had shown to be at least as effective as IM administration, and significantly more effective then oral treatment [11]. Shapiro et al. retrospective analyzed 682 FET cycles in which IMP was used for luteal support and 238 FET cycles in which vaginal progesterone gel was used. They found that the implantation, clinical pregnancy, and live birth rates were not significantly different between IMP and vaginal progesterone gel group [12]. Guo et al. reported that there were no significant differences in the clinical outcomes between the patients receiving dydrogesterone and intramuscular progesterone as luteal phase support in either natural cycle FET or HRT FET [13]. Lee et al. found that the pregnancy outcomes of natural cycle FET were similar with or without luteal phase support [14].

In the present study, the authors found that there was no difference in implantation rate, clinical and ongoing pregnancy rates, and abortion and ectopic pregnancy rates among oral, vaginal and intramuscular progesterone groups. Consequently oral progesterone in FET cycles is convenient and has similar pregnancy outcomes compared with IM or vaginal administration.

Both hCG, which stimulates steroid production in the corpus luteum, and progesterone administration in the luteal phase are effective and significantly improve the clinical pregnancy rate [15]. However, in this study, the authors found that there was no difference in the clinical outcomes between groups C1 and C2.

In this study, the authors did not compare neonatal outcome among the groups and different clinical outcomes among different types of cycles. Aflatoonian et al. showed that if the pregnancy reached 20 weeks of gestation, FET did not adversely affect neonatal outcome in terms of birth weight prematurity, LBW, stillbirth, neonatal death, and major malformation compared with fresh ET [6]. Early studies showed a trend towards similar pregnancy rates and live birth rates with the administration of FET during a spontaneous cycle compared to FET during an artificial cycle or using GnRH in the artificial cycle [16, 17].

Conclusion

The present results showed that oral progesterone in the FET cycles as luteal support had similar clinical outcomes in terms of the implantation, clinical and ongoing pregnancy rates, and abortion and ectopic pregnancy rates compared with intramuscular and vaginal routes. An oral route has the advantage of convenient manipulation and better compliance, so it probably has a good prospect for clinical application.

References


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Obstetric and neurodevelopmental outcome in fetal cerebral ventriculomegaly

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Summary
Purpose: The purpose of this study is to establish the obstetric and early neurological outcomes of fetuses diagnosed with intrauterine ventriculomegaly (VM). Materials and Methods: This retrospective study included 27 fetuses with VM diagnosed by ultrasound (US) and referred for in utero magnetic resonance imaging (MRI). US and MRI reports and laboratory test results were obtained including chromosome analysis, congenital infections, and first and second trimester screening tests. Infants were evaluated for clinical outcome for six to 24 months of age. Results: Twenty (51%) fetuses had mild and 19 (49%) fetuses had severe VM. Accompanying central nervous system (CNS) anomalies were statistically significantly more common in severe VM group. The outcome of mild VM group was statistically significantly better than in the severe VM group. Conclusions: The authors conclude that ventricular dimension is a significant prognostic factor to determine the outcome of fetal cerebral VM. The presence of accompanying CNS anomalies is more common with severe VM and may be considered as an unfavorable indicator for a better outcome.

Key words: Fetal; Outcome; Ventriculomegaly.

Introduction
Ventriculomegaly (VM) is defined as enlargement of the ventricular system due to increased intracranial content of cerebrospinal fluid. Etiology of the abnormality is unknown in the majority of the cases but it may be congenital infections, spina bifida, obstruction of aqueduct flow, Arnold Chiari malformation, Dandy-Walker malformation, chromosomal anomalies or genetic defect.

Fetal VM is the most common sensitive indicator of brain developmental disorders detected by prenatal sonography and reported to occur in 0.3 to 2.5 per 1,000 live births [1]. VM is diagnosed when the atrial width of the lateral ventricles exceed ten mm. It is defined as mild VM when the ventricular width is ten to 15 mm and severe above 15 mm. It can be unilateral when only one ventricle measures ten mm and above. Asymmetric VM is the term used to define that the difference of lateral ventricle width at the atrium is greater than two mm, but both of them are enlarged over ten mm.

The degree of ventricular enlargement and the presence of other central nervous system (CNS) anomalies are two clinical considerations in a fetus with VM [2, 3]. Prenatal ultrasound (US) is the standard modality used to evaluate the CNS, hence it has some limitations even in the most experienced hands. Reverberation artifacts, cranial ossification in the third trimester, oligohydramnios, breech presentation, maternal obesity, and gas are some of the limitations of an optimized visualization. In addition to these limitations, posterior cerebral fossa, myelinisation, cerebral sulcation, and gyration cannot be assessed adequately with US. When the US diagnosis is indefinite or there is a potential for an anomaly that cannot be exactly defined by US, magnetic resonance imaging (MRI) is used as an alternative imaging modality, given the fact that VM has a poor outcome in fetuses with accompanying anomalies [4-11]. Chromosomal anomalies, viral infections, particularly cytomegalovirus infection, early diagnosis, progressive and bilateral VM, and cerebral parenchymal atrophy are other poor prognostic factors according to literature. The aim of this study is to establish the obstetric and early neurological outcomes of fetuses diagnosed with intrauterine VM.

Materials and Methods
This retrospective study included the fetuses of VM diagnosed using US and referred for in utero MRI to Radiology Department of Dokuz Eylul University Hospital during the period 2007 through 2009. Terminated pregnancies according to perinatology council’s decision because of associated CNS anomalies and progressive or severe VM were not included in the study. The study
was approved by the Independent Bioethics Committee for Scientific Research of the present institutional review board.

**Prenatal radiologic imaging**

Using a two-dimensional grayscale ultrasonographic system and a 3-7 MHz convex probe, the atrium of the lateral ventricle was measured in the hemisphere distal to the transducer in the axial plane at the level of the thalamus. The calipers were positioned inside the echoes generated by the ventricular walls at the smooth caudal termination of the glomus of the choroid plexus according to standard technique [12].

Prenatal MRI was performed on 1.5 Tesla superconducting system unit by using body coil. Fetal brain examination was performed in axial, coronal, and sagittal planes using T2 weighted single shot sequence (time of repetition (TR): 839; time of echo (TE): 80; flip angle: 90 degrees; slice thickness: three mm; matrix: 256; field of view (FOV): 250; rectangular field of view (RFOV): 100; number of acquisition (NEX): one; slice gap: 0). MRIs were reviewed by a 15-year experienced pediatric radiologist. Measurement of the lateral ventricle size at the level of atrium was obtained using electronic calipers and accompanying CNS anomalies were recorded.

**Prenatal clinical evaluation**

VM was defined as mild when the dimension of the atrium of lateral ventricle was between ten to 15 mm and severe when it was above 15 mm. Laboratory test results were obtained including chromosome analysis, congenital infections, and first and second trimester screening tests.

**Postnatal follow-up**

Physical and neurological examinations of the infants were performed by the same consultant neonatologist for six to 24 months of age using Denver Developmental Screening Test, audiometric test, weight, height, and head circumference measurements. Postnatal clinical examinations included mental status, strength, deep tendon reflexes, posture, and tone. Transfemoral US, brain computed tomography or brain MRI was performed if necessary. Neurophysiologic evaluation of infants who could not be followed up in this protocol was performed by a parental questionnaire via phone calls and by collected patient files. They were recalled to the hospital for additional investigation if necessary.

Cases were classified as good outcome and poor outcome groups. Infants with normal neurodevelopment were classified as good outcome group. The ones that died in the antenatal period and with mild or severe neurodevelopmental delay were classified as poor outcome group.

**Statistical analysis**

Collected data was assessed by using Pearson Chi-Square test and Fisher’s Exact test. A p value < 0.05 was considered to indicate statistical significance. All statistical tests were performed using the SPSS software, version 20.0.

**Results**

A total of 27 fetuses were included in the study. Fourteen (52%) had mild and 13 (48%) had severe VM at the time of the initial diagnosis. Mean lateral ventricular atrial diameter was 13.1 mm and 21.1 mm in mild and severe VM groups, respectively. Asymmetric VM was detected in four cases. Four cases were found to have unilateral VM.

In five of 13 severe VM cases, other fetal CNS anomalies were identified at prenatal MRI examination, including two germinal matrix hemorrhages, a cerebellar hypoplasia, an agenesis of corpus callosum, and a delayed cerebral sulcal development. Two of the 14 mild VM cases had accompanying CNS anomalies which were a delayed cerebral sulcal development with cerebellar hypoplasia and an agenesis of corpus callosum. Accompanying CNS anomalies were statistically significantly more common in severe VM group (p < 0.005).

Immunoglobulin (Ig) G and Ig M antibodies against toxoplasma, rubella, cytomegalovirus and herpes virus (TORCH) were assayed in maternal serum in all cases and there were no evidence of intrauterine infection. One case with mild VM had high risk at first trimester screening test. Two cases of severe VM included one case of mild VM that was high-risk at second trimester screening test. None of them had any chromosomal abnormalities.

Three cases died in utero. Two of the 24 live born infants died in the early neonatal period. Surviving infants were followed up between six to 24 months. Four cases had severe neurodevelopmental delay. Two cases were diagnosed as having a mild neurodevelopmental delay at follow up. Sixteen cases had normal neurodevelopment. Table 1 summarises prenatal fetal MRI findings and prognosis of the fetuses.

Sixteen cases (59%) with normal neurodevelopment were classified as good outcome group, while a total of 11 cases (41%) which died in the antenatal or postnatal period with mild or severe neurodevelopmental delay were classified as poor outcome group.

The mild VM group had a better outcome than the severe VM group and the outcome of mild VM group was statistically significantly better (p < 0.005). Table 2 shows the outcome of mild and severe VM groups.

**Discussion**

In the present study, cases with mild VM had a better outcome than the severe VM group and the outcome of mild VM group was statistically significantly better in accordance with the literature. Previous reports have revealed that lateral ventricular atrial width is strongly related with the outcome of VM [1, 13, 14]. Kirkinen et al. followed up 25 fetuses with VM at 10.1 (standard deviation ± 2.6) years of age and found that the fetuses with severe handicaps on long-term follow up had more severe ventricular dilatation than the fetuses with good long-term outcomes [15]. Breeze et al. in their research studied obstetric and neonatal outcomes in 20 severe VM cases. Nine out of ten live born babies had abnormal outcomes [16]. However, in an isolated mild VM outcome research, Gomez-Arriage et al. and Kutuk et al. found that 72.2% and 64% of infants showed completely normal postnatal outcomes, respectively [17, 18]. Sixty-four
cases of VM from birth up to four years were followed up in another study which revealed that the degree of antenatal VM was related to pediatric neurological morbidity, and when lateral ventricular atrial diameter was over 15 mm, it was associated with an increase in abnormal neurological development [13]. Accompanying fetal anomalies are one of the poor prognostic factors in VM [10, 19-21]. It is reported that associated structural anomalies significantly worsen the outcome, and fetal and neonatal deaths are much more frequent in this group [22]. In the present study, five (71%) of seven live born fetuses with accompanying CNS anomalies had poor outcome. On the other hand, only six of 20 fetuses (30%) in the isolated VM group had poor outcome. Although the outcome of fetuses with accompanying CNS anomalies was twice as worse than the isolated VM group, and this finding correlates well with the previous reports, we must consider that VM was severe in four of the five fetuses with poor prognosis in the associated anomaly group. Thus it can be speculated that the poor prognosis of the fetuses with accompanying anomalies could also be associated with the severity of VM rather than with the accompanying anomalies itself. To establish the consequence of accompanying CNS anomalies, it must be mentioned that case no. 4, which had mild VM but delayed cerebral sulcal development and cerebellar hypoplasia, died in utero at 30 weeks. On the contrary, cases no. 25 and 26 with isolated severe VM had normal neurodevelopment. However it also must considered that these two cases were followed up to six months which may be a short period of time to determine an exact outcome. Case no. 1 was the unique and mysterious case of this study with isolated mild VM, which had severe neurodevelopmental delay. The authors assume that more detailed investigations are required to disclose this case.

The correlation between accompanying CNS anomalies and the degree of VM has also been studied by researchers. In a study of fetuses with VM, the pregnancy

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Ventriculomegaly</th>
<th>Asymmetric/ unilateral</th>
<th>Accompanying or unilateral</th>
<th>Prognosis of central nervous system anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Severe neurodevelopmental delay</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>3</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>4</td>
<td>Mild</td>
<td>-</td>
<td>Delayed cerebral sulcal development and cerebellar hypoplasia</td>
<td>Died in utero at 30 weeks</td>
</tr>
<tr>
<td>5</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>6</td>
<td>Severe</td>
<td>-</td>
<td>Germinal matrix hemorrhage</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>7</td>
<td>Severe</td>
<td>-</td>
<td>Germinal matrix hemorrhage</td>
<td>Severe neurodevelopmental delay</td>
</tr>
<tr>
<td>8</td>
<td>Mild</td>
<td>Unilateral</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>9</td>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>Severe neurodevelopmental delay</td>
</tr>
<tr>
<td>10</td>
<td>Severe</td>
<td>-</td>
<td>Cerebellar hypoplasia</td>
<td>Died in utero at 38 weeks</td>
</tr>
<tr>
<td>11</td>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>Died after postpartum three hours</td>
</tr>
<tr>
<td>12</td>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>13</td>
<td>Severe</td>
<td>-</td>
<td>Agenesis of corpus callosum</td>
<td>Mild neurodevelopmental delay</td>
</tr>
<tr>
<td>14</td>
<td>Severe</td>
<td>Asymmetric</td>
<td>-</td>
<td>Died after postpartum two hours</td>
</tr>
<tr>
<td>15</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>16</td>
<td>Mild</td>
<td>Unilateral</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>17</td>
<td>Severe</td>
<td>Asymmetric</td>
<td>-</td>
<td>Severe neurodevelopmental delay</td>
</tr>
<tr>
<td>18</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>19</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>20</td>
<td>Mild</td>
<td>-</td>
<td>Agenesis of corpus callosum</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>21</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>22</td>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>23</td>
<td>Severe</td>
<td>Asymmetric</td>
<td>Delayed cerebral sulcal development</td>
<td>Mild neurodevelopmental delay</td>
</tr>
<tr>
<td>24</td>
<td>Mild</td>
<td>Unilateral</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>25</td>
<td>Severe</td>
<td>Asymmetric</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>26</td>
<td>Severe</td>
<td>Unilateral</td>
<td>-</td>
<td>Normal neurodevelopment</td>
</tr>
<tr>
<td>27</td>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>Died in utero at 21 weeks</td>
</tr>
</tbody>
</table>

### Table 2. — Outcomes of mild and severe VM groups (n, %).

<table>
<thead>
<tr>
<th></th>
<th>Good outcome</th>
<th>Poor outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild ventriculomegaly</td>
<td>12 (85.7%)</td>
<td>2 (14.3%)</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Severe ventriculomegaly</td>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>16 (59.3%)</td>
<td>11 (40.7%)</td>
<td>27 (100%)</td>
</tr>
</tbody>
</table>

\(p = 0.004\).
outcome and neurodevelopmental outcome at an age of more than 24 months were evaluated [23]. It was suggested that cases with ventriculic width above 12 mm were more often associated with malformations and had a normal neurodevelopmental outcome less frequently [23]. Griffiths et al. in their prospective study of fetuses with isolated VM found that severe VM was associated with an approximate ten-fold increase in the risk of another brain abnormality being present when compared with fetuses with mild VM [24]. Broomley et al. followed 36 live born infants with VM and revealed that fetuses with mild VM had a lower incidence of accompanying anomalies and a better outcome than fetuses with more severe ventriculic dilatation [10]. In the present study, five out of 13 severe VM cases had accompanying CNS anomalies and accompanying CNS anomalies were statistically significantly more common in severe VM group in accordance with the literature.

In previous studies, asymmetric lateral ventricles and bilateral enlargement are approved to be one of the poor prognostic factors in VM. Ouahba et al. in their outcome research of isolated mild VM found that asymmetrical bilateral enlargement of ventricles was associated with poor outcome [25]. In the present study, only one (case no. 25) of four (20%) live born infants with asymmetric VM had a good outcome. Although the results were remarkable, they were not significant because a larger number of cases is needed for statistical significance. All four unilateral VM cases had good outcome, including case no. 26 which had severe unilateral ventricular dilatation. The present result is in accordance with the previous research by Leitner et al. who studied outcome of isolated mild VM and found that outcome of unilateral VM is generally positive on both the neurodevelopment and cognitive tests [26].

The present authors acknowledge three limitations in this study. Firstly, the small number of the study group may limit the strength of the results. Secondly, the follow-up period ranged between six and 24 months, which is a short period of time to assess an exact outcome for fetal VM. Finally, the present follow-up protocol was heterogeneous because of social conditions in this country.

Conclusion

Recently many researches have been performed to study the outcome of fetal cerebral VM. Risk and degree of neurodevelopmental delay are still not definitive but also not unclear. Considering the literature and the results of the present study, the authors conclude that ventricular dimension is a significant prognostic factor to determine the outcome of fetal cerebral VM. The presence of accompanying CNS anomalies is more common with severe VM and may be considered as an unfavorable indicator for a better outcome.

References


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The genetic background of climacteric symptoms in women during menopause

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² Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan
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Summary

The subject of the study is the evaluation of the correlation between the polymorphism of candidate genes in the etiology of depression and the occurrence of the symptoms of the climacteric syndrome in women during menopause. The group subjected to the study comprised of 203 women aged between 42-65 years: 71 of them still menstruated (premenopausal group) and 132 at least one year after the last period (postmenopausal group), admitted to the Department of Gynecological Endocrinology at the University of Medical Sciences in Poznan with symptoms of the climacteric syndrome. All the examined women were evaluated according to the degree of severity of the climacteric syndrome symptoms using the Kupperman index and the concentration of FSH, LH hormones, 17β-estradiol, PRL, total testosterone, and DHEAS in peripheral blood serum. Among the candidate genes in the aetiology of depression the following were selected for the research: the serotonergic system receptor genes: 5HTR2A, 5HTR1B, 5HTR2C, TPH1, TPH2, and MAO-A; the genes of noradrenergic and dopaminergic systems (COMT, NET), the genes of the GABAergic (GABRB1) system, a gene of the estrogen receptor (ESR1), and the genes of the enzymes crucial in the methyl cycle (MTHRF, MTR, and MTHFD1). With regards to the correlation between the examined polymorphisms and the occurrence of the symptoms of the climacteric syndrome, the associations analysis indicated a connection between GABRB1.TaqI polymorphism and the occurrence of vertigo in premenopausal women (0.0198; after correction: 0.0497 CC to CA). The correlation was also found regarding the examined polymorphisms and the concentration of the examined hormones in blood serum: TPH1.MaeI polymorphism and the LH concentration in the postmenopausal group (0.004; after correction: 0.014 CC to CA), NET Eco147I polymorphism, and the 17β-estradiol concentration in the postmenopausal group (0.0208; after correction: 0.048 GG to GA) and HTR2AMspI polymorphism and PRL concentration in all examined women (0.03; after correction: 0.038 TT to CT).

Key words: Polymorphism; Depression; Climacteric syndrome; Menopause.

Introduction

The symptoms of the climacteric syndrome are a major medical problem reported by women during menopause [1, 2] and the main factor determining the quality of this part of life [1]. In general, those symptoms last from a few months to a few years, but some women experience them for 30 years or more [1, 3, 4]. Some of the risk factors when it comes to the occurrence of the symptoms of the climacteric syndrome are: low concentration of 17β-estradiol in blood serum, African-American race, a high body mass index (BMI), nicotine smoking, and the presence of other symptoms of the climacteric syndrome in the so-called “domino effect” [3–8].

With reference to the role of the genetic factors in the etiology of the symptoms of the climacteric syndrome, individual reports connect the occurrence of the symptoms of the climacteric syndrome to specific polymorphic variants of the genes of the enzymes taking part in the metabolism of estrogens such as CYP1B1 [9, 10] and estrogen receptors [11, 12]. It seems possible that other genetic factors might also play a significant role in the etiology of the symptoms of the climacteric syndrome. This study undertakes to evaluate the correlation between the genes important in the etiology of depression and the occurrence of the symptoms of the climacteric syndrome. The selection of the candidate genes was dictated by the frequent occurrence of depression during menopause [13], as well as a positive effect of antidepressants in alleviating the symptoms of the climacteric syndrome [14].

Materials and Methods

The group subjected to the study was comprised of 203 women, aged between 42–65 years admitted to the Department of Gynecological Endocrinology at the University of Medical Sciences in Poznan with symptoms of the climacteric syndrome. Among them 71 still menstruated (premenopausal group) and 132 were at least one year after the last period (postmenopausal group). All examined women were evaluated according to the degree of severity of...
women participating in the research. These were: FSH, LH, 17β-estradiol, PRL, total testosterone, and DHEAS in peripheral blood serum. The concentration of the hormones: FSH, LH, 17β-estradiol, PRL, and total testosterone were carried out using the immunoenzymatic method. The ranges of intra- and inter-assay coefficient of variation (CV) amounted to 1.2–3.3% and 2.0–5.6%, respectively.

The concentration of DHEAS was determined using the radioimmunological method: the ranges of intra-assay CV amounted to 5.1%, and inter-assay CV to 11%.

For genetic research, the genes important in the etiology of depression were selected: the genes of serotonergic system: receptor genes: 5HTR2A, 5HTR1B, 5HTR2C, tryptophan hydroxylase gene TPH (isoform 1 and 2), and the gene of monoamino oxidase A (MAO-A); the genes of the noradrenergic and dopaminergic systems: a gene of catechol-O-methyltransferase (COMT) and the gene of noradrenaline transporter (NET); the gene of GABAergic system: the gene for the β1 subunit of the GABAa receptor (GABRB1); the gene of the estrogen receptor α (ESR1); the genes of the enzymes key in the methyl cycle: MTHFR, MTR, and MTHFD1.

Genetic research was conducted based on the genome DNA, isolated from peripheral blood of the patients using the salting out method. Isolated and purified DNA was used as a matrix for amplification of exons of the genes under research.

Table 2. — Characteristics of the starters used for the amplification of the genes under research.

<table>
<thead>
<tr>
<th>Gene (fragment)</th>
<th>Polymorphism (SNP number)</th>
<th>Starter sequence</th>
<th>PCR product length</th>
<th>Temp. of annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HTR2A (exon 1)</td>
<td>c.102C&gt;T (SNP 6313)</td>
<td>F: CAAGGTGAAGTTGTGAC</td>
<td>458pz</td>
<td>62°C</td>
</tr>
<tr>
<td>5HTR1B (exon 1)</td>
<td>c.861G&gt;C (SNP 6296)</td>
<td>F: GTGGGCTTCTCTCTCATGCTA R: GCAGGCTCTTGGCAGATAG</td>
<td>516pz</td>
<td>60°C</td>
</tr>
<tr>
<td>5HTR2C (exon 4)</td>
<td>Cys23Ser (SNP 6318) F: AGCAGTGTGGTGATGAGC R: CCATAAAGGATGTCCAGGAG</td>
<td>397pz</td>
<td>57°C</td>
<td></td>
</tr>
<tr>
<td>MAO-A (exon 14)</td>
<td>c.1460C&gt;T (SNP 1137070) F: CGAGCGCTACAGGAGGTAAG R: GTGGCAGGAGCTTGTATTTGTA</td>
<td>615pz</td>
<td>63°C</td>
<td></td>
</tr>
<tr>
<td>COMT (exon 14)</td>
<td>c.649G&gt;A (SNP 4680) F: ACCAGGGAGGTTGAATACC R: CCTTGCCAGTTTACCCAGAG</td>
<td>582pz</td>
<td>57°C</td>
<td></td>
</tr>
<tr>
<td>NET (exon 9)</td>
<td>c.1287G&gt;A (SNP 5569) F: TCACCCCTCCCAAGCAGTT R: GCCGGCGTTCTTAAAGGGCTT</td>
<td>436pz</td>
<td>56°C</td>
<td></td>
</tr>
<tr>
<td>TPH2 (exon 7)</td>
<td>c.1077C&gt;A (SNP 7305115) F: CATCAGAAGCACAAGCAG GAA R: GTGGCAGGAGCTTGTATTTGTA</td>
<td>683pz</td>
<td>64°C</td>
<td></td>
</tr>
<tr>
<td>TPH1 (intron 7)</td>
<td>218C&gt;A (SNP 1800532) F: ACAGGTGTITTCATTCGGTCTT R: CACCCCATACACACACCCCCAATCA</td>
<td>657pz</td>
<td>54°C</td>
<td></td>
</tr>
<tr>
<td>GABRB1 (exon 5)</td>
<td>Ile791Ile (SNP 6284) F: CCATTTCCTCTCTCTCTCTCT R: AACATAGAAGCACACACCTGATGT</td>
<td>741pz</td>
<td>59°C</td>
<td></td>
</tr>
<tr>
<td>ESR1 (intron 1)</td>
<td>454-351A&gt;G (SNP 9340799) 454-397C&gt;T (SNP 2234693) F: TCCACACATCCATTTCCTGTCAG R: CTTCTATTCACCTTGGCCTG</td>
<td>619pz</td>
<td>63°C</td>
<td></td>
</tr>
<tr>
<td>MTHFR (exon 4)</td>
<td>c.677C&gt;T (SNP 1801133) F: AGGCTGTGCCTGCTTGTGT R: CGCCTGTGCAAGTTCTGGAC</td>
<td>477pz</td>
<td>67°C</td>
<td></td>
</tr>
<tr>
<td>MTR (exon 26)</td>
<td>c.2756A&gt;G (SNP 1805087) F: GTCTGGTGAAGGAGAGAAGAA R: CTGGAAGAATGGGGGTCTGTG</td>
<td>583pz</td>
<td>56°C</td>
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<tr>
<td>MTHFD1 (exon 20)</td>
<td>c.1958G&gt;A (SNP 2236225) F: TTCTCTCATCTCCCTCACAC R: TCTGCTCCAAAATCTGGCTT</td>
<td>416pz</td>
<td>60°C</td>
<td></td>
</tr>
</tbody>
</table>
loading buffer and separated through electrophoresis at the voltage of 100V in 1.5% agarose gel with the addition of ethidium bromide. The products of amplification were analysed in ultraviolet light.

Clinical and hormonal parameters of the groups were compared through the Mann-Whitney test. The correlations between the occurrence of individual symptoms of depression and examined markers were evaluated via the Kruskal-Wallis test. In order to determine the correlations between the genotypes, the Wilcoxon test was used with the FDR correction (False Discovery Rate) [16]. All calculations were done using a free platform R for statistical calculations [17]. The markers were concordant with the Hardy-Weinberg principle (\( p > 0.05 \)).

All experiments were carried out after obtaining the informed consent from all the participating women. The local Ethic Review Committee of Poznan University of Medical Sciences approved the study protocol.

Results

Clinical and hormonal characteristics are presented in Table 3. An average score in the Kupperman scale qualified an examined woman as having the climacteric syndrome of medium severity. An average value of BMI in all groups qualified a woman as overweight. Significant differences were found as regards the concentration of FSH and 17\( \beta \)-estradiol in blood serum between premenopausal and postmenopausal patients (as regards both hormones Mann-Whitney test \( p < 0.05 \)). The associations found between individual symptoms of depression and the concentration of hormones in blood serum against the genotypes are presented in Table 4.

Discussion

Earlier research concerning a genetic origin of the symptoms of the climacteric syndrome covered only hot flashes and polymorphisms of the genes of the enzymes taking part in the metabolism of estrogens and estrogen receptors. It was concluded that the occurrence and the degree of severity of hot flashes is connected to polymorphisms CYP1B1 [18] and the polymorphism 3\( \beta \)HSD [19]. The polymorphism CYP1B1 is connected to a higher concentration of estradiol in blood serum [18]. Polymorphisms of the ER\( \beta \) estrogen receptor are connected to a higher frequency of hot flashes [19].

In the present study, out of all examined polymorphisms of the genes of receptors and enzymes important in the etiology of depression (5HTR2A, 5HTR1B, 5HTR2C, TPH1, TPH2, MAO-A, COMT, NET, GABRB1, MTHFR, MTR, MTHFD1, ESR1), only one correlation was found as regards the symptoms of the climacteric syndrome. A correlation was demonstrated between the polymorphism of the gene of \( \beta 1 \) subunit of the receptor GABA\( \alpha \) GABRB1.TaqI and the occurrence of dizziness in premenopausal women (0.0198; after correction: 0.0497 CC to CA). Polymorphism of this gene shows a strong link with the bipolar affective disorder in women [20], with schizophrenia [21], and autism [22]. The occurrence of the correlation between the polymorphism of the receptor GABRB1 and the presence of dizziness in women during menopause has not been described before in literature, and in the opinion of the authors might be connected to the role of the GABA\( \alpha \) receptor in the activity of steroids at the brain level [23].

This study demonstrates a connection between polymorphism of the tryptophan hydroxylase gene TPH1.MaeI and the concentration of LH in blood serum of postmenopausal women (0.004; after correction: 0.014 CC to CA). Polymorphism 218A>C TPH1 is attributed to a higher suicidal tendencies [24, 25]. Whereas the 779A>C polymorphic variant indicates a correlation with a greater susceptibility to addictions and the increase in the risk of committing suicide by alcoholics [26]. A high level of LH is considered one of the risk factors of hot flashes during menopause [27] and Tataryn et al. [28] claimed that in 85% of cases, hot flashes correlate with the occurrence of LH pulses, without any connection to FSH pulses.

<table>
<thead>
<tr>
<th>Analysed parameter</th>
<th>All patients</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kupperman Index</td>
<td>26±13.1</td>
<td>27.1±13.5</td>
<td>25.4±12.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.6±5.3</td>
<td>26.5±4.2</td>
<td>26.5±5.7</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>67.1±35.8</td>
<td>52.7±40.5*</td>
<td>75.8±29.6*</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>34.9±17.5</td>
<td>32.3±22.4</td>
<td>36.2±14</td>
</tr>
<tr>
<td>17( \beta )-estradiol (pg/ml)</td>
<td>48.1±97.4</td>
<td>83.4±125.8*</td>
<td>26.9±67.7*</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.28±0.19</td>
<td>0.32±0.25</td>
<td>0.26±0.16</td>
</tr>
<tr>
<td>DHEAS (µg/dl)</td>
<td>1.36±0.82</td>
<td>1.6±1.03</td>
<td>1.25±0.66</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>13.8±10.2</td>
<td>15.3±10.2</td>
<td>12.5±8.2</td>
</tr>
</tbody>
</table>

\( * p < 0.05 \)

<table>
<thead>
<tr>
<th>SNP</th>
<th>All patients</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABRB1.TaqI</td>
<td>Dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.0198; after correction: 0.0497 CC to CA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR2A.MspI</td>
<td>PRL (0.03; after correction: 0.038 TT to CT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPH1.MaeI</td>
<td>LH (0.004; after correction: 0.014 CC to CA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET.Eco147I</td>
<td>17( \beta )-estradiol (0.0208; after correction: 0.048 GG to GA)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This study shows a connection between the polymorphism of the noradrenaline transporter gene NET.EcoI147I and the concentration of 17β-estradiol in postmenopausal women (0.0208; after correction: 0.048 GQ to GA). In postmortem examination of the brains of the persons with clinically diagnosed depression, a lower level of protein NET was found [29]. An average concentration of estradiol in blood serum in the examined groups was low, which is characteristic to the menopause period [30] and is a direct cause of the occurrence of the symptoms of the climacteric syndrome [31].

This study also demonstrates a link between a polymorphism of the gene of the serotonin receptor HTR2AMspI and the PRL concentration in blood serum in all patients (0.03; after correction: 0.038 TT to CT). A decreased activity of 5HT2A receptor is one of the mechanisms of antidepressants, and in the brains of the persons suffering from depression a higher density of this receptor was found [32]. This would suggest, that 5HT2A receptor might play a role in disturbing the thermal regulation caused by the estrogen deficiency [33]. It was found, that administering estrogens increases the density of the 5HT2A receptor within the cortex [33]. A correlation of polymorphism c.102T>C (Ser34Ser) 5HT2RA with the occurrence of bipolar affective disorder type 1 was found [34, 35], as well as with the occurrence of suicidal tendencies in patients with depression [36]. Also demonstrated was a correlation of polymorphism 1438A>G of this gene with the occurrence of the bipolar affective disorder in the Korean population [37], with the occurrence of other mental diseases such as the seasonal affective disorder [38], anorexia, and bulimia [39], as well as the occurrence of the lowered libido in patients treated with selective serotonin reuptake inhibitors [40].

References
The genetic background of climacteric symptoms in women during menopause


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The relationship between uterine prolapse and premalignant endometrial pathology

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² Department of Obstetrics and Gynecology, Celal Bayar University School of Medicine, Manisa
³ Department of Obstetrics and Gynecology, Egean Maternity and Women’s Health Training Hospital, İzmir (Turkey)

Summary

Objective: The aim of this study was to stress the importance of performing a thorough uterine assessment before selecting an organ-sparing surgery in patients presenting with uterine prolapse and no other complaints. Materials and Methods: This study included a total of 111 participants who presented with pelvic organ prolapse and underwent hysterectomy for grades 3-4 uterine prolapse. The post-hysterectomy histopathology results were classified as benign (atrophic endometrium, proliferative or secretory endometrium) or pathologic (endometrial hyperplasia, endometrial polyp, adenomyosis, myoma uteri, and endometrium carcinoma). Results: Of the 111 patients enrolled in this study, 23 (20.2%) had endometrial hyperplasia, eight (7.2%) had endometrial polyps, 30 (27%) had uterine fibroids, and 20 (18%) had adenomyosis. Conclusion: There may be premalignant lesions of the endometrium in both premenopausal and postmenopausal women presenting with uterine prolapse and no other symptoms. A chronic inflammatory process resulting from the extra-vaginal location of the uterus may play a role in the development of these lesions. Further studies are needed on this subject.

Key Words: Uterine prolapse; Hysterectomy; Endometrial hyperplasia.

Introduction

Pelvic organ prolapse (POP) is defined as the herniation of the pelvic organs into the vaginal canal or externally through the canal. POP is an important condition affecting women and the prevalence has been reported as 51% in outpatient clinics [1]. POP may simultaneously occur with other pelvic floor disorders, including urinary or fecal incontinence, sexual dysfunction, and chronic pain syndromes. A history of hysterectomy, age, number of previous pregnancies, and body mass index have been described as the most important risk factors for POP development [2]. The prevalence of POP surgery has been estimated at 1.5 to 4.9/1,000 females/year [3, 4]. Patients with POP may receive a hysterectomy or have a uterus-sparing procedure, such as a vaginal sacrospinous hysteropexy, laparoscopic sacrohysteropexy, and laparoscopic ligament suspension [5]. The aim of this study was to stress the importance of uterine assessment before selecting organ-sparing surgery in women presenting with uterine prolapse even if they had no other symptoms.

Materials and Methods

This study was performed in 111 patients who presented to Sifa University Faculty of Medicine Department of Obstetrics and Gynecology clinic for POP and received a hysterectomy for grades 3-4 uterine prolapse between March 2007 and March 2014. The patients were scored according to The International Continence Society [6]. The study was retrospectively conducted by accessing and evaluating the gynecological and clinical records of these cases. The patients were divided into two groups based on the menopausal state at the time of the operation. Patients who had amenorrhea for more than one year after the last menstrual cycle were considered in menopause. Accordingly, 80 patients were classified as postmenopausal, and 31 were postmenopausal (Table 1). Ninety-four patients underwent hysterectomy via the vaginal route and 17 patients via the abdominal route. Patients with a history of hormone use were excluded. Patients with gynecological complaints other than POP were also excluded. Patients with malignant pathologies of the ovaries or cervix were also excluded. The post-hysterectomy histopathological results of the patients were assessed (Table 2). In the histopathological examination, atrophic endometrium and proliferative or secretory endometrium were classified as benign lesions, whereas endometrial hyperplasia, endometrial polyps, adenomyosis, myoma uteri, and endometrium carcinoma were classified as pathological findings (Table 2). Along with the descriptive statistics (mean, standard deviation), a chi-square test was used to compare the mean values between premenopausal and postmenopausal patients. The results were assessed within a 95% confidence interval. A p-value less than 0.05 was considered statistically significant.
**Results**

The mean age of the 111 patients enrolled in the study was 53.8 ± 11.83 (min-max: 41-81) years. The mean ages of the premenopausal and postmenopausal women were 45.8 ± 5.6 (min-max: 41-52) and 60.2±11.3 (min-max 44-81) years, respectively (Table 1). The mean body mass index and the previous number of births in the study population are summarized (Table 1). Twenty-two of 30 patients with a benign post-operative histopathology (senile cystic atrophy or proliferative or secretory endometrium) were postmenopausal prior to the operation, and the remaining eight patients were premenopausal (Table 3).

### Table 1. — Demographic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Postmenopausal</th>
<th>Premenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.2 ± 11.3 (min-max: 41-81)</td>
<td>45.8 ± 5.6 (min-max: 41-52)</td>
</tr>
<tr>
<td>Multiparity (%)</td>
<td>77 (97.5%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Nulliparity (%)</td>
<td>3 (2.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.7</td>
<td>71.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.7</td>
<td>27.9</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>31</td>
</tr>
</tbody>
</table>

### Table 2. — Histopathological results after hysterectomy.

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>111 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign histopathological result</td>
<td>30 (27.2)</td>
</tr>
<tr>
<td>Senile cystic atrophy</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>Proliferative or secretory endometrium</td>
<td>22 (20)</td>
</tr>
<tr>
<td>Pathological histopathological result</td>
<td>81 (72)</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td>23 (20.2)</td>
</tr>
<tr>
<td>Simple</td>
<td>21 (18.9)</td>
</tr>
<tr>
<td>Simple with atypia</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Complex</td>
<td>-</td>
</tr>
<tr>
<td>Complex with atypia</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>Simple</td>
<td>7 (6.3)</td>
</tr>
<tr>
<td>Hyperplastic</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Uterine fibroid</td>
<td>30 (27)</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>20 (18)</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 3. — Classification of the histopathological examination of the hysterectomy materials according to the menopausal state.

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>80</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign histopathological result</td>
<td>22 (27.5)</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>Senile cystic atrophy</td>
<td>5 (6.25)</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Proliferative or secretory endometrium</td>
<td>17 (21.25)</td>
<td>5 (16.2)</td>
</tr>
<tr>
<td>Pathological histopathological result</td>
<td>58 (72.5)</td>
<td>23 (74.2)</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td>16 (20)</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>Simple</td>
<td>14 (17.5)</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>Simple with atypia</td>
<td>1 (1.25)</td>
<td>-</td>
</tr>
<tr>
<td>Complex</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Complex with atypia</td>
<td>1 (1.25)</td>
<td>-</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>7 (8.75)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Simple</td>
<td>6 (7.5)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Hyperplastic</td>
<td>1 (1.25)</td>
<td></td>
</tr>
<tr>
<td>Uterine fibroid</td>
<td>22 (27.5)</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>13 (16.25)</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results

The mean age of the 111 patients enrolled in the study was 53.8 ± 11.83 (min-max: 41-81) years. The mean ages of the premenopausal and postmenopausal women were 45.8 ± 5.6 (min-max: 41-52) and 60.2±11.3 (min-max 44-81) years, respectively (Table 1). The mean body mass index and the previous number of births in the study population are summarized (Table 1). Twenty-two of 30 patients with a benign post-operative histopathology (senile cystic atrophy or proliferative or secretory endometrium) were postmenopausal prior to the operation, and the remaining eight patients were premenopausal (Table 3).

Fifty-eight of 81 patients with a pathological post-operative histopathological diagnosis (endometrial hyperplasia, endometrial polyp, myoma uteri, adenomyosis, or endometrial cancer) were postmenopausal prior to the operation, while 23 patients were premenopausal (Table 3).

Discussion

Pelvic floor insufficiency is an important health issue among middle to advanced-age women. With the prolongation of the average life expectancy and a desire to live a high quality life, this condition has become increasingly more common in gynecology practices. POP is a prevalent condition in the female population, and 11% of women receive surgery for this condition by the time they are 80 years old [7]. Advanced age, delivering a large baby, menopause, and a previous hysterectomy or pelvic prolapse surgery are strong etiological factors for severe POP [8-10].

Uterine prolapse surgery can be performed via abdominal, vaginal, or laparoscopic routes. Although a vaginal hysterectomy is the standard approach, symptomatic uterovaginal prolapse therapy is a special technique for women desiring a uterine-sparing approach. Increasingly more women desire to avoid a hysterectomy. Uterine-sparing surgical operations, such as vaginal sacrospinous hysteropexy, laparoscopic sacrohysteropexy, and laparoscopic uterosacral ligament suspension, may be used in women that do not desire a hysterectomy. However, some unexpected uterine pathologies may be encountered in asymptomatic women with uterine prolapses. Mahajan et al. reported that premalignant lesions can be detected in patients with no pathological symptoms [11]. Therefore, they suggested performing a pap smear and a careful pelvic examination preoperatively. Frick et al. reported that post-operative histopathological examination of uterine tissue after a hysterectomy revealed a high likelihood of premalignant and malignant endometrial pathologies in postmenopausal patients with vaginal bleeding [12].

The present authors also detected other pathological histopathological findings, including myoma uteri (n=22; 27.5%), adenomyosis (n=13; 16.25%), endometrial hyperplasia (n=16; 20%), and endometrial polyps (n=7; 8.75%) in postmenopausal patients. In the premenopausal
patients, the pathological findings were as follows: 25.8% myoma uteri (n=8), 22.6% adenomyosis (n=7), 22.6% endometrial hyperplasia (n=7), and 3.2% endometrial polyp (n=1). There were no significant differences between the premenopausal and postmenopausal women (p > 0.05). In a study of 68 asymptomatic women, Mingels et al. found that the rates of simple endometrial hyperplasia, complex hyperplasia, hyperplasia with simple atypia, hyperplasia with complex atypia, and endometrioid endometrial carcinoma were 15%, 2%, 3%, 3%, and 3%, respectively [13]. In contrast, the present study found that the rates of endometrial hyperplasia without simple atypia, endometrial hyperplasia with simple atypia, and endometrial hyperplasia with complex atypia were 18.9%, 0.9%, and 0.9%, respectively. Furthermore, they did not detect any cases of endometrial carcinoma. Mingels et al. encountered more clinically significant endometrial pathologies than the present study.

It is already known that estrogen levels have a positive correlation with myoma uteri, adenomyosis, endometrial hyperplasia, and endometrial polyps [14-17]. Serum estrogen levels have been reported to be lower in patients with uterine prolapse [5,18]. However, previous studies have shown that some uterine pathologies have a positive correlation with estrogen levels in patients with uterine prolapse, as demonstrated in the present study. This suggests that the present findings may be the result of a locally increased uterine estrogen level rather than the circulating estrogen level. Epidemiological studies have shown that there may be a positive relationship between local tissue inflammation and the development of endometrial carcinoma [19,20]. Increased levels of cytokines and free radicals during this inflammatory process give rise to tissue estrogen production, which facilitates the emergence and development of premalignant and malignant endometrial diseases [19,20]. Externalization of the cervix in grades 3 to 4 uterine prolapse may render the endometrium more susceptible to infections. In addition, endometrial tissue protruding from the vagina may be more exposed to mechanical trauma. The resulting chronic inflammatory process may lead to premalignant and malignant endometrial lesions through the local effects of free radicals and cytokines. These factors may explain the unexpected endometrial alterations in patients with uterine prolapse in the present study as well as in previous studies. The possibility of premalignant and malignant endometrial pathologies should not be overlooked in asymptomatic patients [13]. In patients destined for uterine prolapse surgery, it is essential to assess the uterus and ovaries and perform a Thin Prep Pap test. Transvaginal ultrasonography can be used for endometrial evaluation [21].

The first limitation of the present study was the relatively small sample size. The second limitation is the study’s retrospective nature and the lack of data regarding preoperative blood estrogen levels. However, a systemic estrogen insufficiency may be a predisposing factor for uterine prolapse [18, 22]. The authors believe that this study is important because it stresses the importance of the relationship between premalignant endometrial changes, which are known to be related to estrogen levels, and the inflammatory process. As uterine-sparing surgical techniques advance more with each passing day, additional studies are needed to further understand this subject.

Conclusion

Premenopausal and postmenopausal patients with uterine prolapse and with negative gynecological complaint have a risk of premalignant lesions in the endometrium. The present authors found a high number of women with endometrial pathology in especially the postmenopausal group. It is essential to assess the endometrium before selecting an organ-sparing surgery in premenopausal and postmenopausal patients with uterine prolapse. The entire uterine tissue should be sent for histopathological examination after the hysterectomy is performed. Patients may have premalignant lesions in the endometrium even if their only gynecological complaint is uterine prolapse. A chronic inflammatory process due to the external location of the uterus may play a role in the development of these lesions. Additional studies are needed in this field.

References


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A combination of ultrasound-guided rectus sheath and transversus abdominis plane blocks is superior to either block alone for pain control after gynecological transumbilical single incision laparoscopic surgery

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Summary

Purpose: To investigate the efficacy of the combination of ultrasound-guided rectus sheath (RS) and transversus abdominis plane (TAP) blocks compared with TAP or RS block alone in gynecological single-incision laparoscopic surgery (SILS). Materials and Methods: Bilateral TAP blocks (Group A, n = 12), TAP and RS blocks (Group B, n = 12), and RS blocks (Group C, n = 12) with 40 ml ropivacaine/patient were performed for ovarian tumor SILS. The analgesic effects were evaluated using a numerical rating scale (NRS) at zero, six, 12, 24, and 48 hours post-surgery. Results: Umbilical pain on completion of general anesthesia was significantly less frequent in Group B (1/12) than Group A (7/12) (p = 0.03). The postoperative NRS scores were significantly lower in Group B than Group A at zero (p = 0.02) and six (p = 0.03) hours and Group C at zero (p = 0.001), six (p = 0.02), and 12 (p = 0.004) hours. Conclusion: The combination of RS and TAP blocks reduced early postoperative pain compared with RS or TAP block alone for gynecological SILS.

Key words: Single-incision laparoscopic surgery; Transversus abdominis plane block; Rectus sheath block.

Introduction

Single incision laparoscopic surgery (SILS) has recently become popular and has improved the outcome of gynecological surgery. However, a large-sized port scar due to SILS causes umbilical pain in some patients. Postoperative pain requiring bed rest and persistent gastrointestinal dysfunction are key factors that prolong hospitalization [1]. Therefore, adequate postoperative pain control is important to improve the quality of life and reduce the duration of hospital stays.

Abdominal wall blocks can be achieved by perineural injections, which have several advantages compared with neuraxial blockade. They have less severe consequences of infection or bleeding at the injection site, minimal interference with bladder and bowel function, and decreased incidence of lower extremity motor weakness, allowing early ambulation [2]. Abdominal wall blocks are performed using the classic landmark technique, but ultrasound (US)-guided blocks have recently gained popularity. The use of US, which allows non-invasive real-time imaging of the important anatomical structures, can make blocks safer and improve the placement accuracy [3].

Previous randomized controlled trials demonstrated the efficacy of the transversus abdominis plane (TAP) block as a component of a multimodal regimen in providing analgesia after abdominal surgery [4, 5]. One paper reported that postoperative pain was decreased by administering the TAP block for gynecological laparoscopic surgery with four port scars [6–8]. The effectiveness of this block in pain management after SILS procedures has also been reported [9]. The rectus sheath (RS) block provides analgesia after procedures requiring a midline incision by acting on the terminal branches of the 7th–11th intercostal nerves within RS. RS block has also been utilized for postoperative analgesia in patients who underwent umbilical hernia repair [10, 11]. The aim of this study was to investigate the efficacy of the combination of US-guided RS and TAP blocks compared with either TAP or RS blocks alone in gynecological SILS procedures.
Anesthesia

Without premedication, 500 ml of acetate Ringer’s solution was administered via peripheral venous access. Standard monitoring, including non-invasive blood pressure monitoring, three-lead electrocardiography, and pulse oximetry, were performed (S/5 anesthesia monitor. All patients were assessed using the bispectral index (BIS). Patients in both groups received propofol, 0.3 μg /kg/min of remifentanil, and one mg/kg of rocuronium before tracheal intubation. Propofol was administered via target-controlled infusion pumps with a target plasma concentration of 3.0 μg/ml. After intubation, the lungs were ventilated to an end-tidal carbon dioxide concentration within the range of 30–40 mmHg.

Following the induction of general anesthesia, bilateral TAP blocks were performed with 20 ml of 0.375% ropivacaine in 12 patients (Group A), bilateral TAP and RS blocks with ten ml of 0.375% ropivacaine each were performed in 12 patients (Group B), and bilateral RS blocks with 20 ml of 0.375% ropivacaine were performed in 12 patients (Group C) (Figure 1). The authors used a real-time, in-plane needle insertion technique under US guidance to perform TAP and RS blocks. A portable US device with a linear 6–13-MHz US transducer was used. The blocks were administered with a 22 gauge, 80 mm Tuohy nerve block needle.

The TAP block was administered using a mid-axillary approach. After visualization of the external oblique abdominal muscle (EOAM), internal oblique abdominal muscle (IOAM), and transverse abdominal muscle (TAM) at the level of a mid-axillary line between the 12th rib and the iliac crest, the puncture area and US probe were prepared in a sterile manner. After placing the tip of the needle in the space between IOAM and TAM and confirming negative aspiration of blood, the TAP block was bilaterally administered by infusion of 20 ml or ten ml of 0.375% ropivacaine per side in Groups A and B, respectively.

The area of the abdomen between the lateral border of the rectus muscle and one cm cephalad to the umbilicus and a US probe were prepared in a sterile manner. After visualization of the external oblique abdominal muscle (EOAM), internal oblique abdominal muscle (IOAM), and transverse abdominal muscle (TAM) at the level of a mid-axillary line between the 12th rib and the iliac crest, the puncture area and US probe were prepared in a sterile manner. After placing the tip of the needle in the space between IOAM and TAM and confirming negative aspiration of blood, the TAP block was bilaterally administered by infusion of 20 ml or ten ml of 0.375% ropivacaine per side in Groups A and B, respectively.

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Anesthesia was maintained by propofol and remifentanil titrated to maintain a mean arterial blood pressure of 80–120% of that measured before the induction of anesthesia. The propofol dose was adjusted to maintain BIS between 40 and 60 during surgery. Following skin closure, 100 mg of tramadol and five mg of prochlorperazine or 50 mg flurbiprofen intravenously, or 400 mg of acetaminophen administered by suppository were provided and anesthesia was discontinued before tracheal extubation. To manage postoperative pain, the authors used four types of analgesics; namely, tramadol 12 mg by patient-controlled intravenous analgesia (iv-PCA), flurbiprofen 50 mg intravenously, roxiprosoprophene 60 mg orally, and dicrofenac 50 mg suppositories (Table 1). Patient-controlled analgesia (PCA) was performed by intravenous administration of 300 mg of tramadol and 44 ml of saline in a total of 50 ml using the bolus settings for 12 mg tramadol with a ten-minute lockout interval time without basal infusion.

Assessment

The sites of administration of TAP and RS blocks were visually checked for the presence of hematocoles or infection. The total amounts of remifentanil and propofol administered to each patient were recorded. The presence or absence of nausea and vomiting after extubation and during the first 24 hours post-surgery were recorded for each patient.

Analgesic effects were evaluated by the presence or absence of umbilical pain on the completion of general anesthesia and pain intensity using a numerical rating scale (NRS) at zero, six, 12, 24, and 48 hours post-surgery. NRS was used to assess pain intensity in patients who were able to self-report. All patients were assessed on an 11-point scale numbered from 0 to 10 (high scores indicating intolerable pain).

Statistical analysis

All data are expressed as medians and ranges. Statistical analyses were conducted by a Chi-square test and one-way analysis of variance. A level of $p < 0.05$ was considered to be significant.

Table 1. — Postoperative pain relief medication.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tramadol 2 mg/kg</td>
<td>11</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Theater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaminophen 400 mg</td>
<td>6</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Theater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurubiprophen 50 mg</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ward</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCIA*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ward</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurubiprophen 50 mg</td>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Ward</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roxiprosoprophene 60 mg</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Ward</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac 50 mg</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*PCA: patient controlled intravenous analgesia.
A combination of ultrasound-guided rectus sheath and transversus abdominis plane blocks is superior to either block alone etc.

Results

All patients successfully underwent transumbilical SILS without open conversion. No complications due to the block procedures were encountered. There were no significant differences in age, body height and weight, or in the doses of anesthetic among the three groups (Table 2). The duration of anesthesia and surgery in Group A was significantly longer than that in Groups B and C ($p < 0.05$).

No patients in the three groups showed nausea and vomiting after extubation and during the first 24 hours post-surgery. Umbilical pain on the completion of general anesthesia was significantly less frequent in Group B (1/12) than Group A (7/12) ($p = 0.03$) (Figure 2).

Postoperative NRS in Group B was significantly lower than that of Group A at zero ($p = 0.02$) and six ($p = 0.03$) hours post-surgery and Group C at zero ($p = 0.001$), six ($p = 0.02$), and 12 ($p = 0.004$) hours post-surgery (Figure 3). There were no significant differences in the NRS scores at 24 and 48 hours post-surgery.

Discussion

This retrospective study revealed that a combination of RS and TAP blocks more greatly reduced abdominal pain in the early postoperative period as compared with either RS or TAP blocks alone in patients having undergone SILS for ovarian tumors.

Smith et al. reported that postoperative analgesia evaluated with visual analogue pain scores was significantly lower in patients supplemented with bilateral RS blocks as compared to those without these blocks after diagnostic laparoscopy [12]. This regional anesthetic technique has become increasingly popular and is used to provide analgesia for umbilical and epigastric hernia repair, laparoscopic surgery, and other small midline incisions. El-Dawlatly et al. reported that US-guided TAP blocks substantially reduced the perioperative opioid consumption in patients undergoing laparoscopic cholecystectomy under standard general anesthesia [13]. To the present authors’ knowledge, the adequate method of trunk blocks for SILS remains known because reports regarding this issue have been scarce until now [9].

The anterior abdominal wall, including the skin, muscle, and parietal peritoneum, are innervated by the anterior rami

<table>
<thead>
<tr>
<th>Table 2. — Demographics of 3 groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>Body weight (cm)</td>
</tr>
<tr>
<td>Body height (kg)</td>
</tr>
<tr>
<td>Anesthesia (min)*</td>
</tr>
<tr>
<td>Operation (min)*</td>
</tr>
<tr>
<td>Pneumoperitoneum (min)*</td>
</tr>
<tr>
<td>Remifentanil (mg)</td>
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*Group A was significantly higher than Group B and C ($p < 0.05$).
of T6–L1, which include the intercostal, subcostal, ilioinguinal, and iliohypogastric nerves. The thoracolumbar nerves course through the lateral abdominal wall within TAP. The sensory nerves send out a lateral cutaneous branch near the midaxillary line, and continue within TAP to supply sensation to the abdominal wall as far as the midline. There is extensive branching and communication of the segmental nerves within TAP [14]. The US-guided TAP block results in sensory blockade below the umbilicus (T10–L1) according to the data reported by Shibata et al. [15] and Tran et al. [16]. An injection of local anesthetic within TAP can therefore potentially provide unilateral analgesia to the skin, muscle, and parietal peritoneum of the anterior abdominal wall mostly in the area between T10 and L1 [14].

RS block has been used to provide surgical anesthesia as well as postoperative analgesia for surgical procedures involving a vertical midline laparotomy incision and laparoscopic procedures [17, 18]. Bilateral RS blocks have recently been used for the repair of umbilical hernias in pediatric patients [10, 11]. Because the RS plexus forms a longitudinal band of nerve fibers running cranio-caudally with the deep inferior epigastric artery, the RS block covers several segmental levels [19]. In between the rectus abdominis muscle and the three lateral muscles, including EAOM, IOAM, and TAM, is the linea semilunaris. The RS block targets only the midline, not beyond the linea semilunaris, whereas the TAP block targets the nerves of the anterolateral abdominal wall within the neurovascular plane. When an injection is applied in the posterior aspect of RS, the local anesthetic spreads out and blocks nerves from T6–T11 as they enter RS [14].

Prior to this present study, the authors speculated that the RS block would be preferable for analgesia after SILS. This is because the surgical incision of performing SILS is limited only to the umbilicus. The umbilicus is always innervated by a branch of T10. Therefore, either RS or TAP blocks alone should theoretically cover the umbilical area. The fact that the combination of US-guided RS and TAP blocks reduced early postoperative pain compared with either RS and TAP blocks alone in patients undergoing gynecological transumbilical SILS may suggest that the pain was not limited to the umbilicus and had spread over a wider area. The present authors surmise that patients feel two kinds of pain post-SILS. One is pain continuously caused by the large laparoscopic port scar and the other is pain from the expansion of the peritoneum during surgery. The latter, pneumoperitoneum, might be responsible for early postoperative anterior abdominal pain. The combination of RS and TAP blocks is considered to cover a greater cranial area as compared with the TAP block alone, as well as the area beyond the linea semilunaris, where the RS block is known to have minimal effect. It is speculated that the combination block is successful because the RS block efficiently reduces pain cephalad to the umbilicus and the TAP block reduces pain related to pneumoperitoneum by covering the area of the entire anterolateral abdomen.

El-Dawlatly et al. reported that US guidance enabled the exact placement of local anesthetic for TAP blocks [13]. TAP blocks were previously conducted using a landmark-based technique within the ilio-lumbar triangle of Petit as described by McDonnell et al. [4]. The use of US-guided blocks may decrease the risk of complications, although no study has directly compared landmark-based approaches with US-guided techniques. The key to success with regional anesthesia is to place the correct dose of local anesthetic in the right anatomic location. Using real-time US imaging, the tip of the needle and the spread of local anesthetic can be observed within the potential space [20, 21]. The present authors have routinely performed abdominal wall blocks with US guidance. If the correct spread is not observed, the needle is carefully redirected until adequate placement is achieved. They did not encounter any complications due to RS and/or TAP block procedures in this case series.

This study had some limitations. First, the authors did not include the evaluation of the sensory block in the skin in this retrospective study. Second, they collected the data used in this study from existing medical records. The pain scores and quantities of the three types of analgesics used to manage postoperative pain were documented by different nurses and therefore might have been transcribed inconsistently. Third, Group A had significantly longer durations of anesthesia, surgery, and pneumoperitoneum than Groups B and C. However, this study did include a good baseline comparability of age, body height and weight, and disease similarity among the three groups.

Previous reports have shown that trunk blocks rely on the spread of injectates through an appropriate plane and thus, the volume of local anesthetic used is critical to their success. In particular, adequate volume is more important than a high concentration. The choice of local anesthetic also varies amongst the publications. The most common seems to be ropivicaine or bupivacaine in varying degrees of strength and volume [17]. Future studies will be needed to evaluate variations in the type, strength, and volume of local anesthetics.

Conclusion

The combination of US-guided RS and TAP blocks is useful to reduce early postoperative abdominal pain compared with either RS or TAP blocks alone in patients undergoing transumbilical SILS for ovarian tumors.

A portion of this data was presented in an abstract/poster form at the annual meeting of the American Society of Anesthesiologists (ASA) 2012 in Washington, DC, USA.
Acknowledgments

The authors thank the patients who participated in this investigation and the doctors and nurses from the Department of Obstetrics and Gynecology at Nissay Hospital for their assistance in facilitating this investigation. They also thank Enago (www.enago.jp) for the English language review.

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Role of mean platelet volume and neutrophil/lymphocyte ratio to predict single-dose methotrexate treatment success in tubal ectopic pregnancy

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Summary
Objective: The aim of the study was to evaluate the value of mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) to predict single-dose methotrexate (MTX) treatment success in ectopic pregnancy (EP). Materials and Methods: A total of 115 EP diagnosed and hemodynamically stable women were enrolled in the study and divided into two groups as group 1, the treatment success group (n = 78) and group 2, the treatment failure group (n = 37). The authors compared the groups in terms of MPV and NLR. Results: MPV and NLR levels were higher in MTX treatment successful group than in failure group. The cut-off values of MPV and NLR were determined as 10.1 fL and 1.82, respectively. These cut-off values showed similar sensitivity and specificity in prediction of MTX treatment success. Conclusion: MPV and NLR can be used as reliable markers to predict single-dose MTX treatment success however further studies are needed.

Key words: Mean platelet volume; Neutrophil/lymphocyte ratio; Ectopic pregnancy.

Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and is responsible for pregnancy-related deaths in the first trimester [1]. Methotrexate (MTX) is the most popular agent for the treatment of EP worldwide. There are a greater percentage of women treated medically with single dose (50 mg/m²) MTX which is a folate antagonist and acts on rapidly dividing cells at the implantation site, most notably trophoblast cells [2].

Over the years, many putative markers to predict MTX treatment success have been described in the literature but none of them could reach sufficient achievement, therefore researchers have been attempting to find more effective and sensitive markers to predict MTX treatment success [3]. Recent studies have highlighted that some inflammatory cytokines are increased both at the implantation site and systemic circulation in tubal ectopic pregnancies which play role in angiogenesis, inflammation, and immunity [4]. Neutrophil/lymphocyte ratio (NLR) is an inflammatory response marker in peripheral blood. NLR possesses diagnostic value in certain pathologies characterised by systemic or local inflammatory response, such as diabetes mellitus, coronary artery disease, ulcerative colitis, and inflammatory arthritis [5-7].

Mean platelet volume (MPV) is a marker of platelet count and platelet activity. Platelets have also been suggested to play important roles in immune and/or inflammatory processes [8]. MPV levels are higher in inflammatory conditions such as active rheumatoid arthritis, acute attack of familial Mediterranean fever, and active chronic obstructive pulmonary disease [9].

In tubal EP, either ruptured or not, inflammation occurs in the microenvironment of the fallopian tube which may affect inflammation markers [10]. In this trial the authors aimed to evaluate the value MPV and NLR to predict single-dose MTX treatment success in EP.

Materials and Methods

A total of 115 EPs diagnosed and hemodynamically stable women in Umraniye Medical and Research Hospital between 2011-2014 were enrolled in this retrospective study. The ethical approval was taken from Institutional Ethics Committee. The diagnosis of EP was conducted in two ways, namely, by direct visualization of extra uterine gestational sac or by the occurrence of persistent/rising b-hCG values without intrauterine evidence of pregnancy by sonography or histology. Patients with chronic liver disease, pre-existing blood dyscrasias, pulmonary disease, peptic ulcer disease and immunodeficiency, who had sensitivity to MTX, had an intrauterine pregnancy, an unruptured mass of > 3.5 cm, fetal cardiac activity detected, and a quantitative hCG level of > 15,000 mIU/ml were excluded from the study. Moreover the authors also excluded patients with non-adnexal EP, incomplete records, patients who were lost to follow-up evaluation and who had other infections, inflammatory disease, type 2 diabetes mellitus, prediabetes, smoking, hypertension, hypercholesterolemia, obesity, coronary heart...
disease, metabolic syndrome, stating and some antihypertensive use, and atrial fibrillation.

In this paper, the authors included the patients who received single-dose MTX treatment, had complete medical records that fulfilled the aforementioned criteria, and had days 0, 4, and 7 b-hCG values appropriately recorded after MTX administration.

Before any medication, five to seven cc of peripheral venous blood was collected into sterile ethylenediaminetetraacetic acid (EDTA) tubes from all patients. Hematological parameters were analysed within 30 minutes after collection for minimizing the effect of EDTA on platelets by using a haematology analyser. Leucocyte (/mm³), neutrophil (/mm³), lymphocyte (/mm³), and platelet (/mm³) counts were recorded. NLR was calculated using the results of these parameters. Haemoglobin levels (g/dL) and MPV (fL) were determined. MTX treatment was decided as successful in patients who had a 15% decline of b-hCG levels between the fourth and seventh days of treatment.

To evaluate the efficacy of MTX treatment, the study population was divided into two groups; namely group 1, the treatment success group (n = 78) and group 2, the treatment failure group (n = 37) and were compared in terms of MPV and NLR.

Statistical analyses
Statistical analysis was performed with SPSS 12.0 software. Data are expressed as mean ± standard deviation. Chi-square or Fisher’s Exact test was used to compare categorical variables, and the Wilcoxon Rank Sum test was used to compare continuous variables. A p value < 0.05 was considered statistically significant. Pearson correlation test was also used.

Results
The mean age of the group 1 was 30 ± 4.4 years and the mean age of the group 2 was 28 ± 5.9 years. There was no significant difference among groups regarding the mean age (p > 0.05) The mean hemoglobin levels were 11 ± 1.2 g/dl and 10 ± 2.6 g/dl in groups 1 and 2, respectively (p > 0.05).

Prediction of MTX treatment success with b-hCG
The mean day 1 b-hCG levels of group 1 was 1,348 mIU/ml and the mean day 1 b-hCG levels of group 2 was 3,960 mIU/ml. The mean initial b-hCG level was significantly lower in the treatment success group than in the treatment failure group (p < 0.05). The b-hCG levels increased between days 0 and 4 in 53.9% of cases (62/115); b-hCG levels decreased between days 0 and 4 in 46% of cases (53/115). The number of cases with decreasing b-hCG level on day 4 was significantly more in the treatment success group than in the failure group (36% vs. 14.9%, respectively, p < 0.05) (Table 1).

Prediction of MTX treatment success with MPV
Platelet values and MPV of the groups are shown in Table 2. There were no statistically difference between groups in terms of platelet counts (264 ± 75.2 /mm³ vs. 273 ± 65 /mm³, respectively). In group 1 the MPV levels were statistically higher than in group 2 (9.1 ± 0.4 fL vs. 11.5 ± 0.7 fL, p < 0.05). Optimal cut off value was obtained at a level of 10.1 fL with 62% sensitivity and 66% specificity, respectively.

Prediction of MTX treatment success with NLR
NLR was calculated and assessed in both the groups, and it was found that NLR was higher in MTX treatment successful group than in failure group (2.14 ± 0.04 vs. 1.76 ± 0.1, p < 0.05) (Table 2). Optimal cut off value was obtained at a level of 1.82 with 59% sensitivity and 64% specificity, respectively.

Discussion
Single-dose MTX regimen constitutes a safe and effective treatment modality for tubal EP in selected patients. In the literature there are many studies reporting success rates of single-dose MTX administration varying from 52% to 94% among patients with asymptomatic EP [11]; however which patients will benefit from single-dose MTX treatment is still controversial. This study investigated the value of MPV and NLR to predict single-dose MTX treatment success in EP. The main finding of this study was higher MPV and NLR levels in tubal EPs may be used as predictors of MTX treatment success.

There are many suggested predictors for MTX treatment failure which include high b-hCG concentration (5,000 IU/ml), fetal cardiac activity, larger ectopic size (four cm), and sonographic evidence of free peritoneal fluid [3]. In another study, it is suggested that b-hCG levels, alone or combined with serum progesterone levels, have an important predictive value [12]. In the present study the authors excluded the patients with fetal cardiac activity, larger ectopic size (four cm), and sonographic evidence of free peritoneal fluid, but also found that the treatment failure rate was increasing significantly when the initial serum b-hCG level was higher than 3,000 mIU (p < 0.05).

Previous studies have demonstrated that inflammation and microenvironmental changes occur in fallopian tube in

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Table 1. — MTX treatment success prediction with b-hCG levels.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (MTX treatment successful)</th>
<th>Group 2 (MTX treatment failure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=78)</td>
<td>(n=37)</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>11 ± 1.2</td>
<td>10 ± 2.6</td>
</tr>
<tr>
<td>Day 1 b-hCG (mIU/ml)</td>
<td>1348</td>
<td>3960</td>
</tr>
<tr>
<td>Declining of b-hCG values between days 1 and 4 (%)</td>
<td>36</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of MPV, NLR, and platelet counts between groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (MTX treatment successful)</th>
<th>Group 2 (MTX treatment failure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=78)</td>
<td>(n=37)</td>
</tr>
<tr>
<td>Platelet count /mm³</td>
<td>264 ± 75.2</td>
<td>273 ± 65</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>11.5 ± 0.7</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>NLR</td>
<td>2.14 ± 0.04</td>
<td>1.76 ± 0.1</td>
</tr>
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</table>
tubal EPs [10]. This inflammation is suggested to activate platelets which are well-known sources of growth factors. Some of these factors are vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), transforming growth factor-beta (TGF-β), and they have important roles in causing alterations in MPV [13]. In an activated state, some morphological changes occur and platelets form pseudopodias and become spherical in shape. Turgut et al. also showed that patients with ectopic pregnancy had significantly higher MPV levels [14]. In the present study, the authors found that MPV was higher in MTX treatment successful group when compared to treatment failure group. According to their hypothesis, due to inadequate inflammation and lower MPV levels, angiogenic cytokine levels and blood flow were lower in MTX failure group. For this reason they suggest that MTX may not have reached a sufficient concentration in EP tissue in this group. As far as they know, the present study is the first to evaluate the role of MPV in prediction of MTX treatment success.

NLR obtained by dividing neutrophil count to lymphocyte count, is considered as an other inflammatory marker [5]. In the present study, NLR was calculated and assessed in both groups, and it was found that NLR was lower in MTX failure group. This result proves the inadequate inflammatory state in MTX failure group when compared with MTX successful group. In concordance with the present study, Kim et al. reported NLR as a reliable marker for placental inflammation prediction [15]. The study of Sönmez et al. also emphasized that NLR is a simple and inexpensive method in order to evaluate inflammatory status in patients with acute coronary syndrome [6]. There are several studies assessing NLR in various diseases including ulcerative colitis and hepatic cirrhosis, familial Mediterranean fever, cardiovascular diseases, and malignancies [7].

Early b-hCG changes after MTX therapy were also accepted as a predictor of treatment outcome after MTX. In a trial, it was reported that declining b-hCG values of at least 20% between days 1 and 4 during MTX treatment have been associated with a positive predictive value of 97% for treatment success [16]. In this present study the authors also found a correlation between early b-hCG levels after MTX therapy and treatment success. Adjustment of MPV and NLR may increase predictive value of early b-hCG changes after MTX therapy. This may be a new aim for a new study.

In conclusion, this was a preliminary study to evaluate the predictive value of MPV and NLR in single-dose MTX treatment success of tubal EP and the authors concluded that MPV and NLR can be used as reliable markers to predict single-dose MTX treatment success without any clinical signs, but it is necessary to be studied in different cohort groups.

References


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Diagnosis of endometriosis in women with chronic pelvic pain

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Summary

Purpose of investigation: To assess the accuracy of CA-125 determination associated with clinical history and of the neutrophil/lymphocyte (N/L) ratio for a presumptive diagnosis of endometriosis in women with chronic pelvic pain (CPP). Materials and Methods: This was a cross-sectional study of data from the medical records of women with CPP submitted to laparoscopy from August 1999 to January 2009 at the University Hospital. The performance of the evaluation of CA-125 and of the N/L ratio for the prediction of endometriosis was compared based on the corresponding ROC curves and their 95% confidence intervals. Results: CA-125 levels were significantly higher in women with CPP and endometriosis and their association with a complaint of dysmenorrhea improved their sensitivity. For a cut-off of 20 IU/ml, the predictive value for a diagnosis of endometriosis in women with CPP was 97.6%. Dyspareunia, subfertility, and N/L ratio were not useful for a diagnosis of endometriosis in women with CPP. Conclusion: The association of elevated CA-125 levels with a complaint of dysmenorrhea is adequate in a presumptive and accurate diagnosis of endometriosis in this specific group of women with CPP, permitting an early institution of clinical treatment without the need of previous laparoscopic confirmation.

Key words: CA-125; Diagnosis; Endometriosis; Dysmenorrhea; Chronic pelvic pain.

Introduction

Endometriosis is a chronic and recurrent disease characterized by the presence of endometrial tissue outside the uterine cavity, mainly identified in the pelvic peritoneum and in the ovaries, leading to an inflammatory process, cicatricial reaction, and formation of adhesions associated with clinical signs and symptoms of dysmenorrhea, dyspareunia, chronic pelvic pain (CPP), and subfertility [1-4]. The prevalence of CPP in Brazil is 15.1% among women in menacme [5], and about 30% of these women are estimated to have endometriosis [6].

The gold standard for the diagnosis of endometriosis is laparoscopy, an invasive procedure involving costs and risks [7]. Thus, the time between the onset of symptoms and the indication of laparoscopy is often quite long, 7.4 years on average [8]. The delay of the diagnosis of patients with endometriosis and CPP impairs the treatment of pain. The prolonged time of symptoms, especially pain, causes the onset of events that may impair the diagnosis and limit the therapeutic proposals, such as peripheral sensitization secondary to neurogenic inflammation, central sensitization with a consequent reduction of thresholds to potentially painful stimuli (hyperalgnesia) or potentially not painful ones (allodynia), changes in mood such as anxiety and depression, and a difficult doctor-patient relationship [9-12].

Tumor markers, particularly CA-125, represent an important research line for the diagnosis and follow-up of endometriosis [13], although with limited sensitivity and specificity [14, 15]. In view of the inflammatory state inherent to endometriosis, the neutrophil/lymphocyte (N/L) ratio has been studied in the search for a diagnostic test of low cost and easy application. The N/L ratio can increase the sensitivity of CA-125 for the diagnosis of minimal and mild endometriosis in premenopausal women with no myomas or adenomyosis when a cut-off value of 20.1 is considered, with 59.7% sensitivity and 60.1% specificity [16]. Currently, it is not possible to say whether this association can improve the sensitivity and specificity of CA-125 in diagnosis of endometriosis among the population of women with CPP. Thus, the objective of the present study was to assess the accuracy of CA-125 determination in combination with clinical history and of the N/L ratio for a presumptive diagnosis of endometriosis in women with CPP, in order to begin an early clinical treatment without the need for previous laparoscopic confirmation.

Materials and Methods

This was a cross-sectional study based on the analysis of the medical records of 208 women with CPP submitted to laparoscopy between August 1999 and January 2009 at the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. A total of 168 medical records containing complete data were included in the study. The data considered were: a clinical history with quantification of pain using a visual analogue scale (VAS), preoperative determination of CA-125 by chemiluminescence at the beginning of the menstrual period, and hematologic examination for the determination of the N/L ratio. The sample was divided into two groups: with endometriosis (vi-
visual diagnosis when typical lesions were present and with the aid of anatomopathological examination when lesions were atypical), and without endometriosis. Endometriosis was staged according to the classification of the American Society for Reproductive Medicine (ASRM) 1996 [17]. CPP was defined as pelvic pain not exclusively menstrual, lasting six months or more and sufficiently intense to interfere with habitual activities and to require medical, clinical or surgical, treatment [18].

Statistical analysis
Data were analyzed for normal distribution by the D’Agostino Pearson test. Quantitative variables were compared between groups by the unpaired t-test or Mann-Whitney test. The proportions observed for dyspareunia, dysmenorrhea, and previous abdominal surgeries were compared between groups by the Fisher exact test. To compare the performance of the evaluation of CA-125 and of the N/L ratio for the prediction of endometriosis, the corresponding ROC curves and their 95% confidence limits (95% CI) were calculated.

Results
The mean age of the women with endometriosis was lower (32.9 ± 7.45 years) than that of the control group (35.6 ± 8.82 years) (p = 0.04). CA-125 levels and the proportion of women with dysmenorrhea were significantly higher in women with endometriosis compared to control. In contrast, the N/L ratio, the rates of infertility and the intensity of pain according to the VAS did not differ between groups (Table 1).

The evaluation of the accuracy of CA-125 as a diagnostic test for endometriosis in women with CPP based on the ROC curve revealed an area under the curve of 0.71 (95% CI 0.63-0.77, p < 0.001), with 55% sensitivity and 80.7% specificity, for a cut-off value of 11.4 IU/ml. In contrast, for the N/L ratio, the area under the curve was only 0.51 (95% CI 0.43-0.59, p = 0.75).

When a complaint of dysmenorrhea was associated with CA-125, a curve significantly different from random occurrence was observed, with an area under the curve of 0.64 - 95% CI (0.56-0.71) is observed, with 88.3% sensitivity and 40.4% specificity (cut-off of 11.4).

Discussion
The mean age at diagnosis of endometriosis of the women with CPP in the present study was similar to that reported by Arruda et al. [8], who observed a mean age of 33 years for this diagnosis among Brazilian women with endometriosis. The difference in age observed between the groups with and without endometriosis may
be explained by the lower frequency of severe dysmenorrhea in the group without endometriosis, a fact that may delay the indication of laparoscopy for these patients. Ballard et al. [4] demonstrated that the symptoms associated with endometriosis are relatively uncommon in women without endometriosis. On the other hand, women with abdominal-pelvic pain, menstrual-related symptoms (mainly dysmenorrhea), dyspareunia, and subfertility are at high risk to have endometriosis [4].

The high prevalence of dyspareunia observed in women with CPP in the present study has also been reported by Verit et al. [19]. Dyspareunia and subfertility are symptoms frequently used for the diagnosis of pelvic endometriosis. However, considering their high frequency in this specific population, the presence or absence of dyspareunia and subfertility are of no value for a differential diagnosis. In women with CPP, dyspareunia is frequently associated with possible sequelae of inflammatory pelvic disease, with painful spasms of pelvic floor muscles, and with bladder pain syndrome, exacerbated by the chronic pain situation itself, with a reduction of sensitive and painful thresholds. In contrast to dyspareunia and subfertility, dysmenorrhea was significantly more prevalent among women with endometriosis and CPP, as also reported by others in patients with endometriosis [4, 20, 21].

CA-125 levels were significantly higher in women with CPP and endometriosis than in women without endometriosis, also showing association with disease severity, in agreement with literature data [21]. The evaluation of its accuracy as a diagnostic test by the ROC curve resulted in a curve significantly differing from a random occurrence, but still showed a moderate efficacy as a diagnostic test. The association of a complaint of dysmenorrhea with CA-125 led to improved sensitivity with a cut-off of 11.4, for a better prediction of endometriosis in this group of patients. When the authors used a cut-off of 20 IU/ml for CA 125, a value already previously used in the authors’ service for the diagnosis of endometriosis [22], this value proved to be more accurate for the diagnosis of endometriosis among patients with CPP because of its high predictive value (97.6%).

The N/L ratio was not useful for the diagnosis of endometriosis in these women with CPP. This result agrees with the theory that there is a potential inflammatory state in patients with CPP, regardless of the etiology of the condition [23]. Thus, the N/L ratio could not contribute to the diagnosis of endometriosis in this specific group of patients.

Conclusion

The association of CA-125 levels higher than 20 IU/ml with a clinical complaint of dysmenorrhea was adequate for a presumptive and accurate diagnosis of endometriosis in women with CPP, permitting an early institution of clinical treatment without the need for previous laparoscopic confirmation.

References


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Preeclampsia is a serious complication of pregnancy characterised clinically by maternal hypertension and proteinuria after 20 weeks of gestation, affecting 3–5% of all pregnancies, and results in substantial maternal and neonatal morbidity and mortality [1]. The mechanisms involved in the etiology of this disorder have not been clearly identified. Endothelial dysfunction, which is one of the early stages of atherosclerosis, plays an important role in the pathogenesis of preeclampsia. Insulin resistance and low-grade systemic inflammation may contribute to the pathogenesis of endothelial dysfunction. In addition, placental ischaemia secondary to an initial defective placentation and generalised endothelial cell damage have been proposed to be the pathogenic mechanisms underlying preeclampsia [2].

Adipose tissue is not only involved in energy storage but also functions as an endocrine organ that expresses and secretes a variety of hormones and cytokines, which are collectively named as adipokines. Some of the adipokines, such as leptin, adiponectin, resistin, and ghrelin play roles in the regulation of glucose metabolism and are involved in the development of obesity, diabetes mellitus, inflammation, auto-immunity, and metabolic syndrome [3]. In addition, adipokines have a role in regulating maternal energy metabolism and insulin sensitivity during gestation and have been implicated in pregnancy complications, including gestational diabetes mellitus, fetal growth restriction, and preeclampsia [4-6].

Resistin, also known as adipocyte secreted factor (ADSF), is a cysteine-rich adipokine that was originally described as a molecular link between obesity and insulin resistance in mice, as its serum levels are increased in both diet-induced and in genetic mouse models of obesity [7]. In animal models, resistin is expressed almost exclusively in adipocytes, whereas human resistin is expressed and secreted predominantly from mononuclear cells, and plays important roles in regulating energy homeostasis [8, 9]. Resistin impairs glucose tolerance and opposes the action of insulin in peripheral tissues [10]. Previous studies have suggested roles of resistin in obesity and insulin resistance, although these remain controversial. Some studies have shown positive correlations with body fat mass and insulin resistance [11, 12], whereas others have found no such cor-
relations with body mass index (BMI) or insulin sensitivity [7, 13]. Although resistin was first postulated to contribute to insulin resistance, accumulating evidence suggested that it might also be involved in inflammatory processes. In human, pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as lipopolysaccharides, strongly induce resistin mRNA expression [14]. Moreover, resistin can up-regulate the expression of IL-6, IL-1β, and TNF-α, and thereby enhance its own activity by positive feedback [15]. Resistin has also been linked to inflammation in that serum resistin levels were shown to be associated with many inflammatory markers in patients with severe inflammatory conditions [16].

Given the potential role of resistin as a mediator of insulin sensitivity and inflammation, the present authors evaluated the serum level and placental expression of resistin in normal pregnancies and in those complicated by preeclampsia. They hypothesised that circulating concentrations and placental expression of resistin may be altered in relation to disease severity.

**Materials and Methods**

This cross-sectional study was conducted at Antalya Training and Research Hospital, Antalya, Turkey, between January 2012 and March 2013. The Institutional Ethics Committee approved the study, and the patients who agreed to participate provided signed informed consent.

The study groups consisted of 50 women with mild preeclampsia and 48 women with severe preeclampsia. All subjects in these groups had late-onset preeclampsia diagnosed at gestational week 34 or later. The control group consisted of 50 normotensive healthy pregnant women. The study and control groups were matched for age and BMI. All the pregnant women enrolled in the study were non-smokers, had similar demographic backgrounds, and were admitted for delivery. Additionally, the authors selected only women who delivered via elective cesarean section, thus eliminating any possible influence of labor or premature membrane rupture. The indication for elective cesarean section was a prior cesarean section. Exclusion criteria included multiple gestation, chronic hypertension, diabetes mellitus, vascular or inflammatory diseases, premature rupture of membranes, known fetal structural anomalies, clinical chorioamnionitis, previous exposure to magnesium sulfate, and active labor.

Preeclampsia was defined as the presence of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, six hours apart) and proteinuria (≥ 300 mg in 24-hour urine collection or at least one dipstick measurement ≥ 1+) after the 20th week of gestation as defined by the International Society of Hypertension in Pregnancy [17]. Blood pressure was taken with the woman in the sitting position after a ten-minute rest period. Preeclampsia was classified as severe if the woman had one or more of the following symptoms: blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic, ≥ 3+ protein by dipstick test in two urine samples taken at four hour or more apart or five grams of protein in 24-hour urine sample, epigastric or right upper-quadrant pain, blurring of vision, cerebral disturbance, abnormal liver function, pulmonary edema or cyanosis, low platelet level, and oliguria < 500 ml in 24 hours. Gestational age was determined by the last menstrual period and confirmed by ultrasonographic examination performed during the first trimester. BMI values were calculated using the following formula: weight (kg)/height (m²).

All patients underwent Doppler examination at admission prior to delivery. Doppler examinations were performed using a 2–7-MHz transabdominal transducer in the lateral decubitus position to avoid supine hypotension. Doppler measurements were performed in the absence of fetal movements and during voluntarily suspension of maternal breathing. Spectral Doppler parameters were determined automatically from three or more consecutive
waveforms, holding the angle of insonation as close to 0° as possible. Umbilical artery Doppler velocimetry was performed on a free loop of the umbilical cord located distant from the points of fetal and placental insertions. Both uterine arteries were assessed at the level at which they crossed the external iliac arteries. The mean values of parameters derived from both uterine arteries were calculated and used in statistical analyses.

None of the preeclamptic patients or controls received any medications before blood sampling. A fasting venous blood sample (five ml) was obtained from all participants prior to cesarean section. All samples were kept at room temperature for at least 30 minutes to allow the blood to clot and centrifuged at 2,500 rpm for 15 minutes at 4°C to separate serum. Serum specimens were aliquoted and stored at −80°C until batch assay. For measurement of resistin, a commercially available sandwich immunoassay kit was used according to the manufacturer’s instructions. The sensitivity of the assay was 0.016 ng/ml and the inter- and intra-assay coefficients of variation were less than 15% and 10%, respectively. The assay results are expressed as ng/ml.

The placentas were obtained immediately after delivery in all subjects. Biopsies were taken from the central region of the placentas and stored immediately in 10% formaldehyde. After fixation, samples were embedded in paraffin after routine tissue work-up. Immunohistochemical staining of the placental tissues was performed by incubation with anti-resistin primary antibody (rabbit monoclonal, clone EPR3506, dilution 1:100. Sections were then washed with PBS and incubated with biotinylated secondary antibody for 20 minutes. The antigen-antibody complexes were visualised using DAB and counterstained with haematoxylin.

Expression rates for the positive cells in the specimens were determined by consensus. Figures 1B-F shows various resistin expression patterns. Immunohistochemical staining score ≥ 1,

### Table 1. Comparison of obstetric characteristics, Doppler parameters, and neonatal outcome of study groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=50)</th>
<th>Mild preeclampsia (n=50)</th>
<th>Severe preeclampsia (n=48)</th>
<th>p</th>
<th>p'</th>
<th>p''</th>
<th>p''</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>28.1 ± 5.7</td>
<td>25.6 ± 5.8</td>
<td>28.8 ± 5.8</td>
<td>0.148</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (w)</td>
<td>38.4 ± 0.7</td>
<td>37.9 ± 1.5</td>
<td>37.2 ± 1.2</td>
<td>0.001</td>
<td>0.115</td>
<td>&lt;0.001</td>
<td>0.038</td>
</tr>
<tr>
<td>Gravity</td>
<td>2 (1-7)</td>
<td>1 (1-5)</td>
<td>2 (1-10)</td>
<td>0.072</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0-4)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>0.119</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>108.1 ± 10.4</td>
<td>145.9 ± 11.4</td>
<td>160.4 ± 13.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>60.9 ± 7.5</td>
<td>94.6 ± 7.5</td>
<td>104.2 ± 11.9</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.5 ± 3.1</td>
<td>31.9 ± 5.2</td>
<td>30.1 ± 4.2</td>
<td>0.267</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical artery PI</td>
<td>0.70 ± 0.17</td>
<td>0.75 ± 0.22</td>
<td>1.16 ± 0.71</td>
<td>&lt;0.001</td>
<td>0.345</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Umbilical artery RI</td>
<td>0.52 ± 0.14</td>
<td>0.53 ± 0.13</td>
<td>0.66 ± 0.22</td>
<td>0.005</td>
<td>0.355</td>
<td>0.002</td>
<td>0.026</td>
</tr>
<tr>
<td>Uterine artery PI</td>
<td>0.69 ± 0.29</td>
<td>0.79 ± 0.28</td>
<td>1.07 ± 0.41</td>
<td>&lt;0.001</td>
<td>0.131</td>
<td>&lt;0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Uterine artery RI</td>
<td>0.45 ± 0.14</td>
<td>0.47 ± 0.11</td>
<td>0.57 ± 0.12</td>
<td>0.001</td>
<td>0.399</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3474 ± 332</td>
<td>3107 ± 710</td>
<td>2596 ± 622</td>
<td>&lt;0.001</td>
<td>0.038</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (standard deviation) or median (range). If the Kruskal–Wallis test was positive (p < 0.05), then post-hoc analysis was applied. p, between three groups; p', between mild preeclampsia and control; p'', between severe preeclampsia and control; p''', between severe preeclampsia and mild preeclampsia. BMI, body mass index; PI, pulsatility index; RI, resistance index.
In Figure 2, the box plots represent the distribution of serum resistin levels in all groups. There was a statistically significant between-group difference in serum resistin concentrations \((p < 0.001)\). The mean serum resistin levels were 2.61 ± 0.37 ng/ml in the control group, 3.32 ± 0.61 ng/ml in the mild preeclampsia group, and 3.82 ± 0.35 ng/ml in the severe preeclampsia group. Pairwise comparisons between the groups revealed significantly higher resistin levels in the severe preeclampsia group compared to the mild preeclampsia and control groups \((p = 0.042\) and \(p < 0.001\), respectively). Moreover, a significant difference was evident between the mild preeclampsia and control groups \((p = 0.012)\).

Figure 3 shows the immunoreactivity scores and expression of resistin in the placentas of the three groups. There was a statistically significant between-group difference in placental resistin expression \((p < 0.001)\). Pairwise comparisons between the groups revealed significantly increased resistin expression in the severe preeclampsia compared to mild preeclampsia \((p = 0.003)\) and controls \((p < 0.001)\).

The relationships between serum level and placental expression of resistin with other parameters assessed in all groups are shown in Table 2. Serum resistin levels were negatively correlated with birth weight \((r = -0.534, p < 0.001)\), but positively correlated with gestational age \((r = 0.433, p < 0.001)\), systolic and diastolic blood pressure \((r = 0.609, r = 0.598, p < 0.001\) for both), umbilical artery PI \((r = 0.36, p = 0.001)\) and RI \((r = 0.28, p = 0.013)\), uterine artery PI \((r = 0.322, p = 0.004)\) and RI \((r = 0.304, p = 0.007)\), but negatively correlated with birth weight \((r = -0.321, p = 0.004)\). On the other hand, placental resistin expression was positively correlated with systolic blood pressure \((r = 0.275, p = 0.015)\), uterine artery PI \((r = 0.322, p = 0.004)\) and RI \((r = 0.294, p = 0.009)\).
Discussion

In the present study, the authors investigated for the first time serum level and placental expression of resistin in relation to severity of preclampsia. The results indicated an increase in serum resistin concentrations and placental resistin expression in severe preclampsia compared with mild preeclampsia and healthy controls.

In normal pregnancy, maternal serum resistin concentration is significantly elevated compared to the non-pregnant state, and resistin gene expression in term placental tissue is more prominent than in first trimester chorionic tissue [18]. Many investigators have shown that the placenta is the major source of resistin during pregnancy, and the main production site of placental resistin is the cytotrophoblast, but resistin can also be secreted by extravillous cytotrophoblast and decidua [19]. Resistin plays a role in regulating energy metabolism during pregnancy. It contributes to decrease of insulin sensitivity in the second half of pregnancy, which may be related to the development of postpartum hyperglycemia and be beneficial for rapid fetal growth [20]. Although the mechanisms regulating resistin secretion during pregnancy have not been fully elucidated, insulin seems to be an important regulator of resistin gene expression and protein release; however, both inhibitory and stimulatory effects have been reported [21,22].

In addition to maternal biological functions, resistin plays critical roles in the developing fetus. Resistin is a physiological constituent of amniotic fluid, and its concentration increases with advancing gestation and plays a role in immune response against intra-amniotic infection [23]. In addition, resistin has been shown to be involved in fetal growth regulation, and markedly high resistin concentration in umbilical plasma may prevent neonatal hypoglycemia at birth by facilitating hepatic glucose production [24]. Its metabolic pathways may be impaired in fetuses with macrosomia and growth restriction [25]. Furthermore, resistin has been reported to induce the secretion of vascular endothelial growth factor (VEGF) and the proliferation of the fetoplacental unit, which may be another potential mechanism by which resistin contributes to normal fetal development [26].

Previous studies in women with established preeclampsia reported contradictory results in relation to resistin levels. Haugen et al. [27] reported elevated circulating resistin concentrations in patients with preeclampsia as compared to normal pregnant controls, although placental resistin gene expression was found to be unaltered. However, differences in resistin plasma levels between preeclampsia and normal pregnancies are lost after controlling for insulin resistance. Cortalazzi et al. [28] reported lower circulating resistin levels in preeclampsia as compared to normotensive healthy pregnant women, but gestational age at sampling varied widely (20-37 weeks) compared to the present study. This finding was corroborated by Chen et al. [29], and the authors postulated that lower levels of resistin in preeclampsia may be related to a reduction in placental production of this peptid due to smaller size of the placenta; however, this was not confirmed by determination of placental resistin expression. Hendler et al. [30] found no differences in circulating resistin levels between pregnant women with and without preeclampsia. In addition, the authors observed no correlation between serum resistin level and BMI, in agreement with our observations. Seol et al. [31] reported marked elevation of serum resistin levels in women with preeclampsia compared to those with normal pregnancies, but no significant differences in placental expression between the two groups were observed. In contrast, the present authors found significantly increased placental resistin expression in patients with severe preeclampsia compared to mild preeclampsia and healthy pregnant controls. Some of these discrepancies can be attributed to the differences in study design and sample size, assessment of patients at different gestational ages, lack of adjustment for BMI, and to other confounding factors, such as smoking, maternal age, and parity. Differences in assay methods must also be taken into consideration. Although all studies used commercially available immunoassays, some evaluated resistin in plasma [27, 28, 30], while others investigated serum levels [29, 31].

The results presented here indicated that preeclampsia is associated with higher serum levels and placental expression of resistin. Moreover, this study indicated that deterioration of fetoplacental and uteroplacental blood flow, manifested as increased umbilical and uterine artery Doppler indices, is related to serum resistin level and placental expression. Elevated blood pressure is a major clinical manifestation of preeclampsia and the present authors found that both systolic and diastolic blood pressure were positively correlated with serum resistin concentration. This study also indicated a negative correlation between serum resistin level and placental expression. Elevated blood pressure is a major clinical manifestation of preeclampsia and the present authors found that both systolic and diastolic blood pressure were positively correlated with serum resistin concentration. This study also indicated a negative correlation between serum resistin level and placental expression. Elevated blood pressure is a major clinical manifestation of preeclampsia and the present authors found that both systolic and diastolic blood pressure were positively correlated with serum resistin concentration. The changes in circulating maternal resistin serum concentrations favour a state of insulin resistance, which enhances the availability of glucose to the fetus as well as to maternal vital organs. The present authors suggested that elevated placental resistin expression in preeclampsia might be a compensatory response to increase nutrient delivery to the underperfused placenta.

The present study had several limitations. Mean gestational age was inherently lower in the mild and severe preeclampsia groups compared with healthy controls, although all samples analysed were taken in the third trimester. Furthermore, the authors could not investigate fetal cord levels of resistin, thus this apparently unknown feature will require further investigation to clarify.

Conclusion

The present findings suggest that increased serum level and placental expression of resistin may play a role in the pathogenesis of preeclampsia, and seems to be correlated with disease severity. Further studies are required to confirm the present results.
References


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The clinical significance of CA19-9 in ovarian mature cystic teratoma


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Summary

Objective: To evaluate the clinical significance of CA19-9 in patients with ovarian mature cystic teratoma (MCT). Materials and Methods: A retrospective study was performed on 65 patients with pathologically-confirmed MCT and 80 patients with benign epithelial ovarian tumors. Serum tumor markers for all patients and tissue CA19-9 for MCTs were measured. The relationships between clinical characteristics of MCTs and CA19-9, as well as the correlation between serum and tissue level of CA19-9 in MCTs, were evaluated. Results: The mean serum level of CA19-9 in MCTs was significantly higher than that in benign ovarian epithelial tumors (49.9 ± 73.4 IU/ml vs. 17.08 ± 24.8 IU/ml). CA19-9 was the only tumor marker with a mean serum level above the cut-off value and the elevation rate was 30.76% in MCTs. The positive tissue expression rate of CA19-9 in MCT patients were 50.9 % and were higher than that of preoperative serum levels (50.9% vs. 32.7%). Conclusion: Serum CA19-9 has the highest positivity rate among other tumor markers in MCT. Elevated serum CA19-9 is not an uncommon finding MCT and could be used as a marker in the differential diagnosis of MCT in patients with pelvic mass.

Key words: Mature cystic teratoma; Ovarian epithelial tumor; CA19-9; Tumor marker.

Introduction

Mature cystic teratoma (MCT) or dermoid cysts are the most common type of ovarian tumor accounting for 10-20% of all primary ovarian tumors [1]. MCTs generally occur during reproductive years with a mean age of 33 years and are commonly unilateral (about 85% of cases) [2]. Most cases present as an asymptomatic adnexal mass incidentally detected on routine pelvic examination. A vast majority of these tumors are benign and in about 1% of MCTs, one tissue element shows malignant transformation, most often to squamous cell carcinoma [3].

Serum tumor markers have been used in the management of pelvic masses and ovarian cancer. Clinical usefulness of tumor markers including CA125 and CA19-9 in patients with MCTs has been evaluated in few studies only [4-7]. CA19-9 is a powerful and independent prognostic factor in patients with pancreatic carcinoma [8]. However, the relationship of CA19-9 with MCTs is not well established. The current data are insufficient and includes few trials and mostly case reports [9-11]. In patients with MCTs, increased serum levels of CA19-9 have been reported [2, 11-13] and CA19-9 could be used at postoperative follow-up investigation in benign diseases and as a marker for examining MCTs recurrence [14].

This article aimed to evaluate the serum levels of tumor markers (particularly CA19-9) in patients with MCTs with respect to the clinical characteristics, and the correlation between serum and tissue level of CA19-9 in MCTs.
correlation of positive rate to serum level of CA19-9 was performed.

Statistical analysis was performed with SSPS (version 18.0). Statistical evaluation of the data was performed by the chi-square test, Student’s *t*-test, Mann-Whitney U test, and Pearson’s test. Differences were considered significant when *p* < 0.05 for the two-tails.

### Results

For the patients with MCT, the mean age was 32.5 ± 12.1 years (median 28; range 15–71). Tumor size ranged from 2.3 to 12 cm in diameter, with a median and mean ± SD, 5.6 cm and 5.8 ± 2.33 cm, respectively. The overall bilaterality rate was 20%. Unilateral tumors were more abundantly observed on the right side (29 patients, 55.7%) than the left (23 patients, 44.2%).

There was no statistically significant difference in terms of patient age, menopause, and bilaterality between groups 1 and 2. The mean tumor diameter of group 1 was significantly greater than group 2 (6.92 ± 2.0 vs. 5.2 ± 2.3 cm, *p* = 0.003). The mean serum CA125 levels and the rate of elevated CA125 were significantly high in group 1. There was a weak positive correlation of CA19-9 levels to diameter of tumor (*r* = 0.43, *p* = 0.001) in all patients. In group 1 there was a moderate positive correlation of CA19-9 levels to the diameter of the tumor (*r* = 0.48, *p* < 0.05) (Table 1).

Postoperative assessment of CA19-9 levels in 20 patients of group 1, who had elevated levels preoperatively revealed that mean CA19-9 level was decreased from 131.44 ± 88.5 IU/ml to 66.9 ± 32.64 IU/ml, and the difference was statistically significant (*p* < 0.01).

Among the four tumor markers, CA19-9 was the only tumor marker with the mean serum level above the cut-off value and had the highest positive rate among other tumor markers in MCT. The mean serum levels and positive rate of CA19-9 in patients with MCT were significantly higher when compared to the patients with benign epithelial ovarian tumors (49.9 ± 73.4 IU/ml vs. 17.08 ± 24.81 IU/ml, 30.76% vs. 8.75%, respectively) (*p* < 0.01). Meanwhile, the difference in CA125, CEA, AFP, and SCCA in patients with MCT and benign epithelial ovarian tumor group was not statistically significant (*p* > 0.05) (Table 2).

Immunohistochemical investigation of the teratoma was done in 55 patients with MCT. Immunoreactivity to CA19-9 was recognized as brown staining within cells (Figure 1). The positive expression rate of CA19-9 in tissues of MCT (28/55, 50.9%) was higher than the elevated rate in serum (18/55, 32.7%) and the difference attained statistical significance (*p* < 0.01) (Table 3).

### Discussion

Results of the present study have shown that CA19-9 is more frequently elevated than CA125 and hence could be...
Clinical significance of CA19-9 in ovarian mature cystic teratoma

Table 3. — CA19-9 tissue staining in MCT patients with elevated and normal serum level of CA19-9.

<table>
<thead>
<tr>
<th>CA19-9 tissue staining</th>
<th>Serum CA19-9 (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 37</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
</tr>
</tbody>
</table>

The present results indicate that tumor size was the only clinical finding that correlated with elevated CA19-9 levels, which is similar to Cho et al.’s report [2]. Diameters of the MCTs are significantly larger in patients with increased levels of CA19-9 and there is moderate correlation of CA19-9 levels to tumor size. This may be mainly due to weakened cyst wall and CA19-9 leakage from cystic cavities into the bloodstream. However bilaterality is not associated with elevated serum CA19-9 levels. This is in contrast to the study of Dede et al. in which elevated CA19-9 was associated with high rate of bilaterality with a likelihood ratio of 2.8 [4].

Acknowledgements

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References


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Screening of sexual dysfunction in Saudi women before and after the age of 40 years

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Summary

Objective: The aim was to assess sexual performance by screening Saudi women before and after the age of 40 years. Materials and Methods: A cross-sectional study (March-May 2013), conducted at King Abdulaziz University Hospital (KAUH), with two groups of women under 40 years of age and aged 40 or more years, were randomly selected from OPD. Ethical committee approved the study. After verbal consent, one-paper self-administered questionnaire was distributed, and filled in anonymously and privately. Questionnaire included demographic data, the six-item version of female sexual function index (FSFI) to assess desire, lubricants, orgasm, satisfaction, and pain. If score was 19 or less, this meant that females required further investigations (full assessment using FSFI-19). Results: Out of 194, 49.5% (96) were over 40 years of age and 50.5% (98) were under 40 years of age. Answering the six questions regarding sexual dysfunction indicated that women > 40 years had sexual dysfunction more than women < 40 (statistically significant). A scored of less than 19 was found to be statistically significant in women > 40 years. Post-menopausal women, diabetics, women with urogynecological symptoms and/or psychological disorder required further evaluation. Discussion: Using the six-item version of FSFI and calculating a score less than 19 for screening, women aged more than 40 years, reduction in estrogen, diabetes, urogynecological symptoms, and psychological disorder were all found to be important factors affecting female sexual dysfunction.

Key words: Female Sexual Dysfunction, Screening Saudi.

Introduction

Women commonly have sexual problems; about 40% of women in the United States have some sexual problems and 12% report dyspareunia [1]. Loss of sexual desire and arousal, difficulty to reach orgasm, and distressing pain during intercourse are describe as female sexual dysfunction [2]. Sexual dysfunction may begin as a female becomes sexually active or she may acquire this after a period of normal sexual performance. Phases of the female sexual cycle may have problems and sexual response may be interpersonal and sexual activity be initiated not only by desire but also by other factors such as emotional closeness [3].

There is a local belief that Saudi women at the age 40 have sexual dysfunction, and the aim of the present study was to assess sexual performance by screening Saudi women before and after the age of 40 years, and if there are any other important factors.

Materials and Methods

A cross-sectional study conducted at King Abdulaziz University Hospital (KAUH) between March 3rd, 2013 and May 3rd, 2013 involved two groups of the population; women under the age of 40 years and women with an age of 40 years and more. The authors chose the age of 40 because there is a local belief that most Saudi women have sexual dysfunction after the age of 40.

Candidates were randomly selected from outpatient’s clinic. Simple random sampling used in total, 100 candidates selected in each group. KAUH ethical committee approved the study. Inclusion criteria were the following: adult female, married, and willing to participate in the study. Exclusion criteria were the following: pre-menarche girls, single status, and women that refused to participate.

Sexual issues are very sensitive so the proposal of the study was explained to the subjects and were confidentiality assured. Verbal consent was obtained, and one-paper self-administered questionnaire was distributed by physicians participating in the study, the subjects were asked to fill it out anonymously and privately, and to return it once completed.

The questionnaire was composed of demographic data age, parity, continuing to have a period, use of oral contraceptive pills, history of medical or surgical disease, history of urinary incontinence, history of prolapse, and history of psychological disorders.

The six-item version of the female sexual function index (FSFI) questionnaire was used to assess the desire, lubricants, orgasm, satisfaction, and pain. It was composed of six simple questions, which only took a few minutes to complete. If the calculated score was found to be 19 or less, this meant that females required further investigations and a full assessment using FSFI-19 were indicated [4].

To assess desire there were two questions, the first was the degree of sexual desire: 1 = almost never; 2 = few times; 3 = sometimes; 4 = most of the time; and 5 = almost always. The second was the level of sexual interest: 1 = very low; 2 = low; 3 = moderate; 4 = high; and 5 = very high.

Natural lubrication, orgasm, satisfaction, and pain were scored from 1 to 5. Lubricant use during sex: 1 = never; 2 = few times;
= sometimes; 4 = most of the time; and 5 = always. Orgasm: 1 = very dissatisfied; 2 = moderately dissatisfied; 3 = equally dissatisfied; 4 = moderately satisfied; and 5 = very satisfied. Sexual satisfaction: 1 = very dissatisfied; 2 = moderately dissatisfied; 3 = equally dissatisfied; 4 = moderately satisfied; and 5 = very satisfied. Pain: 1 = always discomfort; 2 = most of the time; 3 = sometimes; 4 = few times; and 5 = never.

Statistical analysis
The Statistical Package for the Social Sciences (PC SPSS version 20) was used to analyze data using chi-square test. The frequency of occurrence of different variables was calculated with a p-value less than 0.01, and odds ratio and 95% confidence interval were used to compare variables with a score less than 19.

Results
Participants in this study were 194 Saudi women (six refused to participate). Out of 194 subjects, 49.5% (96) were above 40 years of age and 50.5% (98) were less than 40 years of age (Table 1). Only one patient less than 40 years of age, had no period in contrast to 56 patients above the age of 40 that were menopausal and with parity (Table 2).

The answers of the six questions regarding female sexual dysfunction indicated that women aged more than 40 years had significant sexual dysfunction compared to women under the age of 40 (Table 3).

Chi-square used to compare the age with the scores of less than 19 in 52 out 96 women aged 40 or more and only 13 out 98 in women of less than 40 years showed that this was statistically significant (p = 0.001), and 35/57 of menopausal female had a score of less than 19, while 20/34 women with non-menopausal only (30/137) group; this was statistically significant with (p = 0.001) (Table 4).

When the odds ratio and 95% confidence interval were utilized to analyze the scores of less than 19 in the six-item version of the FSFI questionnaire, age 40 years or more, menopause and non-menopause, history of medical disease, and surgical operation, prolapse, urinary stress incontinence, and psychological disturbance, were not found to be statistically significant.

Discussion
Does the age affect sexual function in women? This is a controversial issue. Shifren et al. [1] found that the prevalence of sexual problems associated with distress was highest in women aged 45 to 64 years (15%), lowest in women 65 years or older (9%), and intermediate in women aged 18 to 44 years (11%) [5]. In the present study women above the age of 40 years scored lower in all six parameters analyzed than women below the age of 40 years and it was statistically significant. When using the six parameters as screening, women above the age of 40 scored less than 19 in 52 out of 96 and required further evaluation.
Table 4. — Factors affecting sexual dysfunction with a score less than 19 (odds ratio and 95% confidence interval).

<table>
<thead>
<tr>
<th></th>
<th>Score &gt; 19</th>
<th>&lt; 19</th>
<th>Total</th>
<th>Odds ratio (95% Confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 40</td>
<td>44</td>
<td>52</td>
<td>96</td>
<td>0.129 (0.064 - 0.263)</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>85</td>
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<td>98</td>
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<td>65</td>
<td>194</td>
<td><strong>p = 0.001</strong></td>
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<tr>
<td><strong>Period</strong></td>
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<td>22</td>
<td>35</td>
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<td>65</td>
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<td><strong>p = 0.001</strong></td>
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<td><strong>p = 0.001</strong></td>
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<td><strong>p = 0.001</strong></td>
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<td>0.170 (0.043 – 0.663)</td>
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<tr>
<td>No</td>
<td>126</td>
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</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>65</td>
<td>194</td>
<td><strong>p = 0.006</strong></td>
</tr>
</tbody>
</table>

> 19: score more than 19; < 19: score less than 19.

Impaired estrogen level in peri- and postmenopausal women can lead to sexual dysfunction, which may result in a reduction in both vulvovaginal lubrication and vasocongestion during sexual arousal, also vaginal atrophy, and an increased likelihood of sexual pain [6]. In the present study, 35 out of 57 postmenopausal female scored less than 19 compared to only 30 out 137 perimenopausal women. In a longitudinal cohort study, perimenopausal women were monitored according to hormone levels and with a sexuality questionnaire annually for up to eight years and it demonstrated that declining levels of estradiol were associated to decreases in libido and sexual responsivity [7].

Female sexual dysfunction is one of the complications of diabetes in women. An article published in Chinese showed that risk factors of sexual dysfunction in diabetic women are vascular disease, neuropathy, psychological disorder, and endocrine problems [8]. In the present study, diabetic patients scored less 19 in 40/68.

High percentage of patients with urogynecological symptoms suffer from sexual dysfunction and it is our responsibility to provide assistance and education in this type of patients [9]. Fourteen out of 19 women with urogynecological symptoms scored less than 19.

The causes of sexual dysfunction include psychological disorders as depression or anxiety, unstable relationship, stress, fatigue, history of sexual or physical abuse, medications, or conditions that make sexual activity painful, such as endometriosis or atrophic vaginitis [10]. In the present study women with psychological disorders scored less than 19 in eight out of 11, when compared to women with no evidence of psychological disorder and this was statistically significant with a p-value of 0.007.

**Conclusion**

By using the six-item version of the FSFI and calculating a score of less than 19 as screening, age more than 40 years, reduction in estrogen, use of oral contraceptive pills, past medical and surgical disease, urogynecological symptoms, and psychological disorders were found to be important factors affecting female sexual dysfunction but not statistically significant.

**References**


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Introduction

Approximately 60% of all women of reproductive age are affected by endometriosis, which is defined as the presence of endometrial tissue outside the endometrium and the myometrium [1]. The exact etiology of endometriosis has not been fully clarified but it has been suggested that reactive oxygen species (ROS) may increase the implantation, growth, and adhesion of endometrial cells in the peritoneum [2]. Oxidative stress occurs when there is an imbalance in the oxidant-antioxidant system, either through over-production of ROS or a decrease in the antioxidant defense. Following activation of immune cells, especially polymorphonuclear leucocytes and macrophages, the production of ROS is known to increase [3]. Superoxide anion (O₂⁻) appears to be of importance in ROS. Superoxide dismutase (SOD) rapidly decomposes superoxide anion into hydrogen peroxide and oxygen and catalase (CAT) is another enzyme involved in the detoxification of hydrogen peroxide (H₂O₂), a molecule in ROS [4]. It has been extensively reported that Malondialdehyde (MDA) can be used to estimate the effect of oxidative stress on lipids [5]. Oxidative stress has been reported to play an important role in the development and progression of endometriosis [6]. In women with endometriosis, evidence of oxidative stress has been observed in the peritoneal cavity and ectopic tissue. The peritoneal fluid of endometriosis patients has been found to contain high concentrations of lipid peroxidation products [7].

Aloe vera (synonym: Aloe barbedensis Miller) is a plant with yellow flowers and triangular leaves, similar to cactus, belonging to the Liliaceal family, which comprises 360 species [8]. The plant leaves contain abundant amounts of mucilaginous fluid of high viscosity, known as aloe vera gel [9]. Various reports have shown that aloe vera gel stimulates wound-healing and skin hydration, induces hematopoesis, and possesses anti-diabetic, anti-carcinogenic, antimicrobial, anti-oxidant, and anti-inflammatory properties [9, 10]. The antioxidant properties of aloe vera render it suitable for use in conditions of enhanced oxidative stress which may have significant implications for the prevention of diseases [11].

This study aimed to evaluate the effect of aloe vera gel on endometriotic implants in a rat endometriosis model. An investigation was made of the effect of aloe vera gel on the formation and regression of endometriotic lesions.

Summary

Purpose of investigation: To evaluate the preventive and reducing effect of aloe vera gel on surgically-induced endometrial foci in rats. Materials and Methods: Twenty-four reproductive aged female non-pregnant, nulligravid Sprague-Dawley albino rats were used. The rats were randomly divided to three groups (Group 1: control, Group 2: aloe vera endometriosis formation, and Group 3: aloe vera endometriosis treatment). A peritoneal lavage using one-ml saline was taken at all the operations for determination of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT). Forty-eight horns were implanted in 24 rats. Results: All the implants were properly formed after implantation. In Group 3, before aloe vera application, the sum of the volumes was 87.2 ± 20.4 mm³ and after treatment the volumes dropped to 28.9 ± 14.9 mm³ (p = 0.01). As evaluation of aloe vera on the formation of endometriosis in the second operation in Group 2, the sum of the volumes was 2.9±1.4 mm³ and in Group 1, 118.9 ± 20.0 mm³ (p = 0.001). Likewise, similar changes were observed in the histopathological scores. Conclusion: The application of aloe vera was seen to raise antioxidant levels in the peritoneal fluid and to reduce oxidative stress markers. Aloe vera is effective in the inhibition of formation and regression of endometriotic lesions.

Key words: Aloe vera; Endometriosis; Oxidative stress.

Effects of aloe vera gel on the induction of endometriosis and regression of endometrial explants in a rat model

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⁵ Department of Biochemistry, Kahramanmaras Sutcu Imam University, Faculty of Medicine, Kahramanmaras (Turkey)
Materials and Methods

Animal model

Approval for the study was granted by the Local Ethics Committee for Experimental Animals and was performed at the Experimental Animal Production and Research Laboratory of Yeditepe University (YUDETAM), Yeditepe University, Turkey. All experiments were performed in compliance with international guidelines on the ethical use of animals.

Twenty-four reproductive aged female non-pregnant, nulligravid Sprague-Dawley albino rats weighing 200-250 grams bred at YUDETAM were used in this study. The animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment (22±2°C) with 12-hour light–dark cycles.

Experimental design and surgical procedures

The rats were randomized into three groups. Group 1 was the endometriosis control group (n=8), Group 2 was the aloe vera endometriosis formation group (n=8), and Group 3 was the aloe vera endometriosis treatment group (n=8).

The induction of endometriosis was performed in the first operation for all three groups. In the first operation, 0.1 ml of aloe vera gel was applied to the implantation sites of Group 2. After two weeks of estradiol treatment, the second operations were performed and 0.1 ml of aloe vera gel was applied to the implantation sites of Group 3. The third operations were performed for assessment of the effects of aloe vera on the endometriotic foci regression (Group 3) after two weeks of estradiol treatment following the second operation.

First operation: endometriosis induction

All the rats were anesthetized with an intramuscular administration of 60 mg/kg ketamine hydrochloride with seven mg/kg xylazine hydrochloride. Endometriosis was surgically induced using the method described by Vernon and Wilson [12]. After the administration of general anesthesia, the abdominal cavity was opened using a vertical incision. A peritoneal lavage using one-ml saline was taken. The uterine horns were ligated at the uterotubal junction and the cervical end was subsequently removed. The uterine horns were placed in phosphate-buffered saline at 37°C. Four pieces of graft measuring 6 x 3 x 1 mm were made by division of the uterine horns. Without removing the myometrium, two of these pieces were implanted onto the peritoneal surface of the right abdominal wall so that the endometrium was in contact with the peritoneal surface. Both ends of the implants were secured with non-absorbable polypropylene 6-0 suture to the inner part of the abdominal wall. The remaining two pieces were placed in the left inner part of the abdominal wall with same methodology. All tissues were implanted just opposite both vascular bifurcations. In Group 2 rats, the areas of implantations were covered with 0.1 ml aloe vera gel. In Group 1 and 3 rats, the areas of implantations were covered with 0.1 ml saline solution. The midline abdominal incision was closed with the continuous suture technique using 3-0 vicryl sutures. All rats were given 50 mg/kg/day cefazolin sodium intramuscularly for three days after the operation, to prevent any intraabdominal infection. All rats were given 50 µg/kg estradiol twice a week intramuscularly until the second operation.

Second operation: assessment of the endometriotic foci and the effect of aloe vera on the formation of endometriosis

Two weeks after the first operation, the second one was performed to assess the endometriotic lesions for all groups. Exogenous high-dose estrogen created a hyper-estrogenic state and resulted in well-defined endometriotic lesions. The second operations were performed using the aforementioned methodologies. A peritoneal lavage using one-ml saline was applied. Before the endometriotic lesions were biopsied, all the implants were measured in three dimensions (length - width - height in millimeters) with a ruler by the same author.

For histopathological analysis, one of the four implants was removed using a randomization table. In Group 3 rats, the areas of implantations were covered with 0.1 ml aloe vera gel. In Group 1 rats, the areas of implantations were covered with 0.1 ml saline solution. Group 2 rats were euthanized under anesthesia and all measurements and tissue collections were performed as described above.

Third operation-necropsy: evaluation of the effects of aloe vera gel

A third laparotomy was performed at the end of week 4 to measure the implant sizes and to perform peritoneal lavage using one-ml saline. The third operations were performed during estrus. All the rats in Groups 1 and 3 were euthanized under anesthesia and all measurements and tissue collections were performed as described above.

Volume analysis

The spherical volume of each ectopic uterine tissue was calculated using the prolate ellipsoid formula: \[ V (\text{mm}^3) = \frac{0.524}{4} \times W \times T \times L, \] in which W = width, T = thickness, and L = length (all in millimeters) [13].
Histopathological analysis

All tissue samples were embedded in paraffin after routine dehydration, and five-mm thick sections were prepared via microtome. The paraffin-embedded sections from each autograft were stained with hematoxylin and eosin (HE) and semi-quantitatively examined under a light microscope (Figure 1). The persistence of epithelial cells in endometrial implants was scored as follows: no epithelium, 0; poorly preserved epithelium, 1; moderately preserved epithelium with leukocyte infiltration, 2; and well-preserved epithelial layer, 3 [14]. All histological chemical measurements were performed by the same histologist who was blinded to the treatment groups.

Biochemical analysis

All biochemical analyses were made on the peritoneal lavage samples which were taken during the surgical procedures.

MDA determination

For determination, 500 μl of peritoneal fluid sample was added to 750 μL of 440 mM H₃PO₄ and 250 μL of 42 mM and thiobarbituric acid (TBA) solution to have a final volume of 1.5 ml. This solution was incubated for one hour at 100°C, and then an aliquot of 500 μl was added to 500 μl of methanol: 1M NaOH (91:9, v:v) mixture. Mixture was centrifugated at 4,000 rpm for five minutes, 30 μl of supernatant was injected to the high-performance liquid chromatography system.

Measurement of fluorescence of the butanol extract was made at an excitation wavelength of 539 nm and emission wavelength of 533 nm. As the standard solution 1,1,3,3 tetraethoxypropane was used and the values were calculated as micromoles per liter.

SOD activity determination

Total (Cu-Zn and Mn) SOD activity was determined according to the method of Durak et al. [15]. This method is based, on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. After 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged, activity was assessed in the ethanol phase of the supernatant. One unit of SOD definition was made as the enzyme amount, causing 50% inhibition in the NBH reduction rate. Activity was presented as units per liter.

CAT activity determination

For determination of CAT activity, Aebi method was used [16]. The assay principle was based on the determination of the rate constant k (dimension: s⁻¹, κ) of hydrogen peroxide decomposition. The rate constant of the enzyme was determined by measurement of absorbance change per minute. Activities are presented as κ units (rate constant) per liter (κU/L).

Table 2. — The comparison of endometriotic volumes and histopathological scores between the groups during the second operation.

<table>
<thead>
<tr>
<th></th>
<th>Endometriosis control (Group 1) (n=8)</th>
<th>AV endometriosis treatment (Group 3) (n=8)</th>
<th>AV endometriosis formation (Group 2) (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd operation (mean ± SEM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>118.9 ± 20.0</td>
<td>87.2 ± 20.4</td>
<td>2.9 ± 1.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>Histopathological score</td>
<td>2.1 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.01**</td>
</tr>
</tbody>
</table>

Statistical analysis

The statistical analysis was performed using SPSS version 11.5. All variables are expressed as mean and standard error. Differences between groups were evaluated by Kruskal–Wallis variance analysis followed by a post hoc Mann–Whitney U-test. A value of p < 0.05 was considered statistically significant.

Results

All laparotomy sites were intact and none of the animals had an incisional hernia. No deaths resulted in any of the groups.

Forty-eight horns were implanted in 24 rats. All of the implants were properly formed after implantation. In Group 1 (endometriosis control group) and Group 3 (aloe vera endometriosis treatment), there was no significant difference between endometriotic foci and lesions were well vascularized at the second operation (Table 1). There was no statistically significant difference between the two groups (p = 0.28). Histopathological scores during this operation were 2.1 ± 0.3 in Group 1 and 2.2 ± 0.3 in Group 2 (p = 0.79).

When all the groups were evaluated in the second operation, the mean lesion volumes were 118.9 ± 20.0 mm³ in Group 1, 87.2 ± 20.4 mm³ in Group 3, and 2.9 ± 1.4 mm³ in Group 2 (endometriosis formation group) (Table 2). In the determination of the effect of aloe vera on the formation of the endometriosis, statistically significant differences were determined between endometriotic volumes (between Group 1 and Group 2: p = 0.001 and between Group 2 and Group 3: p = 0.002);
and Group 2: \( p = 0.001 \) and between Group 2 and Group 3: \( p = 0.008 \).

At the third operation, mean lesions volumes were 28.9 ± 14.9 mm\(^3\) in Group 3 and 136.6 ± 21.6 mm\(^3\) in Group 1. The difference between the two groups was significant (\( p = 0.01 \)). Histopathological scores during this operation were 2.5 ± 0.3 in Group 1 and 0.8 ± 0.2 in Group 3 (\( p = 0.004 \)).

In Group 1, pre-treatment volume was 118.9 ± 20.5 mm\(^3\) and post-treatment volume was 136.6 ± 21.6 mm\(^3\). A slight increase was observed in the lesions (\( p = 0.04 \)). The changes in histopathological scores were not statistically significant (\( p = 0.47 \)) (Table 3).

In Group 3, before aloe vera implantation, the sum of the volumes was 87.4 ± 20.4 mm\(^3\) and after aloe vera implantation this dropped dramatically to 28.9 ± 14.9 mm\(^3\) (\( p = 0.04 \)). Likewise, similar changes were observed in the histopathological scores, which were 2.2 ± 0.3 and 0.8 ± 0.2 before and after aloe vera treatment, respectively (\( p = 0.04 \)) (Table 3).

At the first operation, the values MDA were as follows: Group 1 0.44 ± 0.22 mmol/L, Group 2 0.43 ± 0.09 mmol/L, and Group 3 0.49 ± 0.08 mmol/L with no statistically significant difference between the groups. In Group 1, MDA increased to 0.65 ± 0.12 mmol/L (\( p = 0.001 \)) at the second operation. The MDA levels of Group 1 were 0.63 ± 0.09 mmol/L at the third operation, but no statistically significant changes were observed between the second and third operations (\( p = 0.20 \)). In Group 2, the values of MDA slightly increased to 0.46 ± 0.20 mmol/L at the second operation but this was not statistically significant (\( p = 0.56 \)). When the values MDA of Groups 1 and 2 were compared, the difference was statistically significant at the second operation (\( p = 0.001 \)). In Group 3, the MDA levels statistically significantly increased at the second operation (\( p = 0.001 \)) and decreased at the third one (\( p = 0.02 \)).

If the SOD and CAT levels were taken into account at the first operation, the values were as follows: Group 1 64.5 ± 2.25 U/L, 2,149 ± 13 U/L, Group 2 67.05 ± 1.15 U/L, 2,228 ± 54 U/L, and Group 3 65.4 ± 4.5 U/L, 2,154 ± 46 U/L with no statistically significant difference between the groups. In Group 1, SOD and CAT levels decreased to 54.1 ± 2.94 U/L, 2,010 ± 24 U/L (\( p = 0.01 \), \( p = 0.01 \)) at the second operation but there were no statistically significant changes between the second and third operations (\( p = 0.74 \), \( p = 0.82 \)). In Group 2 the levels of the SOD and CAT increased significantly to 86.97 ± 1.97 U/L, 2,978 ± 183 U/L, respectively, at the second operation (\( p = 0.001 \), \( p = 0.001 \)).

In Group 3, the values of SOD and CAT significantly decreased to 52.34 ± 7.4 U/L, 1,997 ± 143 U/L, at the second operation (\( p = 0.001 \), \( p = 0.001 \)). The SOD and CAT levels significantly increased to 74.17 ± 5.97 U/L, 2,478 ± 156 U/L at the last operation when compared to the second one (\( p = 0.001 \), \( p = 0.001 \)).

### Discussion

One of the most enigmatic and problematic diseases affecting women of reproductive age is endometriosis, for which there is no ideal medical treatment as yet. Oxidative stress occurs when there is an imbalance in the oxidant-antioxidant system as a result of either over-production of ROS or a reduction in antioxidant defence [17].

With a profound alteration of ROS detoxification pathways, endometriotic cells display high endogenous oxidative stress. In stromal endometriotic cells, the \( \Delta \Sigma \) level has been shown to significantly increase and is produced by the cytosolic NAD(P)H oxidase [18].

In the metastatic potential of tumor cells, ROS play a role in the proliferation and control of tumor growth [19]. Just as in tumor cells, the increased production of endogenous ROS is associated with an increase in the proliferation rate in endometriotic cells. The oxidative stress regulation in endometriotic cells is also close to that described in tumor cells [19]. Therefore as in tumor cells, a significant decrease in intracellular H\(_2\)O\(_2\) concentration inhibits the proliferation of endometriotic cells both in vitro and in vivo [19].

In endometriosis patients, local tissue destruction and disease aggressiveness may be caused by oxidative stress [20]. Aloe vera possesses many pharmacological properties, including anti-inflammatory, immune-stimulant, wound healing, and antitumor, which could be involved in the mediation of ROS levels [21]. It has been claimed that aloe vera protects against pro-oxidant-induced membrane and cellular damage [22].

Altintik et al. reported no toxic effects from the application of aloe vera gel to peritoneal surfaces and in a rat peritonitis model also demonstrated an anti-oxidant and anti-inflammatory effect of aloe vera [22].

In the current study, endometriosis formation was prevented by the application of aloe vera during the endometriosis implantation period. In a study by Kang et al., it was shown that aloe vera decreased the oxidative stress markers in rats with oxidative stress [23].

After the formation of endometriosis based on an evaluation of the application of aloe vera, endometriotic foci were observed to have declined two weeks later, which was shown.

### Table 3. — The mean volume of endometriotic lesions and histopathological scores within the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>2nd operation (mean ± SEM)</th>
<th>3rd operation (mean ± SEM)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (n=8)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Volume (mm(^3))</td>
<td>118.9 ± 20.0</td>
<td>136.6 ± 21.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Histopathological score</td>
<td>2.1 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Group 2 (n=8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm(^3))</td>
<td>87.2 ± 20.4</td>
<td>28.9 ± 14.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Histopathological score</td>
<td>2.2 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

(Wilcoxon Test)
by the significant decrease in volume and the histopathological scores compared to the control group.

At the end of the second operation, examination of the peritoneal fluid of the rats in the two endometriosis formation groups (Groups 1 and 3) revealed higher oxidative stress markers and lower anti-oxidative markers, which was consistent with the endometriosis hypothesis. However, at the end of the third operation, the oxidative stress markers in Group 3 were seen to have significantly decreased. The oxidative stress marker and anti-oxidative stress marker values in the control group remained at relatively unchanged levels at the third operation. This suggests that aloe vera not only prevents the formation of endometriosis, but also prevents the progression of the endometriosis due to oxidative stress.

In a study investigating the effect of aloe vera on peritoneal trauma, fewer peritoneal adhesions were seen to have formed following the pre-trauma application of aloe vera [24]. This effect was thought to have resulted from the anti-inflammatory properties of aloe vera. In the current study group, both in the formation of endometriosis (Group 2) and the endometriosis treatment group (Group 3), the application of aloe vera was found to be effective, which suggests that it enabled the endometriosis via the anti-oxidative pathway. Oxidative stress is known to play a role in the pathogenesis of development and progression of endometriosis [19].

Conclusion

The results of this study have shown that aloe vera gel is effective in inhibiting the formation of endometriotic lesions and in their regression. This effect of aloe vera is thought to be a result of reducing oxidative stress markers and increasing anti-oxidative stress markers, which are known to play a role in the pathogenesis of endometriosis. Further experimental studies are required in different animal models using other methodologies and doses to confirm the safety and efficacy of aloe vera gel in the prevention and treatment of endometriosis.

References


Effect of antenatal betamethasone administration on rat cerebellar expression of type 1a metabotropic glutamate receptors (mGluR1a) and anxiety-like behavior in the elevated plus maze

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Summary
Preclinical studies indicate that endogenous or exogenous glucocorticoids acting during the pre- or postnatal periods produce a significant Purkinje cell dendritic atrophy, especially during late postnatal ages. The present authors hypothesized that the underlying substrate that may contribute in part to this morphological change is the under-expression of the metabotropic glutamate 1a receptor (mGluR1a) because its expression is correlated with Purkinje cell dendritic outgrowth. Therefore, in the current study, they analyzed the impact of antenatal betamethasone on the immunoreactive expression of the mGluR1a and on anxiety-like behavior in the elevated plus maze (EPM). Pregnant rats were randomly divided into two experimental groups: control (CONT) and betamethasone-treated (BET). At gestational day 20 (G20), BET rats were subcutaneously injected with a solution of 170 µg.kg⁻¹ of betamethasone, and CONT animals received a similar volume of saline. At postnatal days 22 (P22) and P52, BET and CONT offspring were evaluated behaviorally in the EPM, and their cerebella were immunohistochemically processed. Contrary to the authors’ expected results, animals that were prenatally treated with a single course of betamethasone did not exhibit under-expression of mGluR1a or behavioral changes consistent with anxiety-like behaviors.

Key words: mGluR1a; Vermal Purkinje cells; Molecular layer; Elevated plus maze.

Introduction
When there is a risk of preterm birth, one of the therapeutic strategies used most frequently is the administration of synthetic glucocorticoids (GCs), i.e., betamethasone or dexamethasone. The rationale of this therapy relies on the fact that these steroidal drugs accelerate the maturation of pulmonary tissue, thus minimizing the risk of respiratory distress and reducing the risk of intraventricular hemorrhage and periventricular leukomalacia [1]. However, the administration of these drugs is not entirely safe for neurological and neuroendocrine development. For example, it has been shown that preterm babies whose mothers were treated with betamethasone showed further alterations in hypothalamic-pituitary-adrenal (HPA) function [2]. Similarly, repeated antenatal betamethasone is associated with higher impulsivity together with fearfulness, tendency to anger, and sadness at two years of age [3]. Consequently, at least with regards to early treatment with GCs. This increased cerebellar vulnerability is most likely related to the fact that the mammalian cerebellar cortex expresses a remarkable density of glucocorticoid receptors, even exceeding that of the hippocampus or cerebral cortex [5, 6], together with the fact that Purkinje cell dendritogenesis is rapid during the perinatal period [7, 8]. Consistent with these data, in the present authors’ laboratory, they have recently observed that the prenatal administration of betamethasone in equivalent therapeutic doses and during a similar ontogenetic stage (gestational day 20) [9, 10] produced a significant alteration in the development of cerebellar Purkinje cells associated with an increase in the calcium sequestering protein calbindin-D28k [11]. It has also been reported that cerebellar Purkinje cell dendritic maturation is related to the expression of type 1 metabotropic glutamate receptors (mGluR1) present in the dendritic membrane. mGluR1 is a family of metabotropic receptors present on various cerebellar cells, including cerebellar Purkinje cells [12], and the membrane density of mGluR1 has been shown to increase concomitantly with dendritic cell outgrowth, in close association with learning phenomena such as long-term...
potentiation [13, 14]. The relevance of these metabotropic receptors for Purkinje cell maturation is evidenced by the fact that in staggerer mutant mice, the abnormal signal between parallel fibers and Purkinje cells is closely associated with alterations in mGluR1 expression and function [15]. Interestingly, the expression of mGluR1 on Purkinje cell dendritic spines is a key developmental process in the normal elimination of exuberant olivo-cerebellar climbing fibers that takes place in dendritic Purkinje cell arborization at gestational day 20 (G20) in rodents [12]. In addition, mGluR1 plays a central role in Purkinje cell plasticity via elevation of free intracellular calcium concentration [16]. On the other hand, GCs appear to interact with mGluRs, and an excess of stress-induced GCs may alter memory formation via changes in the function and expression of mGluRs [17].

Because the direct administration of antenatal synthetic GCs in experimental animals at G20 produces a significant Purkinje cell developmental delay as shown in Golgi-Cox-stained Purkinje cells [11], the present authors hypothesized that one pathophysiological mechanism involved in the impact of GCs on cerebellar maturation could be the under-expression of mGluR1 in the molecular layer where Purkinje cell dendritic tree outgrowth occurs. Furthermore, it should be noted that in rodents [7] as well as in humans [8], Purkinje dendritic maturation occurs during perinatal periods following a progressive outgrowth from the soma (located in the middle cerebellar cortical layer) towards the cerebellar upper molecular layer. Considering those findings, the aim of the current study was to evaluate whether antenatal betamethasone administered at G20 in an equivalent clinical dose (170 µg kg⁻¹) [10] alters the postnatal expression of cerebellar mGluR1α in the cerebellar molecular layer. Furthermore, as the main behavioral sequel of antenatal administration of synthetic GCs are anxiety behaviors during childhood and adolescence [18], the authors further assessed whether betamethasone-treated animals exhibit anxiety-like behavior in the elevated-plus maze (EPM).

Materials and Methods

Multiparous female rats were housed under the following controlled environmental conditions: temperature (20 ± 1°C) and 12:12 light-dark cycle. Food and water ad libitum. Pregnant rats were randomly classified into the following four experimental groups: (i) saline control animals evaluated at 22 postnatal days (CONT-P22; n = 13), (ii) betamethasone-treated animals evaluated at 22 postnatal days (BET-P22; n = 9), (iii) saline control animals evaluated at 52 postnatal days (CONT-P52; n = 12), and (iv) betamethasone-treated animals evaluated at 52 postnatal days (BET-P52; n = 13). To avoid gender-related influences, the behavioral and neuronal assessments were conducted only in male offspring.

On G20, the mothers of the BET-P22 and BET-P52 groups were given two doses of 170 µg kg⁻¹ betamethasone subcutaneously, separated by an eight-hour interval. The authors used this dose because pharmacokinetic and pharmacodynamic analysis of betamethasone in the rat has found this dose to be equivalent to the 12-mg dose, which is usually administered in two courses separated by an interval of 12 hours in cases of human preterm birth risk [10]. G20 was chosen because it corresponds to the approximate ontogenetic stage of a pre-term human (24-32 weeks gestation) [9, 10]. Control groups received an equal volume (one ml) of saline solution.

All offspring were tested on the EPM at P22 or P52. The EPM was constructed of black wood and consisted of two open arms (60 × 6 cm) and two closed arms (60 × 6 × 14 cm) and was immobile on a fixed base, 41.5 cm above the floor. Each animal was placed in the center of the EPM and allowed to freely explore the maze for five minutes. The following two behavioral variables were evaluated: the percentage of entries in the open arms (% = open arm entries/total entries in all four arms x 100) and the time spent in the open arms (% = time spent in the open arms/ time spent in all four arms x 100). Animals that explore the open arms less frequently and for less time are considered anxious compared to age-matched saline controls animals [19]. Placement of all four limbs into one arm of the maze is defined as an effective entry into that arm. Exploratory behavior in the EPM was recorded using a webcam. Behavioral data were recorded in a blinded fashion and processed with ANY-maze 4.60 software. Behavioral analyses were conducted between 09:00 and 15:00 hours, and the apparatus was carefully cleaned with 5% ethanol between each analysis. The animals were placed in the behavioral assessment room ten minutes prior to behavioral testing to allow habituation to the new environment.

Following behavioral assessment, all male animals (P22 and P52) were deeply anesthetized with isoflurane/pentobarbital, and intracardiac perfusion was performed with 0.9% NaCl followed by 4% paraformaldehyde. Brains were removed, post-fixed for one hour, and stored in 30% sucrose at 4°C for seven days for cryoprotection. Finally, the cerebellar vermis was sectioned at 20 µm for immunohistochemical procedures using a Thermo Scientific Microim HM525 Cryostat, and each cerebellar vermal section was digitalized to measure mGluR1α immunoreactivity in the molecular layer of vermal lobule IX.

Sections that were previously attached to the slide were washed twice in PBS for ten minutes each at 90 rpm and then incubated with 0.5% H₂O₂ for 30 minutes at room temperature. After two additional washes with PBS (1X), the authors proceeded to block with 3% BSA and 0.1% Triton X-100 for one hour. The primary antibody used was anti-mGluR1α (1/1000, ab1778), which was incubated in blocking solution overnight at room temperature under agitation (40 rpm). The tissue was then washed three times with PBS (1X) and incubated in 1.5% BSA and 0.2% Triton X-100 for two hours at room temperature and 40 rpm agitation. The tissue was washed again (three times) with PBS (1X). The secondary antibody, an avidin-biotin peroxidase complex, was prepared in 1.5% BSA and Triton X-100 for one hour and added to the substrate coupled with diaminobenzidine for 20 minutes without stirring to visualize the labeled protein. Finally, the sections were washed with distilled water for ten seconds. The sections were then mounted on slides, air-dried, covered with Entellan, and coverslipped. The cerebellar vermal sections were coded and observed using a Motic BA210-Tech Lab microscope. The authors evaluated mGluR1α immunoreactivity (% of controls) using grayscale images with Image-J software (pixel intensity arbitrary values).

Animals were treated and housed in accordance with the “Principles of Laboratory Animal Care” (NIH publication N° 86-23, revised 1985), and the experimental protocols received approval from the local animal ethics committee. The t-test was used for the analysis of behavioral and immunohistochemical data. The results are presented as the mean ± SEM. Differences at p < 0.05 were considered significant.
Effect of antenatal betamethasone administration on rat cerebellar expression of type 1a metabotropic glutamate receptors (mGluR1a) etc.

Results

As shown in Figure 1, animals exposed to betamethasone prenatally on G20 showed no significant change in expression of mGluR1a in either age group studied (P22 or P52), suggesting that betamethasone given at the ontogenetic postnatal stage (G20) and given in a clinically equivalent dose (170 mg.kg$^{-1}$) did not produce the expected changes. Figure 2 shows four micrographs of tissue sections from the cerebellar vermal zone immunostained with anti-mGluR1a under ×10 magnification. Similar to the immunohistochemical data, betamethasone-treated animals exhibited no behavioral changes compared to saline-control animals (Figure 3). Consistent with previous clinical and preclinical studies, body weight was significantly lower in the betamethasone-treated animals at both P22 and P52 (CONT-P22: 60.1 ± 1.5 g; BET-P22: 53.8 ± 1.1 g; CONT-P52: 265.8 ± 3.1 g; BET-P52: 246.9 ± 2.9 g; $p < 0.05$).

Discussion

In the present study, the authors failed to support the hypothesis that prenatal betamethasone administered in clinically equivalent doses (170 mg.kg$^{-1}$) alters the immunohistochemical mGluR1a expression in the rat cerebellar cortex. Likewise, animals prenatally treated with betamethasone showed no significant differences in exploratory behavior compared to their age-matched control animals. There are several reasons why the authors were not able to show changes in the expression of mGlu1Rs or in the anxiety-like behavior in the elevated plus maze. First, in this study, immunohistochemical quantification was performed in the molecular layer of the cerebellum, where nearly all dendritic arborization of Purkinje cells occurs; however, because in that layer two other cell types (stellate cells and basket) are found that similarly express mGlu1a receptors, the Purkinje cell immunohistochemical expression of mGluR1a in the molecular layer of animals treated with betamethasone was likely masked by the immunohistochemical expression of the other microneurons residing in this superficial cortical layer. Second, although many mGluRs play a key role in cerebellar development and plasticity [20], in the present study, the authors analyzed the expression of a single subtype of...
mGluR1 (mGluR1a), but there are other splice variants (mGluR1b-g) that may or may not have been affected by prenatal betamethasone administration and may be responsible for masking the immunoreactivity expression of mGluRs. Third, in order to perform reliable comparisons between the control and betamethasone-treated groups, the authors evaluated the immunohistochemistry expression in the vermal cerebellar lobule IX but not in other vermal lobules that could exhibit different degrees of vulnerability; therefore, it is not possible to know if there were changes in other regions of the cerebellar cortex. Fourth, the ontogenetic stage at which the animals were evaluated (P22 and P52) may have influenced the present results. For example, in the authors’ laboratory, rats prenatally treated with a similar dose of betamethasone did not show changes in MAP-2 immunostaining evaluated at P22 and P52; however, Bruschettini et al. [21] showed that MAP-2 immunoreactivity was decreased in rats subjected to a single course of prenatal betamethasone with MAP-2 changes detected at P150. Future long-term studies could clarify this issue.

The present authors’ interest in the medial cerebellar zone is based on the fact that Purkinje vermal cells, by exerting inhibitory influences on the cerebellar fastigial nucleus, can modulate affective states and, during perinatal development, can exert powerful influences on neurobiological processes involved in the wiring of cerebello-limbic connections. In fact, it has been demonstrated that the vermal cerebellar region is directly and indirectly connected with the hypothal-
amus, hippocampus, amygdala, nucleus accumbens (ventral striatum), and cingulate cortex, all of which are involved in the modulation of affective traits or states [23]. Interestingly, Anderson et al. [24] showed that the cerebellar vermis but not the cerebellar hemispheres of adults sexually abused in childhood exhibits significant alterations according to functional magnetic resonance imaging (fMRI) (T2 relaxometry), confirming the vulnerability of the vermal cerebellar region to early stressful experiences. Of note, early stressful experiences produce a significant and sometimes permanent alteration in the function of the HPA axis that releases excessive cortisol in response to normal stressful experiences during postnatal life [25].

In summary, in the current study, the authors showed that a single course of betamethasone, in a clinically equivalent dose and during a prenatal stage similar to the antenatal period during which a human fetus is habitually exposed, had no effect on mGluR1a expression in the vermal cerebellar molecular layer or on the exploratory behavior in the EPM. Furthermore, because the authors studied two ontogeny stages (P22 and P52), it is not possible to rule out the existence of neurobehavioral changes before the postweaning period or after postnatal day 52, as has been shown in previous studies using the paradigm of prenatal stress [11].

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References


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Is there an association between serum vitamin D levels and endometrial polyps?

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Summary

Background/Aim: Anti-proliferative effects of vitamin D (VD) had been proposed previously. Herein, the authors aimed to evaluate serum 25(OH)VitD3 levels in women with endometrial polyps (EPs) and to determine whether VD deficiency is a risk factor for EP formation. Materials and Methods: This study was designed as a controlled cross-sectional study. Forty three women with polyps constituted the study group whereas 47 of them constituted the control group. The selection criteria for the study group were hysteroscopic detection and histological confirmation of EPs. The main parameters recorded for each woman were; age, body mass index (BMI), parity, smoking status, co-morbidities, dressing style, dairy intake of VD-rich foods, duration of sunlight exposure, skin photo-type, serum levels of 25(OH)VitD3, calcium, phosphor, and albumin. Results: The mean serum 25(OH)VitD3 level was 8.3±7.7 ng/ml in the study group and 9.3±10.2 ng/ml in the control group (p = 0.583). Mean BMI was statistically significantly higher in the study group (p = 0.003). Logistic regression model showed that only significant risk factor for EPs was increased BMI (OR=1.241; 95% CI = 1.070-2.440; p = 0.004). Conclusion: VD deficiency is common among the reproductive age women and obesity is the most important risk factor for polyp formation. The authors believe that there is no relation between VD and EPs.

Key words: Endometrial polyps; Risk factors; Vitamin D.
group included 43 women with polyps, and the control group included 47 women without polyps. The selection criteria for the study group were hysteroscopic detection and histological confirmation of EPs. The inclusion criteria for the control group were absence of endometrial pathology in hysteroscopic observation and absence of endometrial hyperplasia, endometrial carcinoma, and EPs in hysteroscopic and histological evaluation. Patients with thyroid disorders, PCOS rheumatological or adrenal diseases, hepatic or renal failure, diabetes, and hypertension were excluded from the study. Current VD and VD containing multivitamin users, and steroids users were also excluded from the study. The risk factors recorded for each patient were age, body mass index (BMI), parity, abortion, smoking status, educational level, co-morbidities, dressing style, dairy intake of VD-rich foods, duration of sunlight exposure, skin photo-type, monthly income, serum levels of 25(OH)VitD₃, calcium, phosphor, and albumin.

The study was carried out during the autumn period in Ankara (Longitude: 40° 4' N, Latitude: 32° 34' E, Altitude: 891 m), and the average temperature was 7-18.7 °C. Ankara has a continental climate which refers cool, snowy winters and hot, dry summers due to its elevation and inland location. Rainfall occurs mostly during the spring and autumn. The weather was rainy about six days of each month during the study period, and also six hours per day it was sunny during the study period [10].

After following at least eight hours of fasting, venous blood samples were drawn from each participant and were poured into the gel tubes, and they were transferred to the laboratory in a light-proof box not to be exposed to light. After clotting, tubes were centrifuged at 4,100 × g for ten minutes, and each serum was separated and immediately analyzed for VD and other biochemical markers. Serum levels of 25(OH)VitD₃ were measured by using an ELISA Kit. All blood samples were analyzed at the biochemistry laboratory of the present hospital. The intra-assay and inter-assay coefficients of variation were 8.9% and 10.6% for serum assay coefficients of variation were 8.9% and 10.6% for serum assay coefficients of variation were 8.9% and 10.6% for serum assay coefficients of variation were 8.9% and 10.6% for serum assay coefficients of variation were 8.9% and 10.6% for serum.
Is there an association between serum vitamin D levels and endometrial polyps? 541

541 phosphor, albumin, and total protein (p > 0.05). The mean serum 25(OH)VitD3 level were lower in the study group, but it was found to be statistically insignificant (8.3 vs. 9.3, p = 0.583). Only one woman showed an adequate serum 25(OH)VitD3 level in both groups. Thirteen (27.7%) patients had severe VD deficiency (≤ five ng/ml), 20 (42.6%) had moderate deficiency (five to ten ng/ml), and 13 (27.7%) had mild deficiency (11-20 ng/ml) in the study group. These numbers in the study group were 16 (37.2%), 13 (30.2%), and 13 (30.2%), respectively. The demographic and laboratory features of the patients are depicted in Table 1. Partial correlation analysis showed that there was a significantly positive correlation between 25(OH)VitD3, calcium, and phosphor (r = 0.201, p = 0.047; r = 0.205, p = 0.043, respectively), calcium and phosphor (r = 0.317, p = 0.001), and albumin, calcium, and phosphor (r = 0.898, p < 0.001; r = 0.260, and p = 0.010, respectively).

The number of employees, educational level, comorbidity, and previous history of surgery did not differ between two groups. Also dressing style, smoking status, skin photo type, physical activity, and dietary intake of VD rich foods, which may have affected the serum levels of VD, were statistically insignificant determinants. Previous cesarean operation was the most common cause of surgical history. The patients had no significant comorbidities except an asthma and epilepsy patient without history of drug use. The vast majority of the participants was exposed to sunlight between ten to 20 minutes during the daytime (09:00 a.m. - 03:00 p.m.) (Table 2). Logistic regression method showed that higher BMI was the only significant risk factor for EPs (Odds ratio [OR], 1.241; 95% confidence interval [CI]; 1.070-2.440, p = 0.004) (Table 3).

Discussion

In this study, patients with EPs were evaluated for serum levels of 25(OH)VitD3 and compared with age-matched controls, with the aim of determining whether VD is risk factor for polyp formation. The present results suggest that VD deficiency is common among the reproductive age women, and that obesity is the most important risk factors in the development of EPs, whereas VD was found not to be an independent risk factor for EPs.

EPs caused by the proliferation of endometrial glands and stroma are often benign lesions that contain both estrogen and progesterone receptors [4]. There are limited studies on the pathogenesis of EPs. However, it was shown that prolonged endometrial exposure to mitogenic effects of estrogen, unopposed by progestin, is recognized as a contributor to the various proliferative endometrial disorders [14]. EPs also have high expression of aromatase enzyme activity. Maia et al. [15] showed that the presence of aromatase expression was significantly higher in EPs than in normal endometrium. Peripheral aromatase activity is associated with aging and adiposity. Aromatase enzyme activity increases in the presence of obesity and sex hormone-binding globulin (SHBG) decreases; as a result, free estrogen levels increase [16].

It is classically known that insulin is an anabolizing hormone, which plays a crucial role in the cellular prolifera-

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**Table 2. — Distribution of the parameters that may affect serum vitamin D levels between the groups.**

<table>
<thead>
<tr>
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<th>Study group (n=43)</th>
<th>Control group (n=47)</th>
<th>p</th>
</tr>
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<td>Previous surgery</td>
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<td>27/20</td>
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<tr>
<td>Low (600-3000 MET)</td>
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<td></td>
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<tr>
<td>Adequate (&gt; 3000 MET)</td>
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<td>10</td>
<td></td>
</tr>
<tr>
<td>Diary product</td>
<td>36/7</td>
<td>39/8</td>
<td>0.723</td>
</tr>
<tr>
<td>Fish</td>
<td>37/6</td>
<td>36/11</td>
<td>0.157</td>
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<tr>
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</tr>
<tr>
<td>&gt; 20 min</td>
<td>9</td>
<td>11</td>
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Data are presented as numbers.

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**Table 3. — Logistic regression analysis of risk factors for endometrial polyps.**

<table>
<thead>
<tr>
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<th>p</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td>BMI</td>
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<td>0.004</td>
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<td>1.070–2.440</td>
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<td>0.140</td>
<td>1.067</td>
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<td>Vitamin D</td>
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<td>0.827</td>
<td>0.994</td>
<td>0.942–1.049</td>
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<td>Calcium</td>
<td>0.071</td>
<td>0.790</td>
<td>1.085</td>
<td>0.596–1.973</td>
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<td>Comorbidity</td>
<td>0.764</td>
<td>0.382</td>
<td>2.261</td>
<td>0.363–14.072</td>
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<tr>
<td>Previous surgery</td>
<td>0.209</td>
<td>0.647</td>
<td>1.251</td>
<td>0.479–3.266</td>
</tr>
<tr>
<td>Multiparity</td>
<td>0.088</td>
<td>0.766</td>
<td>1.158</td>
<td>0.439–3.057</td>
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<td>Smoker</td>
<td>1.739</td>
<td>0.187</td>
<td>0.476</td>
<td>0.158–1.435</td>
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</tbody>
</table>

OR= odds ratio, CI= confidence interval. p < 0.05 is considered statistically significant.
tion directly or indirectly via insulin like growth factors (IGF). Obesity also plays an important role in the pathogenesis of insulin resistance that can result in the proliferation of tumor cells. Insulin and IGFs stimulate hormone-dependent cell proliferation, which is demonstrated through the growth of breast and colon cancer. Serhat et al. [17] previously reported that obesity is an independent risk factor in the development of EPs. Hypertension and diabetes mellitus were not determined as risk factors associated with EPs. Bakour et al. [18] found that age, gravidity, parity, menopause status, and tamoxifen use were risk factors associated with EPs, whereas hormone replacement therapy was not. Dreisler et al. [19] suggested that being overweight (BMI > 25 kg/m²) and using hormones in the postmenopausal period are positively correlated with polyps. While the incidence of EPs increases with age, they are more common in premenopausal women than in postmenopausal women. A previous study found the prevalence of EPs to be 12% in premenopausal women and 6% in postmenopausal women [20]. In the present study, the authors firstly excluded these confounder factors such as diabetes, hypertension, and menopause status from the study. Also, mean BMI, which is the quantitative value of body fat and obesity, was significantly higher in the study group. The findings in the current study were similar to results found in the literature and compatible with the literature increased BMI was found to be only significant risk factor for EPs.

VD is essential for the development, growth, and maintenance of healthy bones during lifetime of a human. Besides the osteoblast and the small intestine, the VDR has been identified in almost every tissue and cell in the body, including brain, heart, skin, pancreas, breast, colon, and immune cells [21]. VD also inhibits the cell proliferation and stimulates the differentiation of normal as well as malignant cells [22]. Its anti-neoplastic effects had been shown in various type of cancer, which requires a higher dose to reduce cancer cell proliferation. However, supra-physiologic use of active VD is hampered by its calcemic side effects. Therefore, VD derived analogs were developed that are characterized by lower calcemic side effects and stronger anti-neoplastic effects. Some VD analogs have been approved in the treatment of psoriasis that is a hyper-proliferative condition of the skin [23]. There are several publications suggesting that there might be a possible association between nasal and colonic polyps and serum VD levels. A meta-analysis showed that there was a consistent inverse relationship between serum VD levels and colorectal cancer [24]. In the study of Wang et al. [25], VD levels were significantly lower in patients with chronic rhinosinusitis with nasal polyps, which revealed an association with greater nasal polyp. In another study authors concluded that women who have low levels of circulating VD may be at higher risk of distal colorectal adenomas [26]. However, the available data are especially conflicting for colonic polyps. Adams et al. [27] suggested that the established inverse association between circulating VD and colonic adenoma may not apply to hyperplastic polyps. However, Sy and Bautista [28] pointed out a possible threshold effect of 25(OH)VitD3 at < 30 ng/ml associated with increasing odds of being colonic polyps. EPs generally do not lead to serious health problems. They sometimes may cause excessive uterine bleeding, that may consequently cause anemia. The malignant potential of EPs is controversial; but in general, malignant transformation of these lesions is very rare and occurs most commonly in the postmenopausal period. In a previous study, endometrial carcinoma was found in 3.9% of postmenopausal women with an EP [29]. Giordano et al. [30] reported that age, menopausal status, hypertension, and obesity may increase the risk of premalignant and malignant polyps. Petterson et al. [31] suggested a three-fold increased risk of developing endometrial carcinoma in women who previously underwent endometrial curettage and was diagnosed with an EP. In another previous cohort study, the prevalence of EP was found to be 24% in 1,305 endometrial biopsies, and that there was a 0.06% risk of a premalignant or malignant lesion [32]. Kilicdag et al. [33] showed that premenopausal women with PCOS and those with two or more polyps had an increased risk of malignancy. Premalignant or malignant conditions were found in 2.2% of 417 premenopausal women in their study. There were no cases of adenocarcinoma or hyperplasia with atypia confined to EPs diagnosed at the present study. The reason for this may be due to the young age of women.

The vast majority of VD is synthesized in the skin by the sunlight. However, vegetarians, Black race, sedentary life, and women with limited sun exposure as those who live in cold climates and northern latitudes or prefer covered dressing style are under the risk of VD deficiency. The present authors designed a study assessing the factors that may affect serum VD levels. Although 25(OH)VitD3 levels were lower in the study group, the differences between mean 25(OH)VitD3 level and associated factors were not statistically significant. In the present study the authors also found that the mean serum level of 25(OH)VitD3 was very low, but there is no consensus on an optimal serum level to maintain overall health. However, most agree that at least 20 ng/ml serum level is required for bone health [7]. To the present authors’ knowledge, this study carried out on this issue is the first clinical study in the literature. Although it was attempted to be a well-designed, cross-sectional in nature study, it may not reveal a clear cause-effect relationship.

In conclusion, according to this study, VD deficiency is common among reproductive-aged women. However, there is no relation between VD deficiency and EPs. The most important risk factor for EP formation seems to increase in BMI. Future large-scale studies are required to evaluate the effects of VD in the development of EPs.
Acknowledgements

The authors would like to thank all women who participated in this study.

References


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Depressive symptoms’ pattern in postmenopausal women

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¹ Department of Gynecological Endocrinology, University of Medical Sciences of Poznan, Poznan
² Department of Child and Adolescent Psychiatry, University of Medical Sciences of Poznan, Poznan
³ Department of Operative Gynecology, University of Medical Sciences of Poznan, Poznan (Poland)

Summary
The study was conducted to elucidate the problem of depressive symptoms’ pattern in climacteric women. The study included 128 postmenopausal women aged 47-65 years admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, because of climacteric symptoms. The authors assessed the intensity of climacteric symptoms with the Kupperman index and the severity of depressive symptoms with the Hamilton depression scale. They measured BMI of all studied women. The average score of the Hamilton scale in the studied group was 11 points (SD ± 7 points). No depressive symptoms were found in 40 (31.2%) of the study participants. A slight increase in the severity of the depression symptoms was found in 64 (50%) and 23 (29.9%) displayed depressive symptoms at a moderate severity, while one (1.3%) study participant was diagnosed with very severe depressive symptoms. Depressive symptoms observed most often were: somatic symptoms of anxiety and fear experienced in 90 (70.2%), light and interrupted sleep reported in 88 (68.6%), and general symptoms in 88 (68.6%) of the study participants.

Key words: Depression; Menopause; Climacterium.

Introduction
Climacteric women often give depressive symptoms as a reason for seeing a doctor [1]. Their occurrence pose a diagnostic problem for a gynaecologist, especially given that they sometimes resemble symptoms of other somatic dysfunctions. The etiology of the depressive symptoms during menopause is not clear and it is believed that the underlying cause, just as in the case of neurovegetative symptoms of the climacteric syndrome, are hormonal changes. This study was conducted to elucidate the pattern of depressive symptoms in postmenopausal women.

Materials and Methods
The study included 128 postmenopausal women aged 47-65 years admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, because of climacteric symptoms. The mean age of the studied women was 54.6 ± 3.8 years. The mean time since last menstrual period was 5.9 ± 4.6 years. The mean BMI value in the study group was 26.4 ± 5.7 kg/m².

The intensity of climacteric and depressive symptoms was evaluated with the Kupperman index [2] and the Hamilton depression scale [3], respectively, in all study participants. The study was approved by the Ethics Committee, Poznan University of Medical Sciences, and financed by the State Committee for Scientific Research (project no: 50305-01109136-12261-08039).

Results
The mean score of the Kupperman index in the studied group was 25 points (SD ± 13 points); 29 women (22.6%) were diagnosed with a mild climacteric syndrome, 36 (28.1%) with a moderate severity climacteric syndrome, and 34 (26.5%) with a climacteric syndrome of high severity. The occurrence rate of individual symptoms of the climacteric syndrome is presented in Table 1. No statistical correlation was found between the degree of severity of the climacteric syndrome and the age of the studied women or in the time since the last menstrual period. A positive correlation was found between the time since the last period and the degree of severity of arthralgia (p < 0.01).

An average score according to the Hamilton scale in the studied group was 11 points (SD ± 7 points); in 64 (50%) of the patients there was a slight increase in the severity of the depression symptoms, 23 (29.9%) were diagnosed with depressive symptoms of moderate severity, and one (1.3%) with depressive symptoms of high severity. The occurrence rate of individual depression symptoms is presented in Table 2. No statistical correlation was found between the age and time since the last period, with the degree of severity of arthralgia (p < 0.01).

Discussion
In the studied group of postmenopausal women, on the basis to the Hamilton scale, 87 patients (79.9%) of the
study participants) were diagnosed with depressive symptoms, where in 64 (50%) the degree of the symptoms severity was typical of a mild depression, in 23 (29.9%) typical of a moderate depression, and in one (1.3%) typical of a serious depression. The high rate of depressive symptoms found in the study confirms the findings of other authors [4, 5]. Ballinger [6] reports that about 50% of women seeing a doctor due to symptoms of the climacteric period can be diagnosed with depression.

In the studied group, the most frequently diagnosed depressive symptoms were: somatic symptoms of anxiety and fear (70.2% of the study participants), a light, interrupted sleep (68.6% of the study participants), and general ailments (68.6% of the study participants). Typical depressive symptoms such as a depressed mood, sense of guilt, as well as suicidal thoughts and tendencies occurred less often, which proves that the depressive symptoms during postmenopause have a different clinical picture than depressive symptoms during other life periods.

No correlation was found between the age or the time since the last period, and the degree of severity of the depressive symptoms, which rules out aging processes being the main factor in the etiology of the discussed symptoms and supports the role of the factors important in the etiology of the symptoms of the climacteric syndrome.

Conclusion

The occurrence rate of depressive symptoms in postmenopausal women is high. Among the most frequent depressive symptoms in postmenopausal women are: somatic symptoms of anxiety and fear, light and interrupted sleep, and general symptoms.

References


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Table 1. — Climacteric symptoms’ frequency in the studied group.

<table>
<thead>
<tr>
<th>Symptom of the climacteric syndrome</th>
<th>Number of studied women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hot flashes</td>
<td>102 (79.7%)</td>
</tr>
<tr>
<td>2. Sweating</td>
<td>105 (81.9%)</td>
</tr>
<tr>
<td>3. Sleep disorders</td>
<td>86 (67.1%)</td>
</tr>
<tr>
<td>4. Anxiety</td>
<td>100 (78%)</td>
</tr>
<tr>
<td>5. Depressed mood</td>
<td>79 (61.6%)</td>
</tr>
<tr>
<td>6. Dizziness</td>
<td>63 (49.1%)</td>
</tr>
<tr>
<td>7. General weakness</td>
<td>74 (57.7%)</td>
</tr>
<tr>
<td>8. Arthralgia</td>
<td>91 (71%)</td>
</tr>
<tr>
<td>9. Headaches</td>
<td>69 (53.8%)</td>
</tr>
<tr>
<td>10. Palpitations</td>
<td>85 (66.3%)</td>
</tr>
<tr>
<td>11. Paresthesia</td>
<td>78 (60.8%)</td>
</tr>
</tbody>
</table>

Table 2. — Depressive symptoms’ frequency in the studied group.

<table>
<thead>
<tr>
<th>Depressive symptom</th>
<th>Number of studied women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Depressive mood</td>
<td>53 (41.3%)</td>
</tr>
<tr>
<td>2. Sense of guilt</td>
<td>53 (41.3%)</td>
</tr>
<tr>
<td>3. Suicidal thoughts and tendencies</td>
<td>33 (25.7%)</td>
</tr>
<tr>
<td>4. Difficulty falling asleep</td>
<td>71 (55.4%)</td>
</tr>
<tr>
<td>5. Light, interrupted sleep</td>
<td>88 (68.6%)</td>
</tr>
<tr>
<td>6. Waking up early</td>
<td>78 (60.8%)</td>
</tr>
<tr>
<td>7. Less engaged and having difficulties at work</td>
<td>75 (58.5%)</td>
</tr>
<tr>
<td>8. Inhibition</td>
<td>30 (23.4%)</td>
</tr>
<tr>
<td>9. Motor excitability</td>
<td>22 (17.2%)</td>
</tr>
<tr>
<td>10. Psychological symptoms of anxiety and fear</td>
<td>84 (65.5%)</td>
</tr>
<tr>
<td>11. Somatic symptoms of anxiety and fear</td>
<td>90 (70.2%)</td>
</tr>
<tr>
<td>12. Gastrointestinal disorders</td>
<td>22 (17.2%)</td>
</tr>
<tr>
<td>13. General symptoms</td>
<td>88 (68.6%)</td>
</tr>
<tr>
<td>14. Reproductive system disorders</td>
<td>77 (60.1%)</td>
</tr>
<tr>
<td>15. Hypochondria</td>
<td>16 (12.5%)</td>
</tr>
<tr>
<td>16. Loss of body weight</td>
<td>6 (4.7%)</td>
</tr>
<tr>
<td>17. Critical approach</td>
<td>8 (6.2%)</td>
</tr>
</tbody>
</table>
Effect of cervical conization on pregnancy outcome of in-vitro fertilization/intracytoplasmic sperm injection treatment: a retrospective cohort study

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2 National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Jinan
3 The Key laboratory for Reproductive Endocrinology of Ministry of Education, Jinan (China)

Summary

Aims: To investigate the effect of cervical conization on the outcome of in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment. Materials and Methods: The authors performed a retrospective, database-searched cohort study based on patients undergoing controlled ovarian hyperstimulation and IVF/ICSI between 2009 and 2013 in the present hospital. Cervical intraepithelial neoplasia (CIN) or cervical cancer was carefully confirmed by transvaginal ultrasound, hysteroscopy, and biopsy. High-quality case-control study with strict inclusion criteria was conducted. The authors analyzed basic characters and main IVF/ICSI outcomes between both groups. Results: The authors included 48 patients with a history of cervical conization who underwent IVF/ICSI and control group without cervical conization. No significant differences were found in IVF/ICSI outcomes between both groups. No obvious evidence was found indicating that cervical stenosis could impact IVF operation. Conclusions: The present results suggest that cervical conization does not affect IVF/ICSI outcomes. Patients can receive cervical conization before undertaking assisted reproductive technology.

Key words: Cervical conization; In vitro fertilization; Pregnancy; Cohort study.

Introduction

Cervical screening for early identification and treatment of cervical intraepithelial neoplasia (CIN) has reduced incidence and mortality from cervical cancer [1-3]. Cervical conization including cold knife conization and loop electrosurgical excision procedure (LEEP) is an efficient and low-morbidity treatment for CIN [4, 5]. Loss of normal functional cervical structure and healing process in regenerated crater after excision may inhibit sperm penetration and conception [6, 7]. Previous studies have shown conflicting results on the outcome of pregnancy following cervical conization and those studies are limited in certain countries. One meta-analysis by Kyrgiou et al. [8] showed that both cold knife conization and LEEP were significantly associated with preterm delivery and low birth weight. However, Kalliala et al. [9] conducted a cohort study on CIN treatment and pregnancy outcomes of 3,530 women and the results indicated that CIN treatment did not reduce pregnancy incidence and women had more live births after compared to before CIN treatment. Demeter et al. [10] discovered that pregnant patients with CIN who underwent cold knife conization during pregnancy were not at increased risk of adverse pregnancy outcomes, however they were at increased risk of cesarean delivery. Recently, Kyrgiou et al. [11] conducted a systematic review and meta-analysis of cohort studies on fertility and early pregnancy outcomes after treatment for CIN. They found that treatment for CIN had no adverse impact on fertility, although treatment was associated with a significantly increased risk of miscarriages in the second trimester.

Although the impact of treatment for cervical precancer on obstetric sequelae has been extensively described [12, 13], its effect on the subsequent in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) procedure and outcomes for infertile patients has been relatively under-reported. Then the question whether cervical conization could affect IVF/ICSI outcomes appeared. Many investigations focused on just pregnancy outcomes but not on IVF outcomes. It has not been fully illustrated whether this surgical treatment is adverse to pregnancy after IVF/ICSI. There are only few studies about this topic until now.

To this aim, the present authors conducted a retrospective case-controlled study to explore the effect of cervical conization on IVF/ICSI. Additionally they observed whether some side-effects of this surgery, like cervical stenosis, could influence embryo transfer (ET) operation.
Materials and Methods

Study design

The authors performed a retrospective cohort study involving the collection of data from the electronic records of a total of 39,653 IVF/ICSI cycles between January 2009 and December 2013 in the Centre for Reproductive Medicine, Shandong Provincial Hospital affiliated to Shandong University. This study was approved by the Institutional Review Board (IRB) of Shandong University. Written informed consent was obtained from the participants at the time of presentation for IVF/ICSI treatment.

Briefly, the study group (group 1) met the following inclusion criteria: (1) age 44 years or less; (2) fresh stimulated and transferred cycles; (3) with a history of cervical conization including cold knife conization and LEEP. The control group (group 2) without a history of cervical conization was matched to the study group by criteria described previously [14]. The authors attempted matching as closely as possible, and most cases were able to match at least three of these criteria. Researchers performing the matching were blinded to IVF/ICSI outcomes. If multiple patients fitted the criteria, one was selected at random. The following exclusion criteria were used: (1) oocyte donor treatment cycles; (2) presence of other comorbidities which prevented matching, e.g. chromosome abnormalities. All the data for assessment originated at Shandong Provincial Hospital affiliated to Shandong University and were comparable.

Statistical analysis

Statistical analysis was performed using SPSS17.0, taking the matching into consideration between women and controls of each case. For continuous variables (birth weight, age), the difference between the case and the mean of the control was computed and tested with a t-test for paired comparisons. For proportions, the Chi-square test or Fisher’s exact test were applied to obtain group comparisons. Data are presented as mean ± SD (standard deviation). The two-tailed p-value < 0.05 was considered significant. Baseline characteristics that were found to differ between the groups (p < 0.05) were entered into two-category models to control for confounders.

Results

Ultimately, 48 patients with a history of cervical conization undergoing the same number of fresh ET cycles, along with 48 related matched controls, were included in the final analysis (Figure 1). As for study group (group 1), diagnosis was micro-invasive carcinoma in one (2.1%) case, carcinoma in situ of cervix in eight (16.7%) cases, CIN3 in 11 (22.9%) cases, CIN2 in 12 (25.0%) cases, CIN1 in one (2.1%) case, cervicitis (not the right indication for cervical conization) in one (2.1%) case, and status unknown in 14 (29.2%) cases. Pregnancy outcomes are shown in Table 1; 94.6% of patients in both groups were undergoing their first or second ET cycle and 10% of patients in study group had recurrent miscarriage history.

Cervical conization has no significant effect on pregnancy outcomes.

Baseline characteristics of Group 1 (study group) and 2 (control group) are shown in Table 2. There were no significant differences between both groups in terms of age, body mass index (BMI), duration of infertility, or ovarian reserve. Main indications included male, tubal and combination factors, and other unknown reasons.

The outcomes of ovarian stimulation and IVF/ICSI are shown in Tables 1 and 3. There was no significant difference in any of listed ovarian response parameters or embryological parameters between both groups. There was no significant difference in cycle cancellation rate, term deliveries rate, preterm deliveries rate per cycle, clinical miscarriage, non-pregnant, vaginal delivery, and caesarean delivery rate. Although preterm deliveries rate of study group is higher than the control group, there is no statistically
2. **Indication to IVF**

Sarean delivery rate were higher than the control group rates. Although preterm delivery birth (PTB) rate and ce-

routinely. That may also help to evaluate the cervical mor-

phology.

The present study has the following strengths: first, the rel-

ationship of cervical conization and outcomes of IVF seems to be only recently placed on the agenda, hence researches on this issue are insufficient. Nowadays, IVF/ICSI protocols have continued to evolve with efforts to improve outcomes [21, 22]. Treatment success may be related to certain procedural factors and the present authors made efforts to prove whether cervical conization affects IVF/ICSI success. Although they collected only 48 patients’ baseline information and treatment outcomes, this study provides references to clinicians for patient consultation and in choosing optional treatment strategy. Second, the matching procedures are one of the most important strengths of this study. Control groups were specifically chosen so that confounding variables were eliminated. As far as the present authors know, few previous studies were as strictly and systematically controlled for age, number of cycles, comorbidities, and other confounding factors in comparison to this study. Many other studies were un-

controlled (or historically controlled). Third, the authors observed that cervical stenosis did not influence IVF/ICSI procedure. There were no records regarding any difficulty on the ET procedure in the study group.

There are some limitations as well. This is a retrospective study, which by nature can include selection bias. For example, data were collected from one IVF center. The sample size is small, just because the present authors were only able to collect 48 patients that met the study group criterion; as a consequence the results should be interpreted with caution. Also they did not have sufficient data to study the height of the cervical cone or the severity of the CIN lesions or the time window between diagnosis of CIN and ART treatment. For further study, the authors can collect larger

### Table 2. — Baseline characteristics of study group (group 1) and control group (group 2).

<table>
<thead>
<tr>
<th>Item</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>33.2±4.40</td>
<td>33.2±4.36</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.9±3.22</td>
<td>22.3±3.37</td>
<td>NS</td>
</tr>
<tr>
<td>History of infertility(years)</td>
<td>4.6±3.3</td>
<td>4.4±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 serum FSH (IU/ml)</td>
<td>6.5±2.22</td>
<td>6.9±2.00</td>
<td>NS</td>
</tr>
<tr>
<td>Mean day 3 E2 (pg/ml)</td>
<td>39.0±18.19</td>
<td>54.0±48.26</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Indication to IVF**

- Male factor: 3 (6.3%) 7 (14.6%) NS
- Tubo factor: 34 (70.8%) 25 (52.1%) NS
- Combination: 5 (10.4%) 14 (29.2%) NS
- Other: 6 (12.5%) 2 (4.2%) NS

**Table 3. — Ovarian stimulation outcomes in groups 1 and 2.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>48</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Protocol of ovary stimulation, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short agonist</td>
<td>17 (23.7%)</td>
<td>11 (22.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Long agonist</td>
<td>30 (62.5%)</td>
<td>36 (75.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Ultra long GnRH agonists</td>
<td>1 (2.1%)</td>
<td>1 (2.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Starting dose of Gn</td>
<td>192.5±53.7</td>
<td>191.9±56.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total dosage of Gn per cycle (IU)</td>
<td>2009±874</td>
<td>1959±857</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of Gn stimulation (days)</td>
<td>10.6±2.0</td>
<td>10.4±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness on hCG day, cm</td>
<td>1.06±0.2</td>
<td>1.06±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean number of oocytes retrieved</td>
<td>12.8±5.5</td>
<td>12.4±9.2</td>
<td>NS</td>
</tr>
<tr>
<td>Good quality embryo transferred</td>
<td>1.72±0.39</td>
<td>2.62±0.98</td>
<td>NS</td>
</tr>
<tr>
<td>Mean number of embryos transferred</td>
<td>0.92±1.05</td>
<td>0.40±0.63</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 2.** Baseline characteristics of study group (group 1) and control group (group 2).

**Table 3.** Ovarian stimulation outcomes in groups 1 and 2.

Note: values are given as means ± SE or n (%).

FSH = follicle-stimulating hormone; NS = not significant.

**Note:** hCG = human chorionic gonadotropin; Gn = gonadotropin; GnRH = Gn-releasing hormone.

Discussion

The present study compared basic information and pregnancy outcomes of 48 patients with a cervical coniza-

tion history and matched group. According to the strict criterion and analysis, the present authors conclude that cervical conization does not significantly impair IVF/ICSI outcomes, especially miscarriage and preterm delivery rates. Although preterm delivery birth (PTB) rate and ce-

sarean delivery rate were higher than the control group but there was no statistical significance. Recently, Pinborg *et al.* [15] reported that in the ART singleton deliveries, the PTB rate was significantly higher in women with cervical conization than without (13.1 vs. 8.2%) while it nearly doubled in ART twin deliveries. Compared to Pinborg *et al.* study, the present authors did not achieve significant results perhaps due to their small sample size. Also they did not analyze the PTB on ART twin deliveries. However, Acharya *et al.* discovered that LEEP in women with CIN did not significantly increase the risk of low birth weight or preterm birth in subsequent pregnancy in comparison to their controls, which is partly consistent to the present study [16]. There has also been reported that LEEP does not affect mode of delivery in the subsequent pregnancy [17-20]. Pinborg *et al.* [15] also reported that cervical dysplasia did not increase the risk of any of the other adverse outcomes in ART singletons or twins, which is also consistent with the present study.

There were no records regarding difficulty during ET procedure related to patients’ cervical conization history. The present authors perform hysteroscopy before IVF/ICSI routinely. That may also help to evaluate the cervical morphology.

The present study has the following strengths: first, the rel-

ationship of cervical conization and outcomes of IVF seems to be only recently placed on the agenda, hence researches on this issue are insufficient. Nowadays, IVF/ICSI protocols have continued to evolve with efforts to improve outcomes [21, 22]. Treatment success may be related to certain procedural factors and the present authors made efforts to prove whether cervical conization affects IVF/ICSI success. Although they collected only 48 patients’ baseline information and treatment outcomes, this study provides references to clinicians for patient consultation and in choosing optional treatment strategy. Second, the matching procedures are one of the most important strengths of this study. Control groups were specifically chosen so that confounding variables were eliminated. As far as the present authors know, few previous studies were as strictly and systematically controlled for age, number of cycles, comorbidities, and other confounding factors in comparison to this study. Many other studies were un-

controlled (or historically controlled). Third, the authors observed that cervical stenosis did not influence IVF/ICSI procedure. There were no records regarding any difficulty on the ET procedure in the study group.

There are some limitations as well. This is a retrospective study, which by nature can include selection bias. For example, data were collected from one IVF center. The sample size is small, just because the present authors were only able to collect 48 patients that met the study group criterion; as a consequence the results should be interpreted with caution. Also they did not have sufficient data to study the height of the cervical cone or the severity of the CIN lesions or the time window between diagnosis of CIN and ART treatment. For further study, the authors can collect larger
number of samples to analyze. It was reported that LEEP was safer for future pregnancies when compared to cold knife conization [23]. The present authors can evaluate the advantage of LEEP on IVF outcomes in their future work.

Conclusion

With strict inclusion criteria and randomly selected paired controls, the present results suggest that cervical conization may not have a strong adverse effect on IVF/ICSI outcomes. Future studies should carefully explore associations between treatment and subsequent IVF/ICSI outcomes stratifying by size of excision, treatment technique, and number of embryos implanted.

Acknowledgments

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References


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The International Continence Society (ICS) defines urinary incontinence as an involuntary leakage of urine [1]. It has a high prevalence among women of all age groups, and prevalence increases with aging [2]. One in three women experience urinary incontinence, which significantly affects quality of life (QoL) [3]. Stress urinary incontinence (SUI) is caused by pelvic floor muscle (PFM) weakness; risk factors include pregnancy, age, childbirth, body mass index (BMI) and hormonal status. The condition is associated with negative psychological effects including avoidance of sexual activity, social isolation, and decreased physical activity [4]. The ICS recommends conservative treatments as the first line of treatment for incontinence. This mainly involves correction and increase of PFM activities, which has no serious adverse effects and results in improvement or cure in two-thirds of patients [2, 5]. The method of strengthening PFMs proposed by Kegel made non-surgical treatment popular for SUI and led to the development of many other therapeutic techniques [6, 7]. One of these techniques is electromyographic-biofeedback (EMG-BF) training and can be used to learn the method of contracting PFMs, or to increase the performance of training. It enables more selective control of pelvic floor musculature, which results in decrease of micturition, defecation or sexual symptoms [8]. The other is extracorporeal magnetic innervation therapy (ExMI) which is a newer technique based on principles of natural physics. According to Faraday's law of magnetic induction, a current flows in a conducting medium to respond to a changing magnetic field. The ExMI technique employs this rule to induce a controlled depolarization of adjacent nerves and resulting muscle contraction. Previous studies reported 50% improvement in 69% of patients, with 44% dry in two weeks, and objective improvement in 58% of patients. Magnetic stimulation has been applied for non-invasive stimulation of the central and peripheral nervous system. An electrical field is induced by a varying magnetic field in a specified loop in the vicinity. Primary autonomic and somatic innervation in the lower urinary tract, which includes pelvic floor, bladder, vaginal wall, rectum, and urethra, is the root area of sacral nerves S2-S4. Stimulation of these roots is an effective method of regulating pelvic floor and then to control pelvic organs [7, 9].

In the literature, it is seen that pelvic floor muscle training (PFMT), ExMI, and EMG-BF training are all individually effective in the treatment of urinary incontinence [8, 10-13]. However, no previous study has evaluated the best and most effective of these three treatment methods. This study compared three conservative therapy strategies for PFMT: ExMI, EMG-BF, and home exercises.

### Summary

**Purpose:** This study aimed to compare the effectiveness of EMG-biofeedback (EMG-BF), extracorporeal magnetic innervation (ExMI), and pelvic floor muscle training (PFMT) treatments on women with stress urinary incontinence (SUI). **Materials and Methods:** The study included 67 women with SUI. Pelvic floor muscles (PFMs) were evaluated with electromyography and the quality of life (QoL) with Incontinence Quality of Life (I-QoL) questionnaire; afterwards, the subjects were divided into three groups: EMG-BF group (n=23), ExMI group (n=20), and PFMT group (n=24). EMG-BF group and ExMI group were given training in urogynecological physiotherapy clinic. PFMT group were given eight-week home exercises. Each group was assessed before training and after eight weeks. **Results:** All three groups showed a significant improvement in EMG activity values and average QoL scores. The greatest improvement was observed in the EMG-BF training group for QoL scores. **Conclusions:** This study demonstrated that all of the three methods performed with the purpose of increasing PFM strength were effective. The increase in PFM strength reduces incontinence associated symptoms and thus improves QoL.

**Key words:** EMG-biofeedback; Extracorporeal magnetic innervation; Pelvic floor muscle training; Urinary incontinence.
Materials and Methods

Patients were diagnosed by a gynecologist by means of patient history, clinical, and physical examinations. Among 130 patients referred as having SUI, those with prosthesis, other implanted metallic devices, cardiac pacemaker, arrhythmia, pelvic malignancies, under radiotherapy, pelvic floor defect, previous surgery for urinary incontinence, and neurological diseases were excluded and the study that began with 80 patients. Thirteen of these left the study due to reasons such as familial problems, irregular attendance to training, feeling discomfort with probe, and for no reason. A total of 67 patients completed the study. Flow diagram is shown Figure 1. The patients were informed about the treatment. The study was approved by the Ethics Committee of Abant Izzet Baysal University.

The following patient information was recorded: height, weight, BMI, occupational status, education level, medical and obstetrical history (number of gravidity, parity, abortus, dilatation and curettage, type of delivery, and the status of having episiotomy and/or perineal tears), used medications, urinary symptoms such as urgency, incontinence conditions (coughing, laughing, sneezing, lifting heavy items, jumping, walking), and duration of incontinence problem.

The Turkish version of the Incontinence Quality of Life (I-QoL) instrument was used to evaluate the effect of SUI on QoL. The patients were asked to score the degree of bother via a four-point rating scale: 0 = not at all, 1 = slightly, 2 = moderately, and 3 = greatly. The survey comprises 28 questions and overall scores range from 0–84. Total scores were recorded for the analysis [14, 15].

A Myomed 932 biofeedback and EMG unit recorded vaginal squeeze in microvolts (μV) to measure PFM EMG activity during three maximal voluntary contraction (MVC) attempts. The patients were asked to lie in a supine position and bend their knees with their legs slightly apart. A physiotherapist used a vaginal probe to measure PFM EMG activity at rest and during MVC. The mean value of three attempts was recorded [16].

All evaluations were made at baseline and at the end of the procedure. Following the first assessment, patients were informed about each of the three treatment methods (ExMI, EMG-BF, and PFMT as home program) and asked to choose one for their treatment. The treatment method was determined by considering the desires of patients.

For the ExMI procedure, patients were requested to sit fully clothed on a special chair and to remove all metallic objects they were wearing (jewelry, watch etc.). The chair contains a magnetic field generator (therapy head) that is controlled by an external power unit. The power output can be adjusted by the physiotherapist. The size and strength of the magnetic field is determined by adjusting the amplitude of the device. The pulse has a steep leading edge that creates a step gradient magnetic field directed vertically through the seat of the chair. The perineum is located in the middle of the seat when the patient is seated. In this position, the PFM and sphincters are located directly on the primary axis of the pulsing magnetic field. Magnetic field flux can directly penetrate...
The comparison of EMG-biofeedback and extracorporeal magnetic innervation treatments in women with urinary incontinence

All perineal tissues. The procedure only generates a magnetic flux but delivers no electrical charge to the patient’s body. The frequency of the pulsed magnetic field was adjusted to five Hz for ten minutes intermittently. Following a five-minute resting period, the second treatment period was applied intermittently at 50 Hz for 10 minutes [17]. Patients were offered treatment sessions of 20 minutes three times a week for a period of eight weeks.

For EMG-BF training, patients received PFMT from a physiotherapist three times during eight weeks using a device with an intravaginal probe. Patients were asked to contract PFMs during visual and auditory EMG signals. Individualized training programs were prepared and all patients were instructed to perform PFM contractions in an isolated way. Each program was prepared according to repetition number, the patient’s muscle strength, endurance, and tolerance. Before starting the training, each patient’s maximum PFM contraction period was recorded and was increased as the maximal contraction period increased. There were ten-second resting breaks between contraction periods. One session lasted for approximately 20 minutes [18].

As home program, patients were asked to perform exercises as contracting and relaxing for fast-twitch muscles and contracting slowly for slow-twitch muscles (by counting to ten; holding contraction by counting to ten; relaxing slowly by counting to ten). Patients initially performed five sets of ten repetitions. They added five more sets each week until reaching 30 sets per day, and then continued with this number. An eight-week PFM exercise tracking chart was given to patients to help them remember the exercises and to perform the exercises in a more disciplined way [16].

Data were analyzed using SPSS (version 16.0, demo). Quantitative and qualitative data are presented in the form of mean, standard deviation and percentage, and frequency (%), respectively.

Table 1. — Demographic characteristics of patients.

<table>
<thead>
<tr>
<th></th>
<th>ExMI (n=20)</th>
<th>PFMT (n=24)</th>
<th>EMG-BF (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.55±8.62</td>
<td>51.54±7.13</td>
<td>50.86±7.35</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72.46±15.68</td>
<td>71.96±11.33</td>
<td>79.04±14.41</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.57±0.04</td>
<td>1.57±0.06</td>
<td>1.57±0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.14±6.33</td>
<td>29.25±4.90</td>
<td>31.72±5.39</td>
</tr>
</tbody>
</table>

Table 2. — Pre- and post-EMG activity values of groups.

<table>
<thead>
<tr>
<th></th>
<th>ExMI (n=20)</th>
<th>PFMT (n=24)</th>
<th>EMG-BF (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMG Activity Value (μV) Pre</td>
<td>14.55±8.91</td>
<td>20.60±8.54</td>
<td>-3.86 0.00*</td>
</tr>
<tr>
<td>EMG Activity Value (μV) Post</td>
<td>3.87±7.33</td>
<td>20.12±7.83</td>
<td>-4.11 0.00*</td>
</tr>
<tr>
<td>Z</td>
<td>1.78±6.78</td>
<td>19.17±7.51</td>
<td>-4.20 0.00*</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of EMG activity values between groups.

<table>
<thead>
<tr>
<th></th>
<th>ExMI (n=20)</th>
<th>PFMT (n=24)</th>
<th>EMG-BF (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMG Activity Value (μV)</td>
<td>6.05±4.69</td>
<td>32.18</td>
<td>32.18</td>
</tr>
<tr>
<td>X±SD</td>
<td>6.25±5.53</td>
<td>31.77</td>
<td>1.426 2 .49</td>
</tr>
<tr>
<td>Rank mean</td>
<td>33.98</td>
<td>37.91</td>
<td>37.91</td>
</tr>
</tbody>
</table>

Table 4. — Pre- and post-I-QoL instrument values of groups.

<table>
<thead>
<tr>
<th></th>
<th>ExMI (n=20)</th>
<th>PFMT (n=24)</th>
<th>EMG-BF (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument-pre</td>
<td>38.15±27.36</td>
<td>22.65±25.22</td>
<td>-3.74 0.00*</td>
</tr>
<tr>
<td>Instrument-post</td>
<td>24.00±21.26</td>
<td>15.70±19.65</td>
<td>-3.36 0.00*</td>
</tr>
</tbody>
</table>

Table 5. — Comparison of I-QoL instrument values between groups.

<table>
<thead>
<tr>
<th></th>
<th>ExMI (n=20)</th>
<th>PFMT (n=24)</th>
<th>EMG-BF (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>15.50±17.23</td>
<td>33.98</td>
<td>33.98</td>
</tr>
<tr>
<td>X±SD</td>
<td>8.29±9.96</td>
<td>26.56</td>
<td>26.56</td>
</tr>
<tr>
<td>Rank mean</td>
<td>21.73±20.17</td>
<td>41.78</td>
<td>41.78</td>
</tr>
</tbody>
</table>

Wilcoxon matched-pairs test, Kruskal Wallis test, and Mann-Whitney test were used for statistical analysis. A p value ≤ 0.05 was considered as statistically significant.

Results

Table 1 shows patients’ demographic characteristics. There was no difference in demographic characteristics between groups. Patients’ EMG activity values revealed a significant improvement after treatment procedures in all groups according to Wilcoxon matched-pairs analysis (p ≤ 0.00, Table 2). There was no difference in EMG activity values between groups by Kruskal Wallis analysis (Table 3). All three groups showed a significant improvement in average QoL scores based on Wilcoxon matched-pairs test (Table 4). The greatest improvement was observed in the EMG-BF training group according to Mann-Whitney test (Tables 5 and 6).

Discussion

This study revealed that EXMI, EMG-BF, and a home program of PFM exercises improved QoL and increased PFM strength in patients with SUI. There was no difference between the three methods with regards to improving
PFM strength; however, the EMG-BF method provided superior improvements to QoL.

Culligan et al. [19] analyzed the effect of ExMI on PFM strength by perineometry in primiparous women. They reported no difference between women who received active or sham ExMI treatment in the early postpartum period. Voorham et al. [7] analyzed clinical results of ExMI therapy, concentrating on improvements in pelvic floor musculature, urodynamics, and QoL. They reported no change in PFM function by ExMI. Lee et al. [11] reported that PFM exercises combined with extracorporeal biofeedback device were effective in reducing urinary leakage and increasing muscle strength. The literature includes contradictory findings on the effect of ExMI on PFM strength. Some researchers report there is no impact; other reported that there is an increase. In this study, ExMI training increased PFM strength by 38.97%.

PFMT has been evaluated over the years and is recognized as an effective conservative treatment in women with urinary incontinence. A Cochrane review contained 14 randomized controlled trials involving 353 women who received PFMT versus 319 controls, supported PFMT as an effective treatment in reducing the symptoms of stress, urge, and mixed incontinence [20]. Fitz et al. [21] reported that PFM strength increased from two to 3.5 on the Oxford scale in a group of 36 women who received PFMT. It is reported in the literature that regular PFMT can improve the function of PFMs [20]. The present study found that regular PFMT over a period of eight weeks increased PFM strength by 44.87%. It is known that increase in PFM strength reduces the symptoms of incontinence [22]. A previous study used pad and leakage tests and reported that increased PFM strength was correlated with decreased leakage [23]. According to statements made by patients, the present authors conclude that incontinence symptoms among patients in the home program group decreased due to the increase in PFM strength.

Morkved et al. [24] compared PFMT with and without biofeedback for six months for women with stress incontinence. They assessed PFM strength via vaginal balloon catheter, and reported increased muscle strength in both groups; however, between three and six months, a significant improvement was observed only in the biofeedback group. Yoo et al. [25] treated with EMG-BF and electrical stimulation for eight weeks in 86 women with urinary incontinence. Patients were assessed at baseline and at four and eight weeks and three months after treatment. It was found that 57% of patients did not require further treatment. A study of the short- and long-term effects of EMG-BF on PFM strength reported that, after treatment, PFM strength increased and EMG values almost doubled, from 11.3 μV to 21.5 μV [26]. Aukee et al. [27] compared PFMT with and without EMG-BF in 30 women with SUI. It was reported that in contrast to the average increase of 10.5 μV in EMG-BF group, the increase in PFMT was 2.3 μV. A similar study was conducted by Jundt et al. [8] with EMG-BF assessment with palpation increased from 3 to 4 on the Oxford scale and this effect was maintained. Similarly in the present study, patients in the EMG-BF group showed an increase of 7.57 μV in PFM strength after treatment; although there was no significant difference between groups with regard to the effect on PFM strength, the highest increase was seen in the EMG-BF group. The present authors conclude that EMG-BF training ensured visual, auditory and tactile, stimuli, and that the individual format of the training motivated participants.

In clinical trials of incontinence training, QoL has become an important outcome measure [12]. There is a consensus that UI can have negative physical, social, and sexual impacts on QoL, although it is not considered as a direct risk for affected women [21]. The literature includes many reports that PFMT improves QoL among women with urinary incontinence [12, 17, 21, 28]. In this study, all three of the methods improved the QoL among participants. However, the EMG-BF training had the highest positive effect on QoL. Patients in the EMG-BF group showed the greatest improvement in QoL. Although patients in ExMI group was treated in clinic under observation, those in EMG-BF group tended to improve in every session by means of following visual and audial stimulus via Myomed-932 device. The present authors can attribute this to the fact group members attended urogynecological physiotherapy clinic and were each seen individually by a physiotherapist.

Conclusion

In conclusion, this study demonstrated that all of the three methods performed with the purpose of increasing PFM strength were effective. The increase in PFM strength reduces incontinence associated symptoms and thus improves QoL. The authors believe that after women with incontinence are treated with one of these methods, PFMT should be adopted and performed for the whole lifetime.

References

The comparison of EMG-biofeedback and extracorporeal magnetic innervation treatments in women with urinary incontinence


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Effect of a low glycemic diet in patients with polycystic ovary syndrome and anovulation - a randomized controlled trial

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Summary
Objective: To determine whether a low glycemic index diet is better than a normal glycemic index diet in producing ovulatory cycles in women with polycystic ovary syndrome (PCOS) and anovulation. Materials and Methods: A randomized controlled clinical trial involving 37 women with PCOS and anovulation. The authors randomly assigned low glycemic index diets (n = 19) and normal glycemic index (n = 18) diets, and analyzed the number of ovulatory cycles for three months. Results: In patients who consumed a low glycemic index diet, 24.6% (14/57) of the cycles were ovulatory. In those who consumed a normal glycemic index diet, only 7.4% (4/54) of the cycles were ovulatory (p = 0.014). Conclusions: The difference observed in the number of ovulatory cycles could be related to a decrease in the serum levels of circulating androgens, secondary to an improvement in insulin resistance.

Key words: Glycemic index; Ovulatory cycles; Polycystic ovary syndrome; Metabolic syndrome.

Introduction
Polycystic ovary syndrome (PCOS) is considered the most common endocrinopathy in women of reproductive age and can affect 5-10% of all women in this age group [1]. The clinical manifestations of this syndrome include menstrual abnormalities that vary from oligomenorrhea to amenorrhea as a consequence of chronic anovulation that has been associated with an increased serum level of free testosterone [2], which in turn determines a hormonal environment that favors hirsutism and acne [3]. There are also metabolic changes; it is even possible to say that about half of PCOS patients meet diagnostic criteria for metabolic syndrome and the majority present insulin resistance [4]. The association of compensatory hyperinsulinemia has been considered important in the pathogenesis of PCOS [5].

Central obesity is a common finding in patients with PCOS; excess adipose tissue seems to be the main source of insulin resistance in these patients. Furthermore, excess body fat has been associated with an increased risk of anovulation, hyperandrogenism, and anovulatory cycles [6].

In women with menstrual irregularities and insulin resistance, it has been shown that the control of these levels through diet and/or medication, even without a consequent weight loss, can help restore ovulatory function and menstrual cyclicity [7]. Diets based on low glycemic index foods appear to be more effective in reducing weight and body fat [8]. In contrast, high glycemic index diets have been associated with hyperinsulinemia and insulin resistance [9].

The aim of this study is to compare the effect of a low glycemic index and a normal glycemic index diet on ovulation in patients with PCOS and anovulation.

Materials and Methods
This was a controlled clinical trial performed with prior approval by the ethics committee of the present hospital.

Participating patients
The authors included 40 patients with a diagnosis of PCOS, infertility, and anovulation who attended the University Center for Reproductive Medicine (CeUMER) of the Hospital Universitario “Dr. José Eleuterio González”, in Monterrey Mexico from January 2 to December 21, 2012.

The diagnosis of PCOS was made according to the Rotterdam criteria of 2003 [10]. Diagnosis of infertility was made according to WHO criteria [11]. Anovulation was diagnosed by vaginal ultrasound monitoring of ovulation. Monitoring included a baseline ultrasound within the first three days of the menstrual cycle and then on days 11, 13, and 15 of the cycle.

After confirming the diagnosis of PCOS, infertility and anovulation, the patients were eligible if they agreed to participate in the study by signing a written informed consent; they also needed to not be under any diet or have received hormone treatment or weight loss drugs at least three months before the study. Patients with a confirmed diagnosis of diabetes mellitus and women with adnexal cystic masses were not included.
Forty patients were recruited and divided randomly, sequentially, into two groups of 20 patients each, group 1 and group 2. One patient from group 1 and two from group 2 were eliminated from the study; the patient from group 1 for not attending the nutritional assessment, and the two patients from group 2 voluntarily withdrew from the study to seek treatment for ovulation induction. Group 1 had 19 patients and group 2 18 patients.

Nutritional analysis

All patients underwent a nutritional assessment by applying a questionnaire with a 24-hour reminder that asked about food consumed including liquids, both qualitatively and quantitatively, during a period of 24 hours which corresponded specifically to the day before consultation. With this reminder the authors sought to determine the patient’s usual intake with the patient being evaluated using the Nutris system, a computer program developed by the School of Nutrition of the Autonomous University of Nuevo Leon, which provides the composition of the food consumed.

Diet intervention

Patients were subsequently included in a diet program, which was calculated according to the Harris-Benedict formula [12] for determining basal energy expenditure (BEE), adding a physical activity factor to determine the amount of calories for each patient. The diet was calculated individually with a normal percentage distribution of macronutrients: 45% and 50% complex carbohydrates, 30% to 40% fat, 10% to 15% monounsaturated fatty acids, less than 10% of polysaturated fatty acids; less than 10% of saturated fatty acids, 15% to 20% protein and 20 to 35 grams/day of fiber, in addition to a source of omega 3 fatty acids. Patients in group 1 received a diet containing foods with a glycemic index lower than 45 and the patients in group 2 received a diet with a glycemic index of 50 to 75 points. Both groups received a diet with between 1,200 and 1,500 kilocalories per day. The glycemic index of foods was calculated using the International table of glycemic index and glycemic load values [13].

Diet adherence monitoring.

As support to confirm good adherence to the diet, the patient was asked to fill out a food diary with all the foods she ate the day before, what time she had her meals, and the amounts ingested. The patients attended once a month for evaluation during three consecutive months at the start of the dietary intervention.

Ultrasound monitoring of ovulation

Ultrasound monitoring of ovulation was performed in all patients included in this study in each of the three cycles after the start of the diet. Monitoring was performed vaginally by the same operator. Baseline ultrasound imaging was performed between days 1 to 3 and then on days 11, 13, and 15 of each cycle. If any follicle was larger than 14 mm on day 15 of the cycle, ovulation was monitored every other day until day 20. The cycle was considered ovulatory, if at least one follicle showed continued growth with subsequent evidence of rupture, which was considered if free fluid was observed in the pouch of Douglas and there was a reduction in follicle diameter.

Serum analysis

All patients underwent baseline determination of serum free testosterone, FSH, LH, prolactin, glucose and insulin; also, the HOMA index was calculated [14].

Evaluation of hirsutism

The presence and degree of hirsutism was assessed with the Ferriman-Gallwey scale [15]. This evaluation was performed in each patient after agreeing to participate in the study.

<table>
<thead>
<tr>
<th>Table 1. — Patient demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Menarche (years)</td>
</tr>
<tr>
<td>Short cycle (days)</td>
</tr>
<tr>
<td>Long cycle (days)</td>
</tr>
<tr>
<td>Days of bleeding</td>
</tr>
<tr>
<td>SSSI* (age)</td>
</tr>
<tr>
<td>Primary/secondary Infertility</td>
</tr>
<tr>
<td>Time of infertility (years)</td>
</tr>
<tr>
<td>Number of pregnancies</td>
</tr>
<tr>
<td>Births</td>
</tr>
<tr>
<td>Cesarean section</td>
</tr>
<tr>
<td>Abortions</td>
</tr>
<tr>
<td>*SSI: start of sexual intercourse. Group 1 patients with a low glycemic index diet. Group 2 patients with a normal glycemic index diet.</td>
</tr>
</tbody>
</table>

Statistical analysis was performed by calculating the mean, standard deviation, and range for continuous variables, whereas frequencies were calculated for categorical variables. To compare the results, the authors used the chi-square test and Student’s t-test as appropriate. They considered a p-value < 0.05 as statistically significant. SPSS version 21 was used for data analysis.

Results

A total of 19 patients in group 1 and 18 patients in group 2 participated. Mean age was similar in both groups; in group 1 it was 26.06 years and in group 2 it was 26.05 years. No statistically significant difference was found in relation to age at menarche, duration of menstrual cycles, days of menses, age of first intercourse, number of sexual partners, time and type of infertility, number of previous pregnancies, births, cesarean sections, or abortions (Table 1).

The results of the Ferriman-Gallwey score for the evaluation of hirsutism were: a mean of 11.05 for group 1, ranging from three to 26 with a standard deviation of 6.55. Similarly, the mean score for patients in group 2 was 11.28 with a range of five to 23 and a standard deviation of ± 4.53; the difference between the groups was not statistically significant.

In relation to the evaluation of laboratory tests, no statistically significant differences were found in any of the serum parameters evaluated (Table 2).
The results of the nutritional assessment of the intake in the previous 24 hours is reported in Table 3. With the exception of cobalt intake, which the authors found was consumed in greater quantity by patients in group 1, there were no differences in the composition of the diet consumed by the patients in both groups prior to the study.

Regarding the weight of the patients, the authors found that women in group 1 weighed at baseline on average 84.136 kg, with a standard deviation of ± 19.468, ranging from 54.6 to 124 kg. The patients in group 2 weighed at baseline, on average, 83.378 kg, with a standard deviation of ± 14.493, ranging from 62.1 to 115.9 kg. The difference was not statistically significant ($p = 0.894$).

The weight of the patients, on average, after the first month was 83.373 in group 1 and 82.216 in group 2 ($p = 0.835$). The decrease in mean weight was 763 grams and 1,161 grams for group 1 and 2, respectively. The difference was not significant ($p = 0.242$).

The weight of the patients, on average, after the first month was 81.142 and 81.477 kg for groups 1 and 2 ($p = 0.901$). The decrease in weight was 1,994 grams and 1,900 grams, respectively. The difference was not significant ($p = 0.895$) (Table 4).

Table 2. — Basal laboratory test results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dL)</td>
<td>1.56 ± 1.91</td>
<td>2.56 ± 2.48</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>(0.11–8.30)</td>
<td>(0.43–9.0)</td>
<td></td>
</tr>
<tr>
<td>FSH (IU/mL)</td>
<td>5.94 ± 2.13</td>
<td>7.02 ± 1.78</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>(3.0–9.8)</td>
<td>(4.6–11)</td>
<td></td>
</tr>
<tr>
<td>LH (IU/mL)</td>
<td>7.84 ± 1.62</td>
<td>8.96 ± 2.11</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>(4.3–11.0)</td>
<td>(6.0–15.0)</td>
<td></td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>18.63 ± 6.81</td>
<td>20.9 ± 14.99</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>(6.4–34.2)</td>
<td>(8–73.1)</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>91.4 ± 10.04</td>
<td>84.5 ± 9.19</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
<td>(76–111)</td>
<td>(63–100)</td>
<td></td>
</tr>
<tr>
<td>Insulin (IU/mL)</td>
<td>26.7 ± 9.77</td>
<td>28.85 ± 20.4</td>
<td>0.684</td>
</tr>
<tr>
<td></td>
<td>(11–43)</td>
<td>(6–1–100)</td>
<td></td>
</tr>
<tr>
<td>TSH (IU/mL)</td>
<td>2.24 ± 0.80</td>
<td>1.95 ± 0.50</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>(0.92–3.88)</td>
<td>(1.23–2.22)</td>
<td></td>
</tr>
<tr>
<td>HOMA index</td>
<td>5.66 ± 1.98</td>
<td>4.95 ± 1.54</td>
<td>0.234</td>
</tr>
<tr>
<td></td>
<td>(2.21–8.77)</td>
<td>(3.1–8.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation with ranges in parentheses.

Table 3. — Basal nutritional analysis. According to the questionnaire of intake in the previous 24 hours.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber (g)</td>
<td>12.5 ± 4.58</td>
<td>12.42 ± 9.4</td>
<td>0.978</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>2318 ± 736</td>
<td>2411 ± 1105</td>
<td>0.810</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>285.42 ± 112</td>
<td>307.58 ± 121</td>
<td>0.647</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>81.25 ± 29.2</td>
<td>82.0 ± 53.4</td>
<td>0.966</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>279 ± 116.5</td>
<td>308 ± 258.1</td>
<td>0.724</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>95.2 ± 34.9</td>
<td>97.92 ± 65.4</td>
<td>0.926</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>31.1 ± 13.8</td>
<td>34.08 ± 25.3</td>
<td>0.730</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>14.1 ± 12.1</td>
<td>19.2 ± 17.1</td>
<td>0.409</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>30.0 ± 13.5</td>
<td>29.5 ± 17.3</td>
<td>0.417</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>824 ± 435.4</td>
<td>784 ± 411</td>
<td>0.816</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>895 ± 474.4</td>
<td>941 ± 615</td>
<td>0.893</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>14.5 ± 6.1</td>
<td>17.1 ± 13.1</td>
<td>0.545</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>157.2 ± 100</td>
<td>224 ± 211.0</td>
<td>0.332</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1407.3 ± 1125</td>
<td>1825 ± 1748</td>
<td>0.490</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1564 ± 1007</td>
<td>1805 ± 1508</td>
<td>0.649</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>7.25 ± 3.5</td>
<td>8.25 ± 7.0</td>
<td>0.666</td>
</tr>
<tr>
<td>Retinol (mcg)</td>
<td>448.1 ± 296.8</td>
<td>375.8 ± 247.8</td>
<td>0.524</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>1.33 ± 0.49</td>
<td>1.42 ± 0.79</td>
<td>0.970</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.33 ± 0.77</td>
<td>1.25 ± 0.75</td>
<td>0.792</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>14.1 ± 5.7</td>
<td>15.5 ± 9.6</td>
<td>0.684</td>
</tr>
<tr>
<td>Pyridoxine (mg)</td>
<td>0.83 ± 0.57</td>
<td>1.00 ± 0.73</td>
<td>0.544</td>
</tr>
<tr>
<td>Folic acid (mcg)</td>
<td>173.1 ± 245.9</td>
<td>179.5 ± 285.7</td>
<td>0.954</td>
</tr>
<tr>
<td>Cobalt (mg)</td>
<td>26.9 ± 19.5</td>
<td>12.8 ± 12.4</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation.

Table 4. — Mean weight.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>84.136</td>
<td>83.378</td>
<td>0.894</td>
</tr>
<tr>
<td>One month</td>
<td>83.373</td>
<td>82.216</td>
<td>0.835</td>
</tr>
<tr>
<td>Two months</td>
<td>82.524</td>
<td>82.427</td>
<td>0.983</td>
</tr>
<tr>
<td>Three months</td>
<td>81.142</td>
<td>81.447</td>
<td>0.901</td>
</tr>
</tbody>
</table>

Table 5. — Ovulatory cycles.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Group One</th>
<th>Group Two</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>1/19 (5.2%)</td>
<td>0/18 (0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>6/19 (31.5%)</td>
<td>1/18 (5.5%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>7/19 (38.8%)</td>
<td>3/18 (16.6%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Cycle 1 + 2</td>
<td>7/38 (18.4%)</td>
<td>1/36 (2.7%)</td>
<td>0.05 *</td>
</tr>
<tr>
<td>Cycle 1 + 2 + 3</td>
<td>14/57 (24.6%)</td>
<td>4/54 (7.4%)</td>
<td>0.01 *</td>
</tr>
</tbody>
</table>

*Statistically significant.
Discussion

PCOS is a complex condition that affects women of reproductive age. It not only produces changes related to menstrual cycles, such as oligomenorrhea and amenorrhea secondary to anovulatory cycles with a consequent increase in infertility rates, but also metabolic changes. Insulin resistance that predisposes the development of diabetes mellitus and cardiovascular disease has been documented [4, 16].

Most patients that present PCOS have central obesity, which is usually associated with insulin resistance [6]. For this reason, strategies that reduce obesity have been previously recognized as the first measure in the treatment of these women, which include changes in lifestyle, increased physical activity, and dietetic changes [17].

Despite having used a variety of food schemes for the prevention and treatment of PCOS, the best diet for this purpose has not been clearly determined. Diets high in monounsaturated fat, high-carbohydrate diets, low glycemic diets, as well as high protein and low carbohydrate diets have been previously used with variable results [7, 18, 19].

It is also known that not all patients with PCOS are overweight or obese; therefore the effect of a diet to decrease body weight alone could be inadequate. In this manner, an intervention that includes lowering glycemic load may favorably improve insulin resistance in these patients. An intrinsic mechanism independent from the obesity related mechanism has been suggested in patients with PCOS to explain the pathogenesis of insulin resistance in these patients [20].

In the present study the authors found a higher number of ovulatory cycles in patients who underwent a diet with a low glycemic index. In this group of patients there were three times more ovulatory cycles in comparison with those who followed the glycemic index diet normally. The low glycemic index diets have been proven effective in improving insulin resistance. This is particularly true in studies conducted in patients with chronic diseases [21]; however, to date, reports on its effects in patients with PCOS are limited.

In patients with PCOS and insulin resistance, an elevated ovarian production of androgens is found, which causes anovulatory menstrual disorders related to this syndrome [22]. Marsh et al. [23] demonstrated an improvement in the regularity of menstrual cycles in a group of patients subjected to a low glycemic index diet when compared with a group of patients undergoing a diet for weight reduction, however their study was limited to reviewing menstrual cyclicity. In contrast, the present authors documented a higher number of ovulatory cycles, demonstrated by ultrasound monitoring of ovulation performed vaginally, in patients who consumed a low glycemic index diet. These findings seem to be independent of weight loss. This could suggest that weight reduction per se did not influence the differences found in relation to the number of ovulatory cycles seen in both groups.

The present authors consider the increase in the number of ovulatory cycles in patients consuming a low glycemic index diet may be related to a decrease in the serum levels of circulating androgens, secondary to an improvement in insulin resistance. There are reports that document a minor decrease in androgen binding globulin and a further reduction in the free androgen index in a group of patients with acne who consumed a low glycemic index diet when compared with patients who consumed a high glycemic index diet [24].

In the present study, the authors found that the amount of cobalt consumed by the patients in group I was the only nutrient that showed a significant difference in the diets consumed in both groups before entering the study; however, the authors have no explanation for this finding and do not know if this could have a relationship with the results.

The strength of this study is that it is a controlled clinical trial, conducted jointly by the Departments of Gynecology and Nutrition at a university medical center. Its weaknesses could be the number of patients included in the study and the follow-up time; however, the present authors feel that with the data obtained, they can suggest that future studies be conducted that include hormonal evaluation at the end of the follow-up period. There is no doubt that this will assist to more accurately explain the increase in ovulatory cycles found in this clinical trial.

Conclusions

With the results of this study it can be concluded that a low glycemic index diet could be a strategy available to a large number of women with PCOS, particularly those looking to achieve pregnancy. The recovery of ovulatory cycles through a dietary intervention is an affordable and accessible strategy for any patient, regardless of their socioeconomic status. Cumulative ovulatory cycles show the need of commitment from patients to achieve these results. This strategy could be the first therapeutic intervention in the complex treatment of patients with PCOS. These findings should be confirmed in future studies.

Acknowledgments

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ventional, high glycemic-load diet on biochemical parameters asso-

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Perinatal and neonatal outcomes of maternal heart diseases

N. Aka, Ş. Arpacı, F. Vural, G. Köse
Obstetrics and Gynecology Department, Haydarpaşa Numune Teaching Hospital, Istanbul (Turkey)

Summary
Aims: To explore the perinatal and neonatal outcomes of patients with heart diseases. Materials Methods: Retrospective case control analysis was carried out among 10,527 deliveries, 188 pregnancies complicated by cardiovascular disease (CVD) compared with pregnancies without CVD for obstetric outcomes from January 2000 to December 2012. The effect of cardiac functional classification (NYHA) on maternal and neonatal complications was explored. Results: The incidence of CVD in pregnancy was 1.78%. About 80.3% had rheumatic heart disease (RHD). Maternal and neonatal mortality rate was 1.06% and 2.13%, respectively. The obstetric outcomes of women in NYHA class I/II were similar to normal group. Vaginal delivery was the preferred way of birth unless deterioration of cardiac functions as in the cases of NYHA class III/IV. NYHA class III/IV had significantly decreased birth weight, premature birth, and increased maternal-neonatal mortality (p < 0.05). Conclusion: RHD is still prevalent. The cardiac functional capacity predicts maternal and neonatal outcomes.

Key words: Maternal heart disease; Perinatal outcomes; Neonatal outcomes; Rheumatic fever; Pregnancy.

Introduction
The incidence of pregnancies complicating cardiovascular disease (CVD) has been increased because of the rising prevalence of risk factors such as diabetes, hypertension, obesity, and the improvements in treatment strategies resulting in the growing number of women with heart disease reaching childbearing age [1-4]. Nearly 0.2% to 4% of all pregnancies is complicated by CVD [1-3]. Among heart disease complicating pregnancy, congenital heart disease (CHD) is the most common in developed countries, whereas rheumatic heart disease (RHD) is predominant in developing countries [2, 5, 6].

Pregnancy causes significant changes in the cardiovascular system to meet the metabolic demands of the mother and fetus. Changes in the circulatory systems commence in the first trimester and peak in the second trimester. The most profound changes occur during birth and the early postpartum period. Therefore, the increased circulatory burden in pregnancy may decompensate or unmask previously asymptomatic cardiac diseases [2, 6, 7].

CVD is the most common non-obstetric cause of morbidity and mortality during pregnancy [2]. Preconceptional counseling and management of pregnancy with CVD are important to decrease perinatal mortality and morbidity [1, 2, 6, 7]. Maternal and neonatal events are highly correlated, and women with heart disease have an increased risk of adverse outcomes in pregnancy [2]. However, perinatal mortality and prevalence of cardiac diseases vary in the literature because of the heterogeneity of the population and heart diseases. In this paper, the authors investigated the various types of heart disease complicating pregnancy and the effect of cardiac functional classification (NYHA) on maternal and neonatal complications in a tertiary center.

Materials and Methods
This retrospective case-controlled study was conducted in Haydarpaşa Numune Teaching Hospital in Istanbul. This tertiary center, and the Siyami Ersek Cardiovascular Surgery Hospital, which is one of the largest cardiology hospital in Turkey, are located in the same health complex and work collaboratively.

A total of 10527 deliveries occurred from January 1, 2000 to December 31, 2012 in the present center. Among these deliveries, 188 singleton pregnancies that were complicated by cardiac disease were included in the study. This study was approved by the Haydarpaşa Teaching Hospital Ethical Committee.

The hospital records were evaluated retrospectively. Women age, parity status, spontaneous abortion, prior cardiac event, cardiac lesion, cyanosis, medication, acute rheumatic fever (ARF) prophylaxis, New York Heart Association (NYHA) functional class, and co-morbid conditions were recorded. All pregnancies underwent laboratory evaluation of blood count, biochemistry, electrocardiography, echocardiography and antenatal obstetric ultrasonography.

Women with isolated mitral valve prolapse (moderate or mild mitral regurgitation) or who lost antenatal follow-up, and who labored in another center were excluded. Arrhythmia cases were included if symptomatic sustained arrhythmia required treatment before pregnancy [6,7].

A total of 376 women without cardiac disease were selected from deliveries during the same week of labour of women with CVD. The control selection was performed by 1:2 ratio among maternal age and gestational weeks matched births. Women with labor complications, diabetes, hypertension, or any known medical disease or known obstetric pathology; multiple pregnancies,
Maternal and neonatal complications

Maternal cardiac complications were cardiac failure (n=5, 2.6%), pulmonary edema (n=2, 1.06%), arrhythmia (n=8, 4.2%), hypertension (n=10, 5.3%), preeclampsia (n=4, 2.1%), and cardiac death (n=2, 1.06%). Maternal non-cardiac complications were postpartum hemorrhage (n=2, 1.06%), severe anemia (n=10, 5.3%), and wound infection (n=6, 3.19%). The majority of the cardiac complications were in NYHA III/IV class. Two maternal mortality cases (1.06%) were noted in the CVD group. The first mortal case was VSD accompanied by Eisenmenger’s syndrome and the pulmonary emboli was the cause of mortality. The other case was uncorrected MS by atrial septal defect and the cause of death was mitral regurgitation with anemia and wound infection.

The comparison of perinatal outcomes of pregnancies complicating by CVD according to NYHA classification

The personal characteristics, obstetric history, hospitalization, and perinatal neonatal outcomes are presented in Table 2. The CVD group had a significantly increased duration of hospitalization, maternal and neonatal death. The comparison of pregnancies with and without heart disease.

Pregnancies complicating by CVD increased risk of materno-fetal complications compared to normal controls. The personal characteristics, obstetric history, hospitalization, and perinatal neonatal outcomes are presented in Table 2. The CVD group had a significantly increased duration of hospitalization, maternal and neonatal mortality. The comparison of perinatal outcomes of pregnancies with and without heart disease according to NYHA classification.

The comparison of perinatal outcomes of pregnancies with heart diseases according to NYHA classification is

Table 1. — The distribution of cardiac lesions in CHD and AHD.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CHD (n=23)</th>
<th>AHD (n=165)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>CHD (n=23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD</td>
<td>5</td>
<td>21.7</td>
</tr>
<tr>
<td>VSD</td>
<td>10</td>
<td>43.4</td>
</tr>
<tr>
<td>MVP</td>
<td>5</td>
<td>21.7</td>
</tr>
<tr>
<td>PS</td>
<td>2</td>
<td>8.8</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td>AHD (n=165)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>9</td>
<td>5.5</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>151</td>
<td>91.5</td>
</tr>
<tr>
<td>I. Mitral stenosis</td>
<td>75</td>
<td>49.6</td>
</tr>
<tr>
<td>II. Mitral regurgitation</td>
<td>40</td>
<td>26.5</td>
</tr>
<tr>
<td>III. Aortic regurgitation</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>IV. Aortic stenosis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V. Multiple valvular heart disease</td>
<td>34</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Table 2. — The comparison of perinatal outcomes in case (CVD) and control (normal) groups.

<table>
<thead>
<tr>
<th></th>
<th>Case Mean±SD</th>
<th>Control Mean±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.16±5.51</td>
<td>26.16±5.64</td>
<td>0.087</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.30±1.52</td>
<td>2.06±1.16</td>
<td>0.222</td>
</tr>
<tr>
<td>Parity</td>
<td>1.59±1.17</td>
<td>1.56±0.79</td>
<td>0.272</td>
</tr>
<tr>
<td>Abortion (spontaneous)</td>
<td>1.18±0.39</td>
<td>1.00±0.00</td>
<td>0.054</td>
</tr>
<tr>
<td>Medical abortion</td>
<td>1.06±0.26</td>
<td>1.18±0.60</td>
<td>0.779</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3021.24±611.63</td>
<td>3278.9±465.28</td>
<td>0.001</td>
</tr>
<tr>
<td>1. Min. Apgar score</td>
<td>7.36±1.65</td>
<td>8.26±1.15</td>
<td>0.001</td>
</tr>
<tr>
<td>5. Min. Apgar score</td>
<td>8.96±1.77</td>
<td>9.63±0.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Hospitalisation (days)</td>
<td>2.37±1.51</td>
<td>1.58±0.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin (gr/dl)</td>
<td>11.12±1.13</td>
<td>11.15±1.06</td>
<td>0.812</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.84±3.10</td>
<td>33.75±2.36</td>
<td>0.770</td>
</tr>
<tr>
<td>Birth weight</td>
<td>37.70±2.60</td>
<td>39.01±1.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Neonatal mortality</td>
<td>4 (2.13 %)</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. — The comparison of perinatal outcomes according to NYHA functional classification.

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>NYHA</th>
<th>Class II</th>
<th>Class III/IV</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (grams)</td>
<td>3114.74±628.37</td>
<td>3075.88±498.90</td>
<td>2508.33±720.98</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>1-min Apgar score</td>
<td>7.57±1.68</td>
<td>7.42±1.48</td>
<td>6.44±1.91</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>8.91±2.20</td>
<td>9.25±0.83</td>
<td>8.00±2.45</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Birth week</td>
<td>37.98±2.67</td>
<td>37.94±1.86</td>
<td>35.89±3.88</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Neonatal mortality</td>
<td>2 (2.67%)</td>
<td>0</td>
<td>2 (8%)</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>Maternal mortality</td>
<td>0</td>
<td>0</td>
<td>2 (8%)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The comparison of perinatal outcomes according to NYHA functional classification.

Maternal mortality rate was 1.06% (two cases) in the CVD group; all the cases were in NYHA class III/IV group (8%). The birth week and weight, Apgar scores, type of delivery, and maternal mortality rates were similar for patients in NYHA class I/II and those with normal pregnancies. The neonatal mortality in NYHA class I/II was slightly increased (1.23%).

Discussion

The prevalence of CVD complicating pregnancies in this retrospective cohort was 1.78% and RHD remains dominant. Maternal heart diseases are associated with adverse obstetric outcomes such as prematurity, low birth weight, and maternal and neonatal mortality. The majority of the maternal and fetal complications were observed in NYHA class III/IV. This study demonstrated that functional capacity of the heart determines pregnancy outcomes.

Heart diseases are heterogeneous groups of diseases either congenital or acquired, so the prevalence of the types of disease differs among countries [2, 5, 6]. According to the present 13-year results, RHD remains the dominant cardiac disease complicating pregnancies with valvular lesions (80.3%). Moreover, mitral stenosis is the most common lesion (49.6%), consistent with the observations of other studies from developing countries [8-19]. Acute rheumatic fever and RHD are significant public health concerns around the world and also in Turkey [17-19]. At least 15 million people are estimated to be living with RHD [20]. These results suggested the importance of developing strategies to prevent ARF and RHD.

Several studies have assessed the maternal and fetal outcomes of pregnant patients with heart disease. Maternal heart disease is associated with an increased risk of both maternal and neonatal complications. The hemodynamic imbalance, placental insufficiency, and drugs may compromise fetal growth [5, 7, 21-25]. Many other studies addressed the importance of risk stratification and functional classification on obstetric outcomes [24, 25]. One of the largest series was performed by Siu et al. that studied 562 pregnant women, with valvular heart disease [6]. In their subsequent study [7], evaluated the prospective longitudinal study of pregnancy outcomes in women with heart disease and found neonatal outcomes in 302 pregnancies. They found increased incidence of neonatal complications such as preterm delivery, fetal growth restriction, respiratory distress syndrome, and neonatal death. Similarly in the present cohort, women with CVD had increased risk of neonatal complications such as small for gestational age (42.5%), premature delivery (16.4%) neonatal intensive care unit need (21.2%), and neonatal mortality (2.13%). The study by Wasm et al. [14] and by McFaul et al. [23] found that NYHA III/IV symptoms were the determinants of adverse fetal and maternal outcomes [14, 23]. Siu et al. showed multiple gestations, anticoagulation therapy in pregnancy, left ventricular obstruction, NYHA class III/IV, and maternal age as the predictors of adverse neonatal outcome [7]. The present results similar to previous searches that cardiac performance and status effects outcomes. The CVD group had adverse obstetric outcomes compared to normal group and functional class predicts the outcome. The perinatal outcomes were similar for patients in NYHA class I/II and those with normal pregnancies. However, NYHA III and IV patients had significantly decreased birth week, birth weight, and Apgar scores (p < 0.05).

Severe pulmonary hypertension, severe mitral or aortic stenosis, left ventricular dysfunction (< 30%), Marfan's syndrome with aortic root dilatation, and previous preeclampsia cardiomyopathy with impaired left ventricular function were the high-risk cardiac lesions [2]. Eisenmenger's syndrome has a 50% mortality rate in the pregnant women with CVD [26, 27]. Two mortality in the present series were high-risk cardiac lesions with NYHA class III/IV. It is strongly suggested that prenatal counseling should be done before planning pregnancy and pregnancy should be prohibited in high-risk conditions. One of the other components of prenatal counseling is off-
spring risk of congenital cardiac anomaly. In the present cohort, two offsprings had cardiac anomaly. Therefore, this issue should also be discussed with patients.

The preferred mode of delivery is the vaginal route, with spontaneous onset of labor and provision of effective pain relief [2, 28]. In the present study population, women in the NYHA class I/II and control groups had similar vaginal delivery rates and vaginal route was the primary mode of delivery. However, women in NYHA III/IV had significantly increased C/S rates. Most of the cases were emergency operations secondary to poor cardiac performance or fetal distress that necessitated early delivery. This result is similar to previous reports [29, 30].

The major limitation of the present study was its retrospective nature. To minimize patient selection bias for control group, age, and gestational week matched women without known medical or obstetric problems were included. The records did not have smoking status, obesity, and gestational diabetes. Therefore, the p-value could not reflect the effects of confounding factors on maternal and neonatal outcome. Despite limitations, the present data with cardiac functional classification was well-recorded and had a large population with RHD. However, prospectively designed studies with cardiac risk stratification are needed.

Conclusion

RHD is still prevalent. The functional capacity of the heart determines pregnancy outcomes. Maternal cardiac status affects fetal status such as low birth weight and prematurity. Pulmonary hypertension is the major risk factor affecting maternal mortality. The majority of women in NYHA class I and II can go through pregnancy and labor without a specific intervention. However, women with NYHA III/IV symptoms are associated with adverse perinatal outcomes. All patients’ antenatal care and labour management necessitate multidisciplinary team approach.

References


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Electromyographic activity of the pelvic floor muscles in the third trimester: comparison between primigravidae and secundigravidae

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Summary

Objective: The objective was to compare the electromyographic activity of pelvic floor muscle (PFM) on third semester between primigravidae and secundigravidae who had previous vaginal delivery. Design: Cross-sectional observational study. Sample: Nineteen primigravidae and 21 secundigravidae between 34th and 36th gestational weeks were evaluated. Materials and Methods: Data collection consisted in assessing the PFMs activity by surface electromyography. Main outcome measures: The variables related to electromyographic assessment such peak and average on the rest, maximal voluntary contraction, and sustained contraction. Results: There were no differences on electromyographic activity of PFMs between primigravidae and secundigravidae. However, a significant increase in body mass index and a negative correlation of the newborn weight with the peak value of electromyographic signal during maximal voluntary contraction were observed. Conclusions: The factors that can change the electromyographic activity pattern during pregnancy can be related to maternal body mass increased and newborn weight.

Key words: Electromyography; Pelvic floor; Pregnancy; Urinary incontinence.

Introduction

The pelvic floor muscles (PFMs) suffer an overload during pregnancy due to progressive increase of the uterus and its weight and size, which goes from 70 grams to about 1,000 grams [1]. Gradually, the gravid uterus increases the angle between the bladder neck and the urethra, and may contribute to the onset of the PFM dysfunctions, such as urinary symptoms and pelvic organ prolapses [2].

Maternal age over 35 years and previous obesity are mentioned as factors predisposing to PFM dysfunction [3]. In addition, parity is indicated as a risk factor for the onset of urinary symptoms [4]. Some studies have found higher prevalence of PFM dysfunction in women with previous vaginal delivery compared to those who underwent caesarean section [5, 6]. Although parity and vaginal delivery are considered risk factors leading to the development of PFM disorders, there is evidence that such conditions can also occur in pregnant women, suggesting that more than childbirth, the first pregnancy may be associated with PFM changes [7]. Therefore it is important to search for new diagnostic methods and techniques that can support the prevention or treatment of PFM disorders. However, information about the possible changes on electrical activation of the PFM during pregnancy, and their relationship with pregnancy and previous vaginal delivery are not established.

The aim of this study was to compare the electromyographic (EMG) activity of the PFM in the third trimester between primigravidae and secundigravidae, with previous vaginal delivery.

Materials and Methods

This was a cross-sectional study conducted from July 2012 to October 2013. The study was approved by the Ethics Committee on Human Research of the Federal University of Sergipe, in accordance with Resolution 466/12 of the National Health Council (CAAE: 06190112.9.0000.5546). The study was conducted in two Family Health Units in Aracaju city (Sergipe, Brazil). While attending prenatal program, the pregnant women were invited to participate at the study by the responsible researcher. All women signed the consent form.

The sample size calculation was performed using G* Power 3.1.3 program. The values found on Marques et al. [8] study were used as parameter for the EMG activity of the PFM in primigravidae and secundigravidae. For a power of 0.80 and alpha test error of 5%, 21 patients per group was suggested.

Inclusion criteria were primigravidae and secundigravidae (with previous vaginal delivery), aged between 18 and 40 years, body mass index (BMI) before pregnancy considered normal, based on the World Health Organization (WHO) concept [9], gestational age between 34-36 weeks, low risk, single pregnancy, and who were in prenatal care. Exclusion criteria were risk of abortion, uterine bleeding, previous and recurrent abortion, urinary tract in-
fection and/or inflammation, cognitive impairment, illicit drug, smoking, and alcohol intake.

The evaluation was conducted between the 34th and 36th gestational week, according to the date of last menstruation [10] and/or the first ultrasound performed during pregnancy [11]. The pre-pregnancy BMI was collected through prenatal care card and gestational BMI was assessed based on Atalah table [12]. All data were collected by a single physical therapist with experience in this evaluation.

An anamnesis to inform personal data and obstetrics history was collected. The PFM evaluation was performed by surface EMG, with the following specifications: converting the original signal to the root mean square value, 20 band pass filter to 500 Hz, rate of common mode rejection > 130 dB, and active electrode impedance of 1012 GW. The filter of 60 Hz was used to reduce interferences coming from the power grid. Data were normalized by the peak value of the three maximal voluntary contractions (MVC) [13]. This device records the sum of the electrical potential generated by the muscle fibers depolarization at rest and during voluntary contraction, and its amplitude is recorded in microvolts (µV). It is the most accurate method to measure the integrity for neuromuscular EMG and can be considered as an indirect measure of pelvic floor muscle strength and pressure level during contraction [14].

The positioning during evaluation was supine with hip and knee flexion, and feet flat. The examiner introduced a vaginal sensor with surface capture of stainless steel with 27 mm diameter and 69 mm length and lubricated with water soluble gel on vaginal opening. Two reference electrodes were placed on the right anterior superior iliac crest and on the right lateral malleolus. Self-adhesive electrodes were placed in infraumbilical region of the rectus abdominis for simultaneous measurements of PFM and abdominal muscle activities.

The protocol consisted of 15 seconds of rest for basal activity registration, three MVC maintained by two seconds (with an interval of one minute between each one) [15].

In order to identify performing Valsalva maneuver and/or simultaneous contraction of the hip and buttocks adductor muscles, instead of isolated PFM contraction, the abdomen and the perineal region were observed during the PFM contraction. When there was accessory muscles contraction, PFM contraction was not recorded.

Data were tabulated in Excel and statistically analyzed with the Statistica program and through descriptive techniques. The Shapiro-Wilk test indicated non-parametric tests. The Mann-Whitney test was used to compare the EMG activity of the PFM between groups. Correlation analysis between the weight of the newborn in previous pregnancy and the variables related to EMG assessment of PFM was performed using the Spearman correlation coefficient. The following classification of the correlation coefficients was adopted to interpret the magnitude of the correlations: correlation coefficients ≤ 0.3 (weak correlation), > 0.3 to ≤ 0.7 (moderate), and > 0.7 (strong) [16]. A significance level of 5 % (p ≤ 0.05) was adopted. Data are expressed as mean ± standard deviation.

Results

The study included 40 pregnant women (19 primigravidae and 21 secundigravidae). Table 1 shows the anthropometric characteristics and the gestational age mean at the time of evaluation. The age was significantly greater (p < 0.0001) in secundigravidae. For these women, the age mean at first pregnancy was 23.24 ± 3.39 years and the time between pregnancies was 4.90 ± 3.32 years.

Table 2 presents the variables of EMG evaluation of PFM and abdominal muscles. No significant differences were found between groups.

Among the secundigravidae, 47.6% (n=10) underwent episiotomy in previous delivery. Table 3 presents data from the EMG evaluation of PFM in the third trimester of pregnant women who did and did not undergo episiotomy in the previous delivery. No significant differences between groups were observed.

Regarding the newborn weight in previous pregnancy, the mean was 3.38 ± 0.52 kg. There was a significant correlation, moderate and negative (p = 0.014, r = - 0.53), between newborn weight and the peak value of the electromyographic signal during MVC. No correlations were found in other variables.
Discussion

There was no significant difference in PFM activation in the third trimester when compared to second and first pregnancy, suggesting that parity and previous vaginal delivery were not decisive factors for possible changes in the EMG activity of the PFM.

According Vodusek et al. [17], during vaginal delivery, the pelvic floor region is subjected to fetal head pressure that can lead to distension and compression of PFM, connective tissue, and nerves. However, Liang et al. [18] claimed that the structural changes of the pelvic floor can gradually regress, returning to pre-pregnancy state with consequent restoration of PFM functions. Likewise, Peschers et al. [19] noted that the PFM function was restored ten weeks after vaginal delivery in most women analyzed. This may explain why there were no significant differences between the groups in the third trimester.

Noteworthy, MacLennan et al. [20] found no relationship between mode of delivery with the PFM dysfunction, however when considering vaginal delivery with perineal trauma, the occurrence of disorders was higher. Some authors indicate episiotomy as a protective procedure for PFM structures [21]. However, according to a systematic review, selective compared to routine episiotomy, presents a lower risk of posterior perineal trauma, required less suturing, and fewer complications on cicatrization [22]. In the present study, 47.6% of secundigravidae had been submitted to this procedure in the previous birth, but no significant differences were found in the EMG signal of the PFM between women who were and were not submitted to episiotomy.

However, according to Chaliha and Stanton [23], excessive stretching or overloading of PFM tissues can lead to irreversible changes in tissue properties by altering the urethral support and continence mechanisms.

In this sense, the practice of cesarean section has been advocated as a protective procedure for PFM dysfunctions [24]. Allen et al. [5] observed abnormalities in pudendal nerve conduction in primiparous undergoing vaginal delivery, compared to those undergoing elective cesarean section. Moreover, Chaliha et al. [25] demonstrated that 9% of women undergoing elective cesarean section showed PFM dysfunctions before labor, such as urinary incontinence and detrusor muscle instability. Therefore the pressure of the fetal head on the PFM, which occurs in the second stage of labor, is not primarily responsible for the changes/damage in PFM. This reinforces gestational effects on PFM changes and, thus, is insufficient evidence to confirm that cesarean section is a protective factor against PFM dysfunctions.

Factors such as maternal age and BMI are important variables that must be considered when evaluating the PFM during pregnancy [26]. Fritel et al. [3] concluded that women over 35 years have a higher risk for developing PFM disorders, because the physiological aging is accompanied by an increased in density of PFM fiber denervation. According to Smith et al. [27], women with pudendal nerve conduction less than 2.4 ms has a 97% chance of developing urinary incontinence because this factor correlates with low urethral closure pressures. In the present study, the age of secundigravidas was statistically higher than primigravidas. However, the two groups showed maternal age below 35 years, which may have minimized the influence of this variable on urinary symptoms.

Considering BMI values, the primigravidas showed a normal range, but the secundigravidas, the value exceeded the maximum limit of 28.5 kg/m². However, it is noteworthy that among the first pregnancy, 31.6% had values above appropriate, with half of those with obesity and, among secundigravidas, the prevalence was 38.1%, with half of women presenting obesity. According to Kirby [28], obesity may contribute to the PFM impairment.

Another factor that seems to predispose to changes in PFM during pregnancy is the weight of the newborn. Eftekhar et al. [6] found higher prevalence of disorders in PFM of secundigravidas who had babies with weight greater than 3,000 grams, regardless of the type of delivery. As in the present study, birth weight was correlated significantly and negatively with the peak value of the EMG signal during MVC and this information suggests a possible influence of birth weight on EMG activity of the PFM.

Besides the changes in BMI and birth weight gradually increasing the overload on PFM, hormone action, primarily by the hormone relaxin, can lead to tissue remodeling by reducing his tension and decreasing the pelvic stability [29]. With this, the endopelvic fascia is gradually elongated and weakened due to chronic stress, which can influence the pattern of electrical activity of the PFM [30].

During EMG evaluation of the PFM, simultaneous measurements of the rectus abdominis activity were performed. Sapsford and Hodges [31] observed that in healthy subjects, voluntary activity in the abdominal muscles results in increased pelvic floor muscle activity and this response is preprogrammed. Pereira et al. [32] analyzed the co-activation of the abdominal muscles and PFM during isometric exercises in nulligestae, primigravidas, and primiparous not pregnant. The authors stated that only nulligestae had a significant co-activation of these muscles, and no correlation was found between the deficit in muscle synergy and mode of delivery. In the present study, no differences were noted between the abdominal average values between the first and second pregnancy, and in both groups, occurred an increased in abdominal activation during sustained and maximal voluntary contractions. Thus, the synergy of these muscles remained independent of previous parity and mode of delivery.

A limitation in this study was the lack of a group of nulligestae women as a normal pattern in the comparison of EMG activity of the PFM with pregnant women, which could help to elucidate the impact of pregnancy on the PFM. Besides several factors involved in pregnancy and childbirth that can alter the pattern of EMG activity of the
PFM, while influencing their support and continence function, there was no difference in the EMG signal and, consequently, in the PFM between first and second pregnancy. This finding suggesting that parity and previous vaginal delivery of secundigestae were not determining factors to possible changes in the EMG activity of the PFM. Therefore it appears that factors related to gestational process, such as increased maternal body weight and birth weight, may be responsible for changes in EMG activity of the PFM during pregnancy due to the overload on pelvic floor structures.

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Are cytokine levels in serum, endometrial tissue, and peritoneal fluid a promising predictor to diagnosis of endometriosis-adenomyosis?

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Summary
Aim: The basic aim was to find a non-invasive procedure to diagnose and monitor endometriosis-adenomyosis. Materials and Methods: A prospective study was carried out. The authors conducted a series of 60 consecutive patients who underwent diagnostic laparoscopy for benign gynecologic conditions. Endometrial, peripheral blood and peritoneal lavage samples were analyzed. IL-6, IL-16, TNF-alpha, and LIF levels were measured and compared. Results: The authors analyzed clinical data of 52 patients (26 endometriosis, 13 adnomayosis, and 13 control group). Peritoneal fluid IL-6 is significantly higher in stage IV endometriosis group than the control group (p = 0.001). In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group (p = 0.008). In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group (p = 0.000 and p = 0.002). Conclusions: Significant immune-inflammatory changes were observed. When the underlying molecular mechanisms will be investigated, this will elicit studies on the immunotherapeutic treatment of endometriosis. Further studies are needed to assess various potential therapeutic interests for biomarkers in a large, well-defined patient population.

Key words: Cytokines; IL-6; Endometriosis; Peritoneal fluid; Tumor necrosis factor-alpha (TNF-α).

Introduction
Endometriosis is a condition where the functional and morphological endometrial glands and stromal structures are found outside the uterus. It is a disease associated with pelvic pain and infertility and mainly affects women during their reproductive age. Hormonal influence could be the important predisposing factor as endometriosis is extremely uncommon before menarche and after menopause. It occurs most often in pelvis, peritoneum, ovaries, posterior cul-de-sac, and uterosacral ligaments. Retrograde menstruation, metaplasia, lymphatic and hematogenous outspread, mechanical transplantations, and some other theories have been introduced for its pathogenesis [1]. Histological examination is needed for a definitive diagnosis. Laparoscopy is currently considered to be the gold standard investigation in patients suspected to have endometriosis, but this is an invasive and relatively costly procedure and there may be significant delays in diagnosis [2]. Efforts to reduce this delay are required. Laboratory evaluation has a minor role in the diagnosis of endometriosis, although studies are underway investigating serum markers, genetics, and endometrial sampling [3]. The term of adenomyosis is the presence of endometrial glands and stroma inside the myometrium. Previously adenomyosis is also called endometriosis interna. The mechanism, clinical symptoms, and treatment of adenomyosis is completely different than endometriosis.

Despite high prevalence of endometriosis, little is known about the etiopathogenesis. Immune-inflammatory changes may be associated with its pathogenesis [4]. The inflammatory response to endometriosis, tissue repair, and revascularization is referred to macrophages and cytokines. Cytokines are proteins that play role in cell proliferation, activation, motility, adhesion, chemotaxis, and morphogenesis. The relation of some cytokines like interleukins (IL-1, IL-2, IL-6, IL-8, and IL-18) and tumor necrosis factor-alpha (TNF-alpha) to the pathogenesis of endometriosis has been previously studied [5]. Many studies focusing on this subject have the limitations of lack of peritoneal fluid sampling and lack of patients with adenomyosis. Mostly assessments are with only one biomarker or in small numbers of series. This present prospective study is designed to overcome these limitations. Cytokines were studied in endometrial tissue, peritoneal fluid, and serum of cases with endometriosis-adenomyosis. These were compared with control groups.
The basic aim was to find a non-invasive procedure to diagnose and monitor endometriosis. Regarding this entity, in this study the authors analyzed the levels of IL-6, IL-16, leukemia inhibitory factor (LIF), and TNF- alpha in serum, endometrial tissue, and peritoneal fluid.

Materials and Methods

A prospective study was carried out between April 2009 and August 2009 in the Department of Obstetrics and Gynecology, Selcuk University Meram Medical School. After approval by the Institutional Ethics Committee the authors conducted a series of 60 consecutive patients who underwent diagnostic laparoscopy for pelvic pain, primary infertility, dysmenorrhea, dyspareunia, and for benign gynecologic conditions. Informed consent was obtained from all patients. None of the patients had autoimmune diseases, pelvic inflammatory diseases, and the history of pregnancy in the last six months. They were diagnosed preoperatively by means of physical examination, transvaginal pelvic ultrasonography, and endometrial biopsy. The endometrial samples in heparinized tubes were studied on the same day by flow cytometry. In patients with suspicions of adenomyosis, magnetic resonance imaging is also used. Peripheral blood samples are obtained before surgery. Blood samples were centrifuged at 400 g (20 minutes) and stored at -80 °C until assayed. Laparoscopy was performed with standard laparoscopic procedures in the follicular phase under general anesthesia in all. Primarily the peritoneal lavage sampling was obtained. These samples were centrifuged at 400 g (20 minutes) to separate the cells from the peritoneal fluid supernatants were removed and samples were stored at -80 °C until assayed for cytokine content. The diagnosis and staging of endometriosis was done by visual evaluation according to the classification of American Fertility Society. The location, size, and the stage of endometriosis was documented and noted to the operation findings. The tubes, ovaries, pouch of Douglas, and intestines were evaluated respectively. The assessment of benign gynecologic conditions confirmed with intraoperative frozen section. In subsequent follow-up some patients who were especially diagnosed with adenomyosis underwent hysterectomy for benign gynecologic conditions. Therein definitive diagnosis with histological examination revealed adenomyosis on the hysterectomy specimen and these were included to the adenomyosis group. Patients with blood in the peritoneal fluid or pelvic infection or those who had been diagnosed with a gynecologic malignancy were excluded from the study. Patients were divided into two major groups according to histopathological diagnosis: group I - endometriosis, group II - adenomyosis. Patients not classified either in adenomyosis or in endometriosis groups were decided as control group. The demographic characteristics and complaints of patients were evaluated. IL-6, IL-16, TNF- alpha, and LIF levels were measured and compared.

Statistical analysis

Data of the study were analyzed with SPSS version 13.0. Results are expressed as median and range with 95% confidence intervals. To compare discontinuous variants Chi-square test was used. Mann Whitney U test or t-test was used to compare continuous variants. Bonferroni corrected Mann Whitney U test was used for double comparisons. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 60 women who were underwent diagnostic laparoscopy included in this prospective study. Because of blood presence in abdominal cavity, four cases were excluded and four samples could not be used because of technical problems during the process of storing and melting. The authors therefore analyzed clinical data of 52 patients; 26 of these were diagnosed pelvic endometriosis. Adenomyosis was present in 13 cases. Thirteen cases who were not classified in adenomyosis or in endometriosis groups were generated as the control group. The results of clinical classification of endometriosis according to American Fertility Society (AFS) and Revised American Fertility Society (RAFS) were stage I endometriosis in six (12%) patients, stage II in four (8%) patients, stage III in three (5%) patients, and stage IV in 13 (25%) patients, respectively. Patients' demographic features and the distributions of complaints are demonstrated in Table 1. There were no significant differences between the groups with respect to demographic data (age-body mass index), the cytokine levels (IL-6, IL-16, TNF-alpha, and LIF) of blood and endometrial tissue and also LIF levels in the peritoneal fluid. According to this study there was no relation found between the complaints of patients and cytokine levels. The authors did find a statistically significant difference between the groups when comparing the levels of IL-6 in the peritoneal fluid. Peritoneal fluid IL-6 was significantly higher in stage IV endometriosis group than in the control group ($p = 0.001$). In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group ($p = 0.008$). In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group ($p = 0.000$ and $p = 0.002$).

Discussion

Promising new therapies for the treatment of endometriosis are continuing to develop. Many studies have
been performed to investigate the underlying molecular mechanisms. The precise pathogenesis of endometriosis is still unclear but it is well-documented today and chronic pelvic inflammation is a common feature in affected women. Significant immune-inflammatory changes have been observed; the ectopic endometrial cells resist apoptosis and produce proinflammatory, angiogenic, growth, and tissue remodeling factors, which may contribute to the ectopic growth of endometrial tissue [6]. Activated peripheral mononuclear cells as well as endometriotic cells in situ are hypothesized to secrete various cytokines with pleiotropic biological activities [7]. It is likely that the growth regulation in vivo of endometrial and endometriotic cells is controlled by a complex combination of cytokine and growth factors [8]. Although benign in structure, endometriosis exhibit differential invasive, adhesive, and proliferative behavior.

Still the diagnosis and staging of endometriosis can only be established by invasive procedures: laparoscopy or open surgery [2, 9]. CA-125 has been widely used for detection of endometriosis and monitoring of progressive disease. However, the sensitivity of this biomarker alone is unsatisfactory [9,10]. In the study of Patacchiola et al., they did not succeed in identifying a clinically useful non-invasive diagnostic biomarker or panel of biomarkers [10].

In the present prospective study IL-6, IL-16, LIF, and TNF-alpha cytokines in the blood, peritoneal fluid, and endometrial tissue were compared. With some aspects this study includes many preliminary assessments. With regards to this topic, this is the first study to evaluate the levels of TNF-alpha and IL-16 in the endometrial tissue of patients in all stages of endometriosis and adenomyosis. As a consequence, the comparison of the present results with literature is restricted.

There were no significant differences between the groups with respect to demographic data (age-body mass index), the cytokine levels (IL-6, IL-16, TNF-alpha, and LIF) of blood and endometrial tissue and also LIF levels in the peritoneal fluid. In this study peritoneal fluid levels of interleukin-6 with endometriosis stage IV were significantly higher than controls (p = 0.001). The stage of disease may considerably alter the cytokine levels. Increased peritoneal fluid levels of interleukin-6 in patients with active endometriosis may be related to endometriosis-associated infertility and to the pathogenesis of endometriosis [11]. IL-6 is a pro-inflammatory cytokine involved in the activation of T cells. IL-6 in the endometrial tissue and serum of patients with adenomyosis and endometriosis was not statistically different than the control group. To the present authors’ knowledge, this is the first report in the literature of patients with adenomyosis that compared IL-6 levels in endometrial tissue and serum between controls.

Mihalyi et al. conducted a case–control study in 294 infertile women. In this study plasma levels of IL-6, IL-8, and CA-125 were increased in all women with endometriosis and in those with minimal–mild endometriosis, compared with controls [12]. In contrast to the present findings, significantly higher levels of interleukin-6 were observed in the serum of subjects with endometriosis in the report of Othman et al. [13].

No statistically significant results were obtained when comparing the level of LIF in the serum, peritoneal fluid, and endometrial sampling of patients with endometriosis (all stages), adenomyosis, and control group (p > 0.005). This is the first study to evaluate the levels of LIF in the serum of patients with all stages of endometriosis and also adenomyosis.

In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group (p = 0.008). The primary role of TNF-alpha is in the regulation of immune cells and known to be critically involved in the regulation of infectious, inflammatory, and autoimmune phenomena. By promoting the growth of endometriotic cells, TNF-alpha in the peritoneal fluid may be an essential factor in the pathogenesis of endometriosis [7]. TNF-alpha cytokine with pro-inflammatory and pro-angiogenic roles could potentially be involved in the diagnosis of endometriosis. The diagnostic accuracy of serum IL-6 and peritoneal fluid TNF-alpha levels in endometriosis showed a 90-100% sensitivity and a 67-89% specificity in the study of Bedaiwy et al. [14].

In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group (p = 0.000 and p = 0.002). In the study of Koga et al., significantly higher concentrations of IL-16 in the peritoneal fluid of women were observed with only advanced endometriosis (stages III/IV) [14]. On the other hand, no relation was found between serum levels of IL-16 and endometriosis in the study of Zhang et al. [15]. IL-16 in peritoneal fluid may play a role in the pathogenesis of endometriosis by initiating or sustaining inflammatory responses in the peritoneal cavity [16].

Some factors have undoubtedly contributed to the conflicting results in literature. Phase of the menstrual cycle and stress factors before surgery might have affected plasma biomarker levels. None of the measured cytokines showed significant correlation with the complaints of patients. To detect and monitor endometriosis, a group of biomarkers may be more useful rather than a single indicator. In combination with imaging techniques, such a panel of biomarkers may indicate which women necessitate a laparoscopy and eliminate countless unnecessary operations [17].

In attempt to find a non-invasive procedure to diagnose and monitor endometriosis and adenomyosis, the present authors designed a prospective study. When the underlying molecular mechanisms will be investigated, this will elicit studies that will provide immunotherapeutic treatment of endometriosis. Further studies are needed to analyze various potential therapeutic interests for biomarkers in a large, well-defined patient population.
References


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Effect of Wnt/β-catenin signal pathway on matrix metalloproteinase-7 and vascular endothelial growth factor gene expressions in endometriosis


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Introduction

Endometriosis is the common gynecological disease caused by the malposition of active endometrial tissues outside of uterine cavity. Due to the different positions and courses of disease, clinical manifestations are diverse. Though it is benign in histopathology, there are malignant behaviors such as proliferation, invasion, metastasis, and high recurrence rate [1, 2]. Studies showed that attachment-invasion-angiogenesis was the basic pathological process of lesion occurring to endometrium and implantation [3], while the mechanism is still not clear.

Classical Wnt signal pathway is that Wnt1-10 acting on transmembrane protein Frizzled (FZD1-10) and low density lipoprotein receptor related protein 5/6 (LRP 5/6) to produce dishevelled protein (DVL) activation and transfer the signal into cells to lessen the phosphorylation of β-catenin by inhibiting the activity of glycogen synthase kinase 3β (GSK-3β). The dephosphorylated β-catenin transferred into nucleus and combined with nuclear factor TCF/LEF, which could activate the targeted genes [4, 5].

Wnt/β-catenin signal pathway is key to regulate growth, development, and differentiation of cells and plays an important role in the processes of occurrence, invasion, and metastasis of tumor [6, 7]. The biological characteristics of endometriosis is similar to that of a tumor [8]. So far, studies on the molecular mechanism of endometriosis mainly focus on the related signal pathway, cellular adhesion molecule (CAM), metastasis-associated genes, matrix metalloproteinase (MMP), its inhibitory factors, and angiogenesis [9]. Some studies found that Wnt/β-catenin signal pathway may participate in the adhesion, invasion, and angiogenesis of ectopic endometrium [10]. In the present study, small RNA interference technology was used to block Wnt/β-catenin signal pathway. The authors discuss the role of Wnt/β-catenin signal pathway in endometriosis through the analysis of the expressions of MMP-7 and VEGF.

Materials and Methods

This study was approved by the University Hospital of Hubei University For Nationalities ethics committee.

Sample preparation

Five patients (average age 46 years old) with endometriosis and operation indications were selected. They did not receive hormone kind medication from six months before operation. Secretory
Establishment of endometriosis nude mice models

Forty-two BALB/c nude mice (female) weighing 16-20 grams were provided by the experimental animal center of Hubei Minzu University [SCXK (Hubei) 2008-0005] which also offered SPF BALB/c nude mice. Laparotomy was conducted to observe the formation of lesions in pelvic and abdominal cavity. Ectopic endometrium was confirmed by both preoperative diagnosis and postoperative pathology. Endometrium was scraped in the hysterectomy and rinsed three times with cold sterile phosphate buffer solution, while discarding the blood and mucus. Then it was cut into small sections of about 0.2~0.3 cm³ in sterile petri dish and put into the phosphate buffer. Then 200 U/ml green enzyme and 200 μg/ml streptomycin were added.

Preparation of siRNA transfection complex and drug administration

β-catenin siRNA(h) (se-29209), β-catenin negative control siRNA (se-36869) and siRNA transfection reagents (se-29528) were synthesized. β-catenin siRNA(h) and β-catenin negative control siRNA (se-36869) were each 3 μg [125 μg/(kg·d)], respectively, mixed with siRNA transfection reagents (se-29528) according to the ratio 1:25 to make siRNA transfection complex and negative control transfection complex. Then they were diluted in 100-ml serum-free optimen medium for preparation. In addition, control mixture liquid was synthesized by transfection reagents and serum-free optimen medium. On the 10th day after endometrial implantation, the nude mice were divided into interference group, negative control group, and control group (n=8) and rose in different cages. 100 μl siRNA transfection complex, negative control transfection complex, and control mixture were respectively injected once every day and for five times in total.

HE staining and immunohistochemistry

In the 24 hours after the last intraperitoneal injection, nude mice were sacrificed. Laparotomy was conducted to observe the formation of lesions in pelvic and abdominal cavity. Ectopic endometrium specimens were collected and washed in PBS solution. The tissues were divided into two parts, and one part was stored in fridge at 70°C. The other part was fixed in 10% neutral formaldehyde solution and embedded by paraffin and cut into slices, which were then stained with HE and immunohistochemical staining. Ectopic endometrium paraffin block was continuously cut into five-μm slices and stained by HE method. Under the light microscope, the cellular structure of ectopic endometrium tissues was observed.

Rabbit anti-human β-catenin monoclonal antibody (1:100), rat anti-human MMP-7 monoclonal antibody (1:100), and rabbit anti-human VEGF monoclonal antibody (1:100) were used as SP kit were provided. Endogenous peroxidase was blocked by 3% H₂O₂ and antigen was repaired by microwave heating and 10% healthy sheep serum was used to incubate and primary antibodies were dropped, and the reaction was conducted at 4°C overnight. After oscillation cleaning by PBS buffer solution, secondary antibodies marked by HRP were dropped. Subsequently, the slices were colored by DAB and again stained by HE, and finally conventionally mounting. PBS buffer solution was used as negative control instead of primary antibody and the known positive cells were used as positive control. HMIAS-2000 automatic medical colored image analysis system was adopted and the average optical density values (A) of stained positive cells of β-catenin, MMP-7 and VEGF were determined. Ten visual fields were selected from each section and each visual field was measured three times to calculate the average value.

Detection of β-catenin, MMP-7 and VEGF at the transcriptional level

Reverse transcription PCR (RT-PCR) and real-time quantitative PCR were used to determine the expressions of β-catenin, MMP-7, and VEGF. TRIZOL method was used to extract the total RNA of ectopic focus tissues of nude mice. According to the instruction of reverse transcription kit, cDNA was obtained by RT with mRNA as a template. Then CDNA was taken as the template, target genes were obtained through PCR amplification method with β-actin as the reference gene. All the primers were synthesized. The sequences of primers were as follows. β-actin forward: 5’-CCTGTACGCAAA-CACAGTGC-3’, reverse: 5’-ATACTCTGTC TTGCTGATCC-3’; β-catenin forward: 5’-AGGAAG CTTCCAGACACCG-3’, reverse: 5’-CGCACTGCATTTTAGCT CC-3’; MMP-7 forward: 5’-AGAGTGAGGTGCCAG ATG-3’, reverse: 5’-CTTGCC TACATCGT CAGAT-3’; VEGF forward: 5’-CCCACCACAT ACATACT T-3’, reverse: 5’-CTCCTCAACT CAAGTCCA A-3’.

According to the instructions of qPCR Mix kit, the reaction system was designed. Reaction continued according to the following procedure: 95°C for 120 seconds; 95°C for ten seconds; 60°C for 30 seconds; 70°C for 45 seconds, for a total of 40 cycles. Fluorescence signal could be detected and 2-ΔΔCt analytical method was used to process qR-PCR data.

Statistical analysis

SPSS17.0 software was used to make statistical analysis. Quantitative indexes were analyzed by One-Way ANOVA and comparisons between two groups were examined by t-test. Statistical significance was defined as p < 0.05.

Results

Establishment of endometriosis nude mice models

A total of 28 nude mice were selected for experiment, and one died of anaesthesia unexpectedly, three died due to postoperative infection, with only 24 modeling successfully. On the 15th day after operation, each nude mouse had one to three lesions, distributed in the anterior peritoneum and side front and side peritoneum, adipose tissues, and deep pelvis. The transplanting endometrium attached to the tissues of pelvic and abdominal cavities which was obvious and with a hard texture. Rete vasculosum developed well in the adhesion region. The lesions were fused to tissues of the nude mice and adhered to surrounding tissues, which were difficult to be scraped. There was no free endometrium in abdominal cavity and all the transplanting endometrium grew naturally. There was no difference in the positions and forms among each group according to visual inspection.

Histomorphology observation

There were obvious endometrial glands and mesenchymal cells in ectopic focus. Gland cells were cuboidal or flat. The margin of lesion was linked to mesothelial cell layer of peritoneum. Blood was abundant and inflammatory cells such as B lymphocytes and plasma cells seeped with obvious hyper-
Effect of Wnt/β-catenin signal pathway on matrix metalloproteinase-7 and vascular endothelial growth factor gene expressions etc.

Figure 1. — HE staining of ectopic endometrium (×400).

Figure 2. — Immunohistochemistry detection of β-catenin, MMP-7 and VEGF proteins (SP×200). a, b, and c are the protein expressions of β-catenin, MMP-7, and VEGF respectively, and d is the quantitative analysis of average optical density. ➀ siRNA interference group; ➁ negative control group; ➂ blank control group).
plasia. Interstitial, glandular, inflammatory, and fat cells co-existed. Degeneration, necrosis, and peripheral inflammatory cells' infiltration was found in all the ectopic endometrium. The differences of the lesions under the light microscope in each group was not significant (p > 0.05) (Figure 1).

Expressions of β-catenin, MMP-7 and VEGF proteins
The results of immunohistochemistry showed that the positive expressions of β-catenin, MMP-7, and VEGF proteins were brown and mainly located in glandular epithelial cells with cytoplasm stained. The expression quantities of β-catenin, MMP-7, and VEGF in siRNA interference group were much lower than those in negative control group and blank control group (p < 0.05), while there was no statistical significance in the expression difference between negative control group and blank group (p > 0.05) (Figure 2).

Expressions of β-catenin, MMP-7 and VEGF mRNAs
According to the detection of RT-PCR (Figure 3), and qRT-PCR (Figure 4), the relative expression levels of β-catenin, MMP-7, and VEGF mRNA in siRNA interference group were much lower than those in negative control group and blank control group (p<0.05), and the difference between negative control group and blank control group was of no statistical significance (p>0.05) which is consistent with the expressions of proteins.

Discussion
The symptoms of endometriosis are mainly pain, infertility, and others, which mostly occurs in childbearing age women with an incidence up to 5%-10% [11]. Endometriosis is benign, but it has the ability of distant metastasis and implantation, similar to malignant tumors. Sampson proposed the theory of retrograde menstruation implantation [12], arguing that ectopic endometrium originated from endometrium tissues which transferred to the outside parts of uterine cavity through retrograde menstruation, lymphatic spread, vascular dissemination, and iatrogenic planting and then implanted. Endometrial invasive implantation is a complex process caused by multiple factors. The increase of local estrogen is the key to successfully implanting ectopic endometrium [13]. Studies have showed that estrogen can regulate Wnt/β-catenin signal pathway through estrogen receptor in cytoplasm and consequently promote the transcription of target genes in nucleus [14, 15]. In recent years, it has been found that Wnt/β-catenin signal pathway may participate in the formation and development of endometriosis [10]. Some studies showed the expression of Wnt7a mRNA in ectopic lesions and pelvic peritoneum of patients with endometriosis was much higher than that in healthy cases [16, 17]. β-catenin is an important regulatory factor of Wnt signal pathway [18].

VEGF and MMPs play important roles in the processes of adhesion, invasion, and angiogenesis of EMs ectopic endometrium. Classical Wnt/β-catenin signal pathway is closely related to angiogenesis, vascular remodeling and the distribution of blood vessels in different categories and different organs [19]. EMs is an angiogenesis dependent disease. Endogenous angiogenesis factors stimulate ectopic endometrium and its surrounding tissues to form new vessels, conducive to the implantation and survival of ectopic endometrium [20]. Machadoet al. [21] found that the expression of VEGF in patients with endometriosis involving rectum increased significantly. The expression of VEGF in ascites of patients with EMs and ectopic endometrium was high, the hyperplasia of vascular endothelial cells was active, and angiogenesis was exuberant [22]. Goteri et al. [23] found VEGF took an important part in endometriosis and provided nutrition through new vessels, promoting the diffusion of ectopic focus. Moggio et al. [24] proved that the increase of
VEGF in peritoneal fluid of patients with EMs led to the enhancement of the ability to receive ectopic endometrium in local parts of pelvic cavity. Some experiments with nude mice models showed that VEGF antibody could effectively interfere the formation of vascular network and inhibited the development of disease through preventing angiogenesis of endometriosis [25].

Wnt/β-catenin signal pathway can upregulate the expression of VEGF significantly. Some studies showed that Wnt/β-catenin signal pathway could increase the expression of MMPs by regulating cyclooxygenase [26]. In this study, on the basis of successfully establishing endometriosis nude mice models, RNA interference technique was used to targeting silence the expression of β-catenin gene in order to block Wnt/β-catenin signal pathway. Results showed that the expressions of VEGF and MMP-7 decreased significantly, indicating Wnt/β-catenin signal pathway was key in the occurrence and development of endometriosis and promoted the expressions of VEGF and MMP-7 in endometriosis. MMPs, as the most important proteolytic system in extracellular matrix for degradation, can promote the infiltrated growth of ectopic endometrium and impede the formation of ectopic lesions through inhibiting the secretion of MMPs [27]. MMP-7 belongs to matrilysin class of MMP and can degrade III, IV, and V collagen protein as well as fibronectin and laminin [28].

References
Attitudes towards abortion in Italian women: socio-economic trends and epidemiological features

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Summary

Purpose of investigation: The aim of this study was to analyze trends and attitudes towards abortion in Italian women in the last decades. Materials and Methods: The authors analyzed number, socio-economic trends, and major clinical-epidemiological features of induced abortion in Italy (1980-2009). Results: Up to 1996 abortion rates were higher among married women, but from 1996 to 2009 they were higher among single women. The reduction of abortions has been observed in all age-groups, except in women from 15 to 19 years of age. Abortions were higher among younger women, women without previous abortions, nulliparous women, women with junior and senior high school diplomas (2005-2006), women with an academic degree (2007-2009), and professional women. Conclusion: In Italy, despite the decrease of the abortion rates, voluntary termination of pregnancy is still present and the spread of contraception is scarce. More information about contraception is necessary to help lower the incidence of both unintended pregnancy and abortion.

Key words: Abortion; Contraception; Italian women; Socio-economic status; Occupational status.

Introduction

Abortion is a consequence of an unintended, unwanted or unplanned pregnancy at the time of conception, resulting from contraceptive failure or non-use. Induced abortion is practiced almost all over the world and the proportion of all pregnancies ending in abortion is higher in the more developed than in the less developed regions [1]. In most European countries, from a culture of abortion, we are turning to a culture of contraception and prevention and this change is probably due to the commitment of women’s organizations, scientific societies, and the media, which are increasing awareness and information about contraception [2]. Moreover, nowadays in Europe, we are observing a reduction of sexual intercourse, of fecundability, and of fertility [2], which could be responsible for the decrease of the abortion rates. A change in the way of supporting women through counseling and the possibility to offer a suitable contraceptive method to help women avoid an unintended pregnancy has also been noted [2]. To date, an increase in the prevalence of premarital sexual activity associated with a rising mean age for marriage could be important reasons for increasing abortion rates among young women. Furthermore, this group seems to be more susceptible to having sex without contraception, and to engage in unprotected intercourse [3]. Over the years, the proportion of couples using contraceptives has increased steadily and this change has been most pronounced in the developing countries due to a decline in the number of children desired and the intention to avoid unintended pregnancies [1]. In general, condom and pill use are prevalent among younger women, whereas the use of long-acting and permanent methods increases with age [4]. In 2009, according to the United Nations, the mean global percentage using contraception in women who were married or in union was 62.7%, but despite these findings each year, many unintended pregnancies occur, suggesting that contraception still needs to be promoted [5].

Materials and Methods

In this descriptive retrospective study the authors analyzed the number, the socio-economic trends, and the major clinical and epidemiological features of induced abortion performed in Italy, since the first available data after its legalization, which occurred in 1978 in accordance with the law n.194. The study is based on Italian National Institute of Statistics (ISTAT) data, which since 1979 commenced the statistical survey of abortion cases (data found in the National Account section of http://www.istat.it/it/ archivio/ivg) [6]. The data are collected through an individual abortion statement, which is filled by the physician who performs the abortion. In this statement, information about the woman, the abortion, and the operation is summarized. In particular, between 1980 and 2009, the authors analyzed the mean age at the time of the abortion, the number of abortions, the standardised rate of abortion, the rate of abortion among single and married women, the percentage of abortion among immigrant women, and the rate of abortion among different age-groups. The analysis has been carried out considering the specific rate of the abortion age and of the marital status at the time of the operation and the standardised rate of abortion. Finally, in accordance with the availability of the ISTAT data, from 2005 to 2009, the authors correlated the num-
number of abortions to the number of previous pregnancies, previous voluntary abortions, educational level, and employment status.

Results

In this study the authors evaluated a population composed of women with different marital status (single, married, divorced, widows, with a non-indicated marital status), and women in reproductive age (aged 15-49, but also 14 and 50 years of age). First of all the authors assessed the abortion trend in Italy from 1980 to 2009. The number of abortions, after an initial peak between 1980 (207,644) and 1984 (228,377), began to decrease from 1985 to 2009 (114,793), with a total reduction of 44.72%. Thus, throughout the period of analysis, the trend showed a general reduction of abortions (Figure 1). Therefore, from a standardised rate of abortion of 15.9 per 1,000 women in reproductive age in 1980, we reached 8.55 per 1,000 in 2009. The previsional number of abortions for the following years, even if there are not conclusive data, showed a downward trend. Regarding the marital status, the authors compared the abortion rates of single and married women. In the singles group in 1981, the rate was 11.4 per 1,000 women, while in 2009 it was 8.45 per 1,000 women. In the married group in 1981 the rate was 17.7 per 1,000 women and in 2009 it was 6.83 per 1,000 women. In both groups, single and married ones, there was a reduction of the specific rates of abortion from 1981 to 2009. Until 1996 the rates were slightly higher in the married group, but form 1996 to 2009, even if it decreased, abor-
Attitudes towards abortion in Italian women: socio-economic trends and epidemiological features

tions were higher in the single ones (8.45 per 1,000 vs. 6.83 per 1,000) (Figure 2). The mean age at abortion was 29.2 years in 1982 and 29.98 years in 2009, with 0.78 points of increase, but remaining essentially unchanged throughout this period. The reduction of abortions from 1980 to 2009 was observed in all age-groups, except in women 15 to 19 years of age, in which a small increase was reported, from a rate of 6.2 per 1,000 women in 1980 to 6.62 per 1,000 women in 2009. In general, younger women showed a major tendency to abortion. The lowest abortion rates were reported in the age-groups 15-19, 40-44, and 45-49 years. On the other hand, the highest rates of abortion were observed in women aged 20-39 years (Figure 3). In the recent years, the percentage of immigrant abortions increased from 6.56% in 1995 to 33.81% in 2009. After the year 2000, the Rumanian women began to differentiate themselves from other immigrant women and while before there was no dominant citizenship, from 2,858 abortions in 2000, the Rumanian reached 11,049 in 2009 (13.6% and 29.3% of all abortion among immigrant women, respectively). Finally, focusing on the time interval from 2005 to 2009, the authors analyzed the number of abortions related to eventual previous pregnancies, previous abortions, educational level, and employment status. They observed that the number of abortions was higher in women without previous pregnancies (Figure 4). A similar pattern was examined in women without previous abortions (Figure 5). The abortion rate was higher in women with junior and senior high school diplomas, occupying the first and the second positions, respectively, and it is interesting to note that while in 2005 and 2006 in the third and the fourth position the authors found women without educational qualification or primary school certificate and women with an academic degree, respectively, in 2007, 2008, and 2009 exactly the opposite (Figure 6), with an increase of abortions among women with an academic degree. The present data also showed higher abortion rates among professional women than among housewives, students, and non-professional ones (Figure 7).
Figure 5. — Induced abortion and previous induced abortions (2005-2009).

<table>
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<tr>
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<th>2009</th>
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Figure 6. — Induced abortion and educational level (2005-2009).

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<th>2008</th>
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Figure 7. — Induced abortion and employment status (2005-2009).

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Discussion

The use and the spread of contraceptive methods in Italy are widely inferior than in other European countries, even than Eastern Europe, which has the lower rate of contraception [7]. There is a lower use of oral contraceptives (OCs) and intrauterine devices (IUDs) and a great use of such inconsistent methods as withdrawal [8]. One girl out of five under 15 years of age has already had sexual intercourse and the majority of women recurring to an emergency contraception is under 25 years of age, single, and without previous pregnancies [9]. A recent survey conducted in Italy reported that 53% of the Italian women refused to use contraceptive methods, 38% do not have an adequate knowledge, or do not use them properly [10]. Common reasons for the non-use of contraceptives include underestimation of the pregnancy risk, the concern about side effects, and unplanned intercourse [4]. Only 0.3% of young Italian women under 19 years old have a good sex education, 26.5% sufficient, 72% insufficient [10]. Moreover, recent findings have shown that young women have a more difficult time negotiating the use of user-dependent methods. A survey conducted by Censis in 2000, has shown that 31.6% of the Italian couples use the withdrawal method, 28.4% the condom, 3.2% IUDs, and only 20.9% the pill (the former prevailing in the Northern Italy than in the South) [11]. According to the ISTAT data in 2002, in Italy, almost 50% of women have sexual intercourse without contraception and the mean percentage of users of oral contraceptives has been estimated as 19.1% [12], well below the European mean of 30% [12]; 45% of pregnancies are intended, while in the rest of Europe they are 6.5% [13]. In a study conducted in 2003, 12,138 women were enrolled from five European countries, of which 2,301 were Italians. The study demonstrated that 2.6 million of Italian women used OCs (19%) between 15-49 years old. The same situation was found in Spain (19%), while UK, Germany, and France demonstrated a higher use of contraception with 27%, 34% and 45%, respectively [14]. However, the abortion rate among Italian women under 20 years old in 2009 was 6.9 per 1,000, lower than other European countries. Subsequent to the legalization of abortion in Italy in 1978, an upward trend has been experienced in the first years among Italian women and then the number of abortions declined steadily. The highest abortion rates have been observed between 1982 and 1983 with a second peak for all age-groups in 2004. The first peak was followed by a consistent decrease that can be attributed to the fact that the legalization focused the attention on this theme, making women aware that substituting abortion for contraception was unacceptable. Furthermore, the decrease of voluntary termination of pregnancy beginning from 1982 has been correlated to the diffusion of modern methods of fertility regulation and to an increase in OCs diffusion [15]. Instead, the second peak (2004) has been ascribed to an increase of abortion among immigrants. The contribution of immigrant women to the voluntary termination of pregnancy has increased over the years, especially among Rumanian women. This increase has been reported mostly in the Central and Northern Italy, where the number of immigrants has grown in that period [16]. Data have shown that immigrant women generally have a lower educational level than Italian women [6], which strongly influences their socio-economic position, their reproductive life, their access to health services, and it might explain, according to the growing number of immigrants in the last years [6], these increasing abortion rates. Low educational level and insufficient Italian integration policies may be responsible for this upward trend. Regarding the marital status, the authors noticed a reduction of the number of abortions both in single and married women, but since 1996, abortions began to increase in the former. This evidence is probably due to the transformation of the society in the 1990s. The age at marriage has been increasing and the risk of unintended pregnancy and abortion among sexually active women may also be increasing. Abortions among young and single women often reflect the desire to delay the start of childbearing, whereas among older ones, they reflect the desire to space or stop childbearing [4]. In Italy over the years, the authors observed a reduction of abortions among all age-groups, except among women aged 15-19 years; indeed in this group a small increase has been reported. In general, in this report age-specific abortion rates and the share of all abortions by age-group are generally highest in the mid-range of women’s reproductive years (aged 15-39 years). The present results have shown that women in certain age-groups are more likely than others to have abortions, and are probably in greater need of contraceptive information. The lowest abortion rates were observed among women aged 15-19 and 40-49 years. This trend could be explained by the fact that teens 15-19 years of age are less sexually active comparing them to women in their 20s or older. Moreover, they numerically represent a small group among women who resorted to induced abortion, and even if a slight increase of voluntary termination of pregnancy has been demonstrated in this teens, they are just a small share comparing their rates to those of the other age-groups. On the other hand, the low abortion rates among women of 40-49 years of age in the present study are expected, female fecundity declines with age, which surely contributes to the lower abortion rates among older women. Furthermore, these women are more likely to have a higher awareness about the sexual behaviour and the relationship between a couple. Finally, the authors found higher abortion rates among Italian women without previous pregnancies and previous abortions. This evidence let us hope that the occurrence of an unintended pregnancy and the subsequent induced abortion is a teaching moment for a woman, which could learn from that experience and begin to use a contraceptive method or switch to another more suitable contraceptive if she is already using one. Furthermore, the authors noticed that a leading factor associated with abortion is the socio-economic position. The educational level is an indicator of the social position and the maternal educational level
influences pregnancy outcome and access to health services. Voluntary abortion rates were higher among women with an academic degree and among professional women. This trend is probably linked to the changing of personal and professional needs, in a world where women are always more emancipated and hard workers, with the growing tendency to postpone marriage and childbearing. Thus, the number of years during which women are potentially at risk of experiencing an unintended pregnancy increases proportionately. The incidence of unintended pregnancy and subsequent induced abortion is an important health status indicator in the field of reproductive health. Improving access to contraceptives for all women and couples, and improving the effectiveness of use among those who are already using them, are crucial steps toward reducing the incidence of unintended pregnancy and induced abortion.

Conclusion

Abortion is not only a medical or surgical procedure, it is also a social problem, a social process involving several subjects and raises questions about the right of women, the protection of the unborn, and the religious view of life. Abortion rate reduction in many countries is linked to a more widespread use of contraceptive methods, but also to a reduction in fertility, as observed in some industrialized countries. There is also a change in the way of supporting women, by counseling and offering contraceptive methods to help women avoid an unwanted pregnancy. But despite the availability of effective contraceptive methods, unintended pregnancy continues to be a significant health problem for women throughout the world. Thus, unintended pregnancy contributes to abortion and improving access to sex education and increasing the practice of effective contraception would be the best way to reduce it. Educational programs should include information about pregnancy risk and correct use of contraceptive methods. It is also necessary to improve access to the health services for immigrants and to implement the integration policies. Nowadays in Italy, despite the decrease of the abortion rates, voluntary termination of pregnancy is still present and the use and the spread of contraception are still scarce. Thus, healthcare professionals need to inform their patients about the benefits, risks, and the correct usage of all available contraceptives and to increase awareness of emergency contraception to help lower the incidence of both unintended pregnancy and abortion, taking in consideration that the more contraception is used, the less abortions there will be.

References


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Reproductive performance after hysteroscopic metroplasty in infertile women: complete versus partial uterine septum

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Summary

Objective: To investigate the impact of hysteroscopic metroplasty on pregnancy outcome in women with complete or incomplete uterine septum (US) accompanying infertility. Materials and Methods: Seventy-three patients who had hysteroscopic metroplasty for complete and incomplete US with primary and secondary infertility were reviewed. Obstetric outcomes (number of pregnancies, live births, and miscarriages) up to 36 months follow up period were investigated. Results: Twenty-five patients in complete US and 28 patients in incomplete US became pregnant in 36 months follow up. Postoperative miscarriage rate was significantly lower in patients with complete US ($p = 0.0001$, $p = 0.0001$, respectively). The mean gestational week at the time of birth and mean birth weight of the infants were significantly lower in patients with complete US compared to the incomplete US cases ($p = 0.026$, $p = 0.049$, respectively). Postoperative pregnancy rate was significantly lower in incomplete US patients with primary infertility compared with secondary infertility ($p = 0.037$). Conclusion: Hysteroscopic metroplasty improves fertility and pregnancy performance. This improvement is more prominent in patients with complete US, and incomplete US patients with secondary infertility.

Key words: Complete uterine septum; Incomplete uterine septum; Hysteroscopic metroplasty; Obstetric outcome.

Introduction

Uterine septum (US) is the most common uterine structural anomaly among the fertile population with a prevalence of 1-4.3%. Almost half of the (34-48%) uterine malformations are US [1-3]. During embryological development, fusion of the two paramesonephric Müllerian ducts forms the uterus. Unification of the uterine cavity begins during the first trimester of the embryonic life from the uterine cervix proceeding caudally towards the fundus. A failure of the reabsorption process of the midline septum causes US. The degree of septation is variable; complete septum extends from the uterine fundus to the cervix, while incomplete septum demonstrates resorption of a portion of the caudal aspect of the septum [4].

Hysteroscopic metroplasty (hysteroscopic resection of the US) reduces intra- and postoperative morbidity and accepted as the method of choice for correction complete and incomplete US. Shorter operation time and hospitalization period are other advantages of this operation [5].

The negative impact of US on fertility is an interesting but still debated issue. Grimbizis et al. reported that the US is not an infertility factor, although it may contribute to the delayed natural conception of patients with secondary infertility [6]. However, an improvement in the pregnancy rate was reported after the hysteroscopic metroplasty in women with US and otherwise unexplained infertility, by various authors [7-9].

Many studies have described an increase in the pregnancy and a decrease in the miscarriage rates after hysteroscopic metroplasty in patients with complete US. Some authors believe that incomplete US is a variant of normal anatomy and it no impact on reproductive outcome [10, 11]. On the other hand, some studies reported an increased incidence of miscarriage and of preterm birth rate in women with incomplete US [12-15].

The first aim of the present study was to investigate the impact of hysteroscopic metroplasty on fertility rate and pregnancy outcome in women with complete or incomplete US accompanying infertility. The second aim was to assess if there was a difference in the pregnancy outcome following the hysteroscopic metroplasty, considering primary and secondary infertility.

Materials and Methods

A total of 131 patients who were treated with a hysteroscopic metroplasty in the Infertility Clinic in Etilik Zubeyde Hanım Maternity and Teaching Hospital between January 2007 and De-
December 2008 were reviewed. The Institute's ethics committee approved the study protocol.

For the selection of the primary or secondary infertile patients, inclusion criteria were accepted as follows: women’s age < 35 years, no endocrine-metabolic disorders including diabetes, hypothyroidism, hypopituitarism, hyperprolactinemia, hyperandrogenism, no other coexisting infertility factors including endometriosis, tubal occlusion, ovarian cyst, decreased ovarian reserve (FSH > 12), history of primary or secondary infertility, and partner’s spermogram analysis in normal range. A total of 58 patients were excluded from the study for the following reasons: the indication of the operation for the pregnancy loss (n=41), coexisting pathology as diagnosed on the laparoscopy including endometriosis (n=8), tubal occlusion (n=5), and coexisting male factor infertility (n=12). Eight couples had more than one cause of the infertility. Primary infertility was defined as the inability to conceive after one year of regular unprotected intercourse while secondary infertility was defined as the inability to get pregnant after 12 months of contraception-free intercourse after at least one pregnancy.

The initial diagnosis of US was made with hysterosalpingography (HSG) and confirmed by laparoscopy carried out simultaneously with hysteroscopic metroplasty. US was classified according to the American Society of Reproductive Medicine (ASRM) classification. US was categorized as type a (complete) or type b (incomplete) [16]. Normal sperm parameters according to WHO criteria for sperm analysis were used for evaluation (a sperm concentration of ≥ 20×10⁶, ≥ 50% motility and ≥ 30% normal morphology, ≥ 75% viability with ≤ 1×10⁶ white blood cells).

The operations were performed under general anesthesia in the proliferative phase of the cycle. Hysteroscopic metroplasty was performed using a monopolar cutting knife electrode with laparoscopic supervision (ten-mm fiberoptic resectoscope). The uterine cavity was distended with a 1.5% glycine solution. Continuous irrigating flow with Hamoumat was used. Complete US was incised from the internal orifice of the cervical canal, up to the base of the septum until both of the tubal ostia were visualized at the same level. Tubal patency was tested during the laparoscopic observation. Intrauterine device was not inserted after the procedures. Postoperative diagnostic hysteroscopy and/or HSG were used asses the uterine cavity two to three months after surgery in all patients.

Follow-up after the hysteroscopy was done either in the infertile outpatient clinic or by phone with the form that contained the following data: number of pregnancies achieved, time interval following data: number of pregnancies achieved, time interval of infertility (months) 35.3 ± 26.3 43.4 ± 35.8 NS

Table 1. — The main characteristics and obstetric history of complete and incomplete septum groups before hysteroscopic metroplasty.

<table>
<thead>
<tr>
<th></th>
<th>Complete uterine septum (n=32)</th>
<th>Incomplete uterine septum (n=41)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.1 ± 4.7</td>
<td>27±5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>14 (43.8)</td>
<td>13 (31.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>18 (56.2)</td>
<td>28 (68.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>2.2</td>
<td>17.3</td>
<td>0.035</td>
</tr>
<tr>
<td>Miscarriage rate (%)</td>
<td>86</td>
<td>71.7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (months)</td>
<td>35.3 ± 26.3</td>
<td>43.4 ± 35.8</td>
<td>NS</td>
</tr>
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</table>

Table 2. — Pregnancy rate and pregnancy outcomes after hysteroscopic metroplasty.

<table>
<thead>
<tr>
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<th>Incomplete uterine septum (n=41)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate (%)</td>
<td>78.1</td>
<td>75.6</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriage rate (%)</td>
<td>3.1</td>
<td>26.3</td>
<td>0.035</td>
</tr>
<tr>
<td>Mean gestational weeks</td>
<td>36.4 ± 5.1</td>
<td>39.1 ± 11.1</td>
<td>0.026</td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>2733 ± 994</td>
<td>3202 ± 405</td>
<td>0.049</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>68.7</td>
<td>65.8</td>
<td>NS</td>
</tr>
</tbody>
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Table 1. — The main characteristics and obstetric history of complete and incomplete septum groups before hysteroscopic metroplasty.

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</tr>
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<td>86</td>
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<tr>
<td>Duration of infertility (months)</td>
<td>35.3 ± 26.3</td>
<td>43.4 ± 35.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SD. p < 0.05 was considered as statistically significant.

Results

Overall 73 infertile patients with complete (n=32, 43.8%) and incomplete (n=41, 56.2%) US were evaluated. Twenty-seven of these women (37%) had primary infertility while 46 (63%) had secondary infertility. The mean age of the patients was 26.2 ± 5 years and the mean duration of infertility was 39.9 ± 32 months. Live birth rate was significantly higher in patients with US before hysteroscopic metroplasty (19.5% vs. 3.1%, p = 0.035). The main characteristics and obstetric history of the patients before hysteroscopic metroplasty are presented in Table 1. The range of the patients' follow up period was 24 to 36 months.

After the procedure, two cervical lacerations on the tenaculum application site were observed; one of them required suture. Postoperative evaluation of the patients revealed normal shaped cavity in all patients.

Pregnancy rates were similar in both complete and incomplete US groups. Twenty-five patients in complete US and 28 patients in incomplete US became pregnant in 36 month follow up period. A total of 32 pregnancies was observed in complete US and 38 in incomplete US. Miscarriage rate and live birth rate calculated from total pregnancies. There was a significant decrease in postoperative miscarriage rates in both complete and incomplete US patients after operations in comparison to preoperative period (p = 0.0001, p = 0.0001, respectively). The decrease in miscarriage rate was more prominent in patients with complete US than incomplete US (p = 0.035). The mean gestational week at the time of birth and mean birth weight of the infants were significantly lower in patients with complete US compared to the incomplete US cases (p = 0.026, p =
Table 3. — Pregnancy rate and pregnancy outcomes after hysteroscopic metroplasty.

<table>
<thead>
<tr>
<th>Primary infertile patients (n=27)</th>
<th>Secondary infertile patients (n=46)</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>Pregnancy rate (%)</td>
<td>62.6</td>
<td>84.5</td>
</tr>
<tr>
<td>Miscarriage rate (%)</td>
<td>7.7</td>
<td>14.9</td>
</tr>
<tr>
<td>Mean gestational weeks</td>
<td>36.3 ± 4.5</td>
<td>38.7 ± 1.5</td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>2720 ± 874</td>
<td>3116 ± 418</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>92.3</td>
<td>91.5</td>
</tr>
</tbody>
</table>

NS: non-significant.

0.049, respectively (Table 2). There was no difference in terms of the type of delivery (vaginal or cesarean section) between complete and incomplete septum groups ($p = 0.606$).

The pregnancy outcomes of the 73 patients with US were compared according to the type of infertility (primary and secondary infertility). Postoperative pregnancy rate was significantly lower in patients with primary infertility compared to the secondary infertility ($p = 0.037$). There was no significant difference in the miscarriage rate, live birth rate, mean birth weight, and mean gestational weeks at the time of birth between the patients with primary and secondary infertility (Table 3). The postoperative pregnancy rate was significantly lower in incomplete US patients with primary infertility compared to secondary infertility. (primary: 53.8%, secondary: 85.7%, $p = 0.037$).

Discussion

US is associated with poor pregnancy outcomes with an increased miscarriage and preterm birth rate. The negative impact of US on fertility is an interesting but still debatable issue. The presented data revealed that the reproductive performance of the patients with US was improved after the hysteroscopic metroplasty. However, observed improvements on the miscarriage rates were in favor of patients with complete US. Although no significant difference was observed on the live birth rate, the mean gestational week at the time of birth and mean birth weight of the infant were significantly lower in patients with complete US. Postoperative pregnancy rate was significantly lower in patients with primary infertility compared to the secondary infertility. Of interest, the postoperative pregnancy rate was significantly lower in incomplete US patients with primary infertility compared with secondary infertility. These results suggest that hysteroscopic metroplasty has a more beneficial effect on the achievement of pregnancy in secondary infertility.

Grimbizis et al. reported the incidence of miscarriage in patients with untreated US as 44% [3]. In another study assessing the reproductive outcomes with untreated complete septate uteri demonstrated a miscarriage rate of 27% [17]. Hysteroscopic resection of the septum improved the miscarriage rates in the range from 2.5% to 25% [3, 7, 8, 18]. In the presented study, the miscarriage rates decreased significantly after hysteroscopic metroplasty in complete septum group (from 53% to 3.1%) and incomplete septum (from 61% to 19.5%) group. The decrease in miscarriage rate is more distinct in the complete US group. It can be speculated that the miscarriages observed in incomplete uterine cases might also be related to the other factors.

Abnormal uterine cavity, different structure of the septum, and dysfunction of the covering endometrium are thought to impair the reproductive outcomes in patients with US. In 1995, Fedele et al. reported that septum is a fibro-elastic tissue [19]. On the contrary, Dabirshahri et al. noted that the muscular tissue was more than a connective tissue in the septum structure [20]. The altered proportion of the connective and muscular tissue in the septum may lead abnormal uterine contractility in the uterus. In the study presented by Fedele et al., the maturation defect of the covering endometrium in the US was related to the infertility [21]. Recently, Raga et al. reported that the mRNA expression of the VEGF receptors was significantly lower on the covering endometrium of the septum. Authors suggest that local defect of VEGF receptors may lead to the poor vascular placentation that caused the poor reproductive outcome in pregnancies implanting the septum [22]. Restoration of the uterine cavity using hysteroscopy is intended to restore normal-adequate uterine cavity and uterine function, especially endometrium. In the present results, significantly lower gestational week at birth and mean birth weight of the infant may indicate that uterine volume in the complete US patients after hysteroscopic metroplasty, might be smaller than incomplete US patients.

Efficacy of prophylactic hysteroscopic metroplasty is controversial in infertile women without a history of adverse pregnancy outcome. Pregnancy rates in the literature after hysteroscopic metroplasty range from 38.6% to 81% in cases with infertility however a meta-analysis comparing the improvement in incomplete septum cases with complete septum cases is still lacking [8, 23]. Some studies failed to show a relationship between septate uterus and infertility as a considerable extent of women conceived without resection of the US [3, 4, 24, 25]. However, an improvement of the pregnancy rate was reported after the hysteroscopic metroplasty in women with US and otherwise unexplained infertility, by various authors [7-9].

Grimbizis et al. evaluated 42 US patients with infertility (primary: 26, secondary: 14). Following the hysteroscopic metroplasty, the pregnancy rates were 57.9% and 71.4% in patients with primary and secondary infertility, respectively. Postoperatively, 78.8% of the patients were treated by assisted reproductive technology (ART). Although statistical comparisons were not given, spontaneous pregnancy rate was lower in patients with primary infertility compared to the secondary infertility (15.8% and 35.7%, respectively). The pregnancy rate after ART was 42.1% for primary infertility and 35.7% for secondary infertility [6].
Pabuccu et al. investigated the reproductive performance after hysteroscopic metroplasty in patients with unexplained infertility. The pregnancy rate was given as 75% and 30.1% in patients with complete and incomplete US, respectively [7].

Mollo et al. investigated the impact of hysteroscopic metroplasty on the pregnancy rate in women with US and unexplained infertility. On the 12 months follow-up period, the pregnancy rate was 38.6% in women with US and unexplained infertility while 20.4% in the control group including unexplained infertile patients (p<0.05) [8].

The retrospective nature and lack of control group are two potentially limitations of the presented study. However, observational management of women with US and otherwise unexplained infertility as control group would not be acceptable due to ethical difficulties. The valuable aspects of the presented study are long term follow-up period without additional treatment of the couples and comparing the data of reproductive outcomes in infertile couples with complete versus incomplete US.

In conclusion, hysteroscopic metroplasty improves the pregnancy rates and pregnancy outcome in the patients with US. This improvement is more prominent in cases with complete US, and incomplete US patients with secondary infertility. However, interference of US on women’s fertility and improving effect on the infertility of hysteroscopic metroplasty in patients with unexplained infertility is still open to research and further analysis with larger series of patients is required.

References


The effect of endometrial polyps on pregnancy rates in intracytoplasmic sperm injection cycles

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Summary

Objective: The purpose of this study was to determine reproductive results of intracytoplasmic sperm injection (ICSI) for different endometrial polyps subgroup divided according to polyp size and number. Materials and Methods: Eighty-three primer infertile patients were retrospectively analyzed. Group A consisted of 36 patients having an endometrial polyp with a diameter ≤ one cm; whereas 47 patients were included in Group B who had a polyp with a diameter > one cm or more than one polyps. All patients underwent a hysteroscopic polypectomy and ICSI treatments were started in the following cycle. Results: Pregnancy was achieved in 16 patients (44.4%) in Group A and 23 patients (48.9%) in Group B. The pregnancy ratios did not reveal a statistically significant difference between the two groups. Conclusion: The authors concluded that in patients who have undergone hysteroscopic polypectomy before the intracytoplasmic sperm injection (ICSI) cycle, the pregnancy rates do not depend on the diameter of the endometrial polyps.

Key words: Endometrial polyp; Hysteroscopy; ICSI; Pregnancy.

Introduction

Endometrial polyps are frequently encountered during the reproductive period. Their prevalence in women is 24% and this rate further increases with advanced age [1]. Endometrial polyps usually develop as a result of an anomaly in hormonal receptivity, namely the persistence of the receptors for estrogen and the absence/downregulation of the receptors for progesterone. Hormonal disorders such as disovulation/anovulation, luteal insufficiency, and hyperestronegemia frequently accompany endometrial polyps [2].

Endometrial polyps are easily diagnosed with sonohysterography or office-based hysteroscopy in symptomatic women; they may also be incidentally encountered with transvaginal sonography in asymptomatic women. Endometrial polyps damage the normal endometrial tissue and play an important role in the implantation failure. Mittal et al. [3] have reported that the gland and stroma of the endometrial polyps remained unresponsive to the stimulation by progesterone. This fact seems to cause a defective implantation at the polyp site. In patients diagnosed with an endometrial polyp, the increased plasma concentration of glycodeline was held responsible for the implantation failure [4]. The presence of an endometrial polyp can also affect the implantation and the embryonic development by inducing local inflammatory changes or deteriorating the normal shape of the uterine cavity [5].

It was previously shown that endometrial polyps interfere with fertility during natural conception [6, 7] as well as intrauterine insemination [8]. Their detection during or just before the in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles is more troublesome. In such a case, one of the four different approaches to the endometrial polyp is preferred: 1) cancellation of the cycle followed by polypectomy; 2) removal of the endometrial polyp and embryo freezing with embryo transfer after a few months; 3) negligence of the polyp and completion of the treatment protocol; 4) the utilization of hysteroscopic polypectomy during the ICSI cycle before the oocyte retrieval [9-11]. The abundance of different treatment options may confuse the clinician specialized in assisted reproductive techniques who aims for a better implantation and pregnancy rates overall.

Materials and Methods

In this study all fresh ICSI cycles admitted in Zeynep Kamil Women and Children’s Diseases Training and Research Hospital were retrospectively analyzed. All patients with primary infertility were included into the study. Following a complete infertility workup of the couples, the presence of an endometrial polyp remained as the only possible reason of infertility. The design of the study was approved by the local ethics committee and written consent has been taken from each patient. Prior to the ICSI treatment program, all of the patients underwent transvaginal ultrasonography and sonohysterography exam-
Clinical pregnancy rates and clinical abortion rates were compared between the two groups. The statistical analysis of the data was performed with independent Student t-test. Prior to the transfer, the overall quality of the embryo was evaluated; thereafter each patient received two embryos between the third to fifth days of oocyte retrieval via a soft catheter under normal saline infusion. Frozen embryo transfers were excluded from the study.

All patients underwent a hysteroscopic polypectomy and ICSI treatments were started in the following cycle. The endometrial polyps were removed under general anesthesia with a ten-mm monopolar cutting loop resectoscope. The pathological examination confirmed the diagnosis of endometrial polyp in each patient. Frozen embryo transfers were excluded from the study.

Starting from the 21st day of the spontaneous menstrual cycle, all of the patients received standard GnRH agonist (leuprolid acetate) treatment. On the third day of the resulting withdrawal bleeding, recombinant FSH treatment was started. Recombinant FSH doses were titrated according to the E2 levels and the standard criteria for follicular maturation on ultrasonographic examination and 250 mcg recombinant hCG (r-HCG) was administered when at least three of the follicles had reached a diameter of 17 mm. The oocytes were retrieved 36 hours later under intravenous sedation anesthesia and transvaginal ultrasound guidance. Oocytes were inseminated four to six hours after retrieval by ICSI using fresh ejaculates. Prior to the transfer, the overall quality of the embryo was evaluated; thereafter each patient received two embryos between the third to fifth days of oocyte retrieval via a soft catheter under the guidance of transabdominal sonography.

On the day of oocyte retrieval, the luteal phase support was started as a daily vaginal application of 90 mg progesterone. On the 12th day following the embryo transfer, a serum b-hCG level of 20 IU/L was recognized as biochemical pregnancy; the presence of the gestational sac in ultrasonographic examination six weeks following the embryo transfer was regarded as clinical pregnancy.

Total dose of gonadotropins, peak E2 level, number of oocytes retrieved, endometrial thickness on the day of hCG injection, clinical pregnancy rates and clinical abortion rates were compared between the two groups. The statistical analysis of the data was performed with independent Student t-test and chi-square test.

The values are presented as mean ± SD if not stated otherwise.

Results

A total of 83 patients were included in the study; Group A consisted of 36 patients having an endometrial polyp with a diameter ≤ one cm; whereas 47 patients were included in Group B who had a polyp with a diameter > one cm or more than one polyps. According to various parameters including average age, body mass index (BMI), duration of infertility, required total gonadotropin dosage, number of retrieved oocytes, peak E2 level, and endometrial thickness on the day of hCG application, the comparison of these findings revealed no statistically significant difference between the groups (p > 0.05) (Table 1).

Pregnancy was achieved in 16 patients (44.4%) in Group A and 23 patients (48.9%) in Group B. The pregnancy ratios did not reveal a statistically significant difference between the two groups (p = 0.684) (Table 2). On the other hand, abortion has occurred in three patients (8.3%) in Group A and five patients (%10.6) in Group B; the comparison of these findings revealed no statistically significant difference between the groups (p = 0.724) (Table 2). The analysis of the pregnant women also showed that in Group A, ten patients (76.9%) had undergone cesarian section and three patient gave birth through normal vaginal delivery. In Group B, 12 women (66.7%) gave birth by cesarian section and six women (33.3%) by normal vaginal delivery. The comparison of the living birth rates and the delivery methods revealed no statistically significant difference between the two groups (p = 0.53) (Table 3).

Discussion

Asymptomatic endometrial polyps are often incidentally detected during the treatment cycles of assisted reproductive technology. Polyps are either composed of immature endometrium that is unresponsive to progesterone and responsive to the growth stimulus of estrogen (the most common type) or they can be also composed of a functional endometrium that can respond to the ovarian hormones just like endometrium [12]. Endometrial polyp is the most...
commonly encountered pathological finding during a hysteroscopic evaluation that is performed before the IVF intervention.

In a study by Fatemi et al. the prevalence of endometrial polyp was reported as 6% in a group of 678 patients waiting for IVF/ICSI who had a normal transvaginal ultrasonography and no sign of an intrauterine pathology [13]. During the therapy cycles with assisted reproductive technology, the achievement of a successful embryonic implantation is a rather complex process. The most important factors in this respect are the overall quality of the embryo and the endometrial receptivity [14]. Moreover, the diameter, amount, and location of the polyps may also have an impact on the reproductive results.

If the ultrasonographic examination before the IVF or cryo-embryo transfer indicates a suspicious presence of a polyp, a thorough investigation is warranted, followed by appropriate treatment directed at the polyps. On the other hand, polyps smaller than 20 mm and incidental polyps encountered in patients receiving gonadotropin stimulation during IVF have to be treated with a different approach. In such a case ovarian stimulation can be sustained and fresh embryo transfer can be accomplished. Following the cryopreservation of all embryos and removal of the polyp, embryo transfer can be completed; as a rare alternative, the cycle is cancelled and polypectomy is planned [15].

In several studies the effect of endometrial polyps on the pregnancy rates was investigated in patients subject to IVF, intrauterine insemination (IUI) and spontaneous pregnancy cycle. These studies have yielded different results. In a randomized controlled study (n=215), a statistically significant increase in pregnancy rates was reported in patients who had undergone hysteroscopic polypectomy and intrauterine insemination with gonadotropin dependent ovarian hyperstimulation (63% vs. 28%, p < 0.001) [8]. Additionally, several non-randomized studies have demonstrated an increase in spontaneous pregnancy rates following hysteroscopic polypectomy in women with polyp and infertility due to an unknown cause [5, 6, 16].

HOXA10 and HOXA11 are known as the molecular markers of the endometrial receptivity. Rackow et al. conducted a study and investigated the endometrial polyps which were hysteroscopically diagnosed with help of HOXA10 and HOXA11 and the effects of these polyps on endometrium. In uteri carrying endometrial polyps, an increase in HOXA10 and HOXA11 mRNA levels have been documented, which could have disturbed the implantation process. These findings have suggested a molecular mechanism that is responsible for the decrease in pregnancy rates among the women with endometrial polyps. Therefore Rackow et al. primarily evaluated the uterine cavity in infertile women; if an endometrial polyp was detected, they suggested the application of hysteroscopic polypectomy before the infertility treatment in order to increase the fertility [17].

In a study conducted by Stamatellos et al. [18], the effect of endometrial polyp diameter on pregnancy rates was investigated in spontaneous cycles. The patients were divided into two groups: those patients with an endometrial polyp diameter equal or below one cm constituted the first group and patients with a diameter above one cm or with multiple endometrial polyps comprised the second group. In both groups patients were followed up for three to 18 months after the hysteroscopic polypectomy procedure and the rates of spontaneous pregnancy, term pregnancy, and spontaneous abortion were analyzed. Following the procedure the rates of spontaneous pregnancy and term pregnancy demonstrated an increase; in the group of patients with a small polyp, the rates were calculated as 67.6% and 58.8%, respectively. In the other group with a greater polyp or multiple polyps, the rates were 57.1% and 51%, respectively. The comparison of both groups for the rates of spontaneous pregnancy and live birth following polypectomy revealed no statistically significant difference. The total rate of spontaneous abortion in the first trimester was identified as 6% (five patients) and no significant difference was found between the groups. The present study differed from this research in that the clinical pregnancy and live birth rates following the polypectomy procedure were analyzed during ICSI cycles and not during spontaneous cycle. In the present study the clinical pregnancy rate and the live birth rate were found as 44.4% and 36.1%, respectively, in Group A and as 48.9% and 38.2%, respectively in Group B. Similar to the findings of Stamellos et al. the present authors also found no significant difference in clinical pregnancy and live birth rates between the two groups.

In another study by Lass et al. [19] patients with endometrial polyps encountered during transvaginal ultrasonographic examination in IVF cycle were evaluated. They reported that endometrial polyps with a diameter smaller than two cm do not decrease the implantation or pregnancy rates but increase the abortion rates. Therefore they suggested the application of embryonic cryo-preservation upon detection of an endometrial polyp during IVF cycle. In the present study the authors analyzed whether polyp diameter was related to the pregnancy results in patients in whom they have detected endometrial polyp and performed hysteroscopic polyp resection prior to the ICSI cycle. In the present study the diameter of the endometrial polyp (> one cm vs. < one cm) or its multiplicity had no significant influence on the rates of clinical pregnancy. The rates of abortion also did not demonstrate a statistically significant difference between the groups.

In a study conducted by Preuthipan et al. [20] 190 patients underwent hysteroscopy and women diagnosed with an endometrial polyp were divided into two groups according to the size of the polyp diameter (≤ 2.5 cm vs. > 2.5 cm). Following the polypectomy procedure, cumulative pregnancy rates were analyzed. The pregnancy rates did not show a statistically significant difference between the groups. In
this study clinical pregnancy rates were evaluated in spontaneous cycles, in this respect it differs from the present study.

In another study led by Isikoğlu et al. [21], patients receiving ICSI has been divided into three groups: patients with a polyp diameter ≤ 1.5cm detected during ovarian stimulation, patients without polyp detection, and patients who have undergone hysteroscopic polypectomy. In each group pregnancy and implantation rates were evaluated. The comparison of the groups for pregnancy and implantation rates has revealed no statistically significant difference. In the same study the authors were convinced that endometrial polyps with a diameter ≤ 1.5cm did not have a significant influence on the pregnancy and implantation rates during ICSI. Upon the analysis of various data in the literature, it can be concluded that in patients prepared for IVF or ICSI, polyps, independent from their size and amount, do not have a negative effect on the pregnancy rates; nevertheless it seems that the increase in polyp size has a positive correlation with the rise in the rate of early pregnancy loses.

In conclusion, various approaches exist for the management of patients with endometrial polyps undergoing ICSI cycles. In the present study the authors applied hysteroscopic polypectomy before the ICSI cycle and observed that in these patients the reproductive results did not depend on the diameter of the endometrial polyps. However more randomized controlled trials are needed which will further enlighten the influence of the hysteroscopic polypectomy before the ICSI cycle on pregnancy outcomes.

References

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Cervical cytology ASCUS patients with HPV detection and clinical value

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2 Department of Gynaecology, the Affiliated Cancer Hospital of Xinjiang Medical University, Urumqi
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Summary
Objective: Patients whose cervical cytological exams produced a result of atypical squamous cells of undetermined significance (ASCUS) were asked to undergo human papillomavirus (HPV DNA) genotyping detection to assess the role of HPV infection in ASCUS.

Materials and Methods: This study included 1,219 patients with ASCUS that were randomly divided into two groups. The first group contained 618 patients. These participants underwent colposcopy with cervical biopsy. The remaining 601 underwent colposcopy with biopsy with HPV DNA detection. Results: Out of the 56,000 patients with ASCUS who underwent ThinPrep cytology test (TCT) detection in the authors’ hospitals’ gynecological outpatient clinics, 1,604 were diagnosed with ASCUS (2.86%). Among the 1,219 patients with ASCUS, the rate of detection of cervical intraepithelial neoplasia (CIN) and cancerization was 22.89% (279/1,219). Among the 601 patients who underwent HPV testing, 182 were positive for high-risk HPV (30.28%). Among HPV-positive samples, the most common high-risk types were HPV16, and HPV58. The most common low-risk types were HPV6 and HPV11. The rate of detection among high-risk patients who were positive for HPV and cervical carcinoma with intraepithelial neoplasia was 70.88% (129/182). The rate of detection for HPV-negative patients with cervical cancer with intraepithelial neoplasia was 11.55% (47/407). The rate of detection of high-risk HPV was higher than among patients who had not undergone HPV detection and among patients who were negative for HPV (p < 0.05).

Conclusion: The results of cervical cytological examination showed that the manner of progression from inflammation to cervical intraepithelial neoplasia (CIN) is more probable than cancer. HPV DNA examination is an effective means of categorizing and managing ASCUS.

Key words: ASCUS; HPV genotyping detective; Colposcopy; Cervical intraepithelial neoplasia (CIN).

Introduction
Cervical cancer ranks second in cancer mortality in women worldwide. Medical intervention of human malignancies [1, 2] is the only way to decrease morbidity and mortality and screening and proper treatment of cervical intraepithelial neoplasia (CIN) is the key to effective prevention and treatment of cervical cancer. Cervical cytology method is considered to be one of the most successful methods of early cancer screening. However, vulnerable subjective factors, especially for cervical cytology results, are not clear for atypical squamous cells of undetermined significance (ASCUS). The rate of detection of HPV infection in ASCUS is higher than among patients who had not undergone HPV detection and among patients who were negative for HPV (p < 0.05).

Materials and Methods
From October 2007 to October 2012, a total of 56,000 patients came to a gynecology clinic for treatment. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Cen-
Chinese women (70.64%), 439 ethnic Uyghur women (27.36%), and 32 women of other ethnicities (2.00%). They ranged in age from 18 to 65 years. The most numerous age groups were 31-35 and 36-40 years (Table 1).

**ASCUS histopathological diagnosis**

Among the 1,219 women with ASCUS, all underwent cervical biopsy. The rate of detection of cervical abnormalities was 22.89% (298/1,219); 938 patients had chronic cervicitis CIN I: 32, CIN II: 73 or CIN III: 62, and 14 cervical cancer, accounting for 76.95%, 10.35%, 5.82%, 4.33%, and 1.06% of the total, respectively. Among the 250 Uyghur patients with ASCUS, 176 had chronic cervicitis, CIN I: 32, CIN II: 2 or CIN III: 20, and four had cervical cancer, accounting for 69.12%, 12.80%, 8.40%, 8.00%, and 1.60%, respectively. Among the remaining 22 patients, nine had chronic cervicitis, CIN I: 2, CIN II: 2 or CIN III: 1, or no cervical cancer, accounting for 77.27%, 9.09%, 9.09%, 4.55%, and 0.00%, respectively. No significant differences were found among these three groups (Table 2).

**HPV DNA test**

The HPV DNA detection results of the 601 patients with ASCUS who had HPV testing were as follows: 194 (32%) patients were positive for HPV and 407 (43%) patients were negative for HPV. Among the 450 ethnic Han Chinese participants, the positive rate was 28.89% (130/450). Among the 141 ethnic Uyghurs, the positive rate was 43.26% (61/141) and among the ten individuals of other ethnicities, the positive rate was 30.00%.

Of the HPV-positive specimens, 182 (93%) showed high-risk subtypes, most of these were 16, 18, and 58. Two patients showed a low-risk hypotype, giving a detection rate of 6.96% (12/194). Most of these were HPV 6, 11, and 42.

### Table 1. — Age distribution of 1,604 patients with ASCUS.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Numbers (n)</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>24</td>
<td>1.5</td>
</tr>
<tr>
<td>21-25</td>
<td>180</td>
<td>11.25</td>
</tr>
<tr>
<td>26-30</td>
<td>280</td>
<td>17.46</td>
</tr>
<tr>
<td>31-35</td>
<td>320</td>
<td>19.95</td>
</tr>
<tr>
<td>36-40</td>
<td>380</td>
<td>23.79</td>
</tr>
<tr>
<td>41-45</td>
<td>220</td>
<td>13.72</td>
</tr>
<tr>
<td>46-50</td>
<td>150</td>
<td>9.35</td>
</tr>
<tr>
<td>≥ 51</td>
<td>50</td>
<td>3.12</td>
</tr>
</tbody>
</table>

### Table 2. — Comparison of patients with ASCUS cervical histopathology results between various nationalities, n (%).

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Quantity</th>
<th>Chronic cervicitis</th>
<th>CIN I</th>
<th>CIN II</th>
<th>CIN III</th>
<th>Cervical cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han nationality</td>
<td>947</td>
<td>748 (78.99)</td>
<td>98 (10.35)</td>
<td>50 (5.32)</td>
<td>41 (4.33)</td>
<td>10 (1.06)</td>
</tr>
<tr>
<td>Uyghur nationality</td>
<td>250</td>
<td>173 (69.20)</td>
<td>32 (12.80)</td>
<td>31 (8.40)</td>
<td>20 (8.00)</td>
<td>4 (1.60)</td>
</tr>
<tr>
<td>Other nationality</td>
<td>22</td>
<td>9 (77.27)</td>
<td>2 (9.09)</td>
<td>2 (9.09)</td>
<td>1 (4.55)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>1219</td>
<td>938 (76.95)</td>
<td>132 (10.83)</td>
<td>73 (5.99)</td>
<td>62 (5.09)</td>
<td>14 (1.15)</td>
</tr>
</tbody>
</table>
The most common high-risk hypotype was HPV16, which had a detection rate of 46.91% (91/194). The second most common hypotype was HPV58, and it had a detection rate of 20.10% (39/194). HPV18 had a detection rate of 12.37% (24/194). Three patients had unitary type HPV, two had both HPV16 and HPV33, one had both HPV16 and HPV58, one had both HPV33 and HPV39, and one had HPV16, HPV31, and HPV52.

Women of various ages with ASCUS were examined. HPV examination produced positive diagnoses in 182 patients, giving a rate of 30.28% ($p < 0.05$). Among women under 20 years of age, the HPV infection rate was 0, for those aged 41-45 and 45-50, it was higher (Table 3).

Relationship between high-risk HPV infection and pathological diagnosis

The comparison of between high-risk HPV detection group and HPV detection group undone pathological histology: 601 patients underwent cervical biopsy with colposcopy but did not undergo HPV detection. Pathological results indicated that 510 of patients who underwent HPV detection had chronic cervicitis CIN I: 51, CIN II: 27, or CIN: III and that six of them had cervical cancer, accounting for 69.88%, 14.14%, 8.15%, 6.49%, and 1.33% of the total, respectively. After examination, the two groups showed no significance differences (Table 4).

Negative pathological histology in the high-risk-HPV-positive group: If the histopathological results are assumed to be correct, then the rate of detection of high-risk-HPV-positive cervical cancer with intraepithelial neoplasia was 70.88% (129/182), and the rate of detection of low-risk-HPV-positive cervical cancer and intraepithelial neoplasia was 41.67% (5/12), and the rate of detection of patients with intraepithelial neoplasia but not with cervical cancer was 11.55% (47/407). There were statistically significant differences among these three groups ($p < 0.05$) (Table 5). This indicates that, among patients with ASCUS, the rate of detection of high-risk-HPV-positive cervical cancer, intraepithelial neoplasia and cervical cancer was higher than among other individuals.

Histopathology of high-risk-HPV-positive and HPV-negative individuals

If the histopathological results are assumed to be correct, then the rate of detection of high-risk-HPV-positive cervical cancer with intraepithelial neoplasia was 70.88% (129/182), and the rate of detection of cervical cancer with intraepithelial neoplasia but without HPV was 17.48% (108/618). These two groups showed statistically significant differences ($p < 0.05$) (Table 6). This indicates that,
among patients with ASCUS, the rate of detection of high-risk-HPV-positive patients with cervical lesions was higher.

Discussion

Cervical cytology screening is key in controlling and preventing cervical cancer. ASCUS is the most common cervical cytology. The diagnosis is primarily exclusive, but can hint at the presence of dangerous lesions. ASCUS may have a relationship with inflammatory stimuli, poor producers, IUD insertion, old age, gender reassignment surgery, and genital renovation or beautification surgery. It is may also have a relationship with CIN and carcinoma of the cervix [13].

This study involved 1,219 patients with ASCUS. Patients underwent cervical biopsy under colposcopic guidance. Histopathological results showed the rate of detection of CIN and cervical cancer was 22.89% among 938 individuals with chronic cervicitis CIN I: 132, CIN II: 73, CIN III: 62. There were 14 patients with total cervical cancer patients, accounting for 76.95%, 10.83%, 5.99%, 5.09%, 1.15% of the total, respectively. These data suggest that the results of colposcopic biopsy can be chronic cervicitis or CIN. Results can also be early invasive carcinoma if the cytological report is ASCUS. A way of triaging and managing patients with ASCUS would increase the rate of detection of cervical lesions and reduce the number of unnecessary cervical biopsies. This would in turn conserve medical resource and reduce costs.

HPV infection is a basic contributing factor to the development of cervical cancer. It is divided into high-risk HPV and low-risk HPV according to their carcinogenicity. Persistent infection with high-risk HPV is considered a primary cause of cervical cancer and precancerous lesions [14]. The results of the present study showed a significant difference between the rate of high-risk HPV among women of different ages with ASCUS. The rate of high-risk HPV infection was found to be age-related. The rate of HPV infection was considered 0 if the woman was younger than 20 years, but it was considered high if the woman was between the ages of 41 and 50, indicating that high-risk HPA mainly centers on women over 40. It coincides with the peak incidence of cervical cancer among women in their 50s. Most HPV-positive specimens collected in the present work were high-risk HPV16, giving a detection rate of 46.91% (91/194). HPV16-positive patients over 40 years of age with ASCUS should be alerted to the possibility of cervical intraepithelial neoplasia and cervical cancer. It has been reported that around 20% of cytological diagnoses are for ASCUS and patients who also have high-risk HPV infections showed the highest rates of cervical intraepithelial neoplasia and cervical cancer [15]. The data show the detection rate for high-risk HPV-positive cervical cancer with intraepithelial neoplasia to be 70.88% (129/182). However, the rate for low-risk HPV-negative cervical cancer with intraepithelial neoplasia was 11.55% (47/407). These groups showed a statistically significant difference (p < 0.05). It remains to be discovered whether the detection rate for high-risk HPV-positive cervical cancer with intraepithelial neoplasia is higher than that of HPV-negative patients with ASCUS. This indicates the importance of HPV testing for ASCUS patients and that TCT results may be better for screening cervical intraepithelial neoplasia and cervical cancer compared to other methods.

Currently, for cases of ASCUS that may have cervical lesions, there are three ways of performing further screening: repeating the cytological examination within three to six months, high-risk HPV detection, and direct colposcopic biopsy [16]. The sensitivity of cytological examination is limited [17, 18]. Approximately half of abnormal patients are ASCUS at the first screening. If cytological examination is considered way of triaging ASCUS patients, then it cannot be considered suitable for the elimination of precancerous cervical lesions. Solomon et al. [19] tested 3,488 patients with ASCUS by using all three methods of colposcopy, immediate examination, repeat cytological examination, and high-risk HPV detection for the purpose of examining and comparing these methods. Results showed that HPV detection was the best choice for triage management of ASCUS and that it was more sensitive and specific in the diagnosis of both CIN III and cervical cancer than other methods [19].

In short, cervical cytological diagnosis of ASCUS patients should be given more attention, and HPV DNA detection is a more reasonable and meaningful triage
approach for patients with ASCUS than cervical cytology testing. It can reduce the number of unnecessary colposcopies performed and therefore lighten the economic burden on patients, conserve medical resources, and reduce the rate of false negative diagnoses of cervical lesions.

References


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Revisiting serum beta-human chorionic gonadotropin concentrations as a predictor for dizygotic twinning after in vitro fertilization

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² Department of Obstetrics and Gynecology, ¹Department of Histology and Embryology, Faculty of Medicine, Hacettepe University, Ankara (Turkey)

Summary

Purpose of investigation: To determine a cut-off value for beta-human chorionic gonadotropin (β-hCG) concentrations to predict dizygotic twinning after in vitro fertilization (IVF) and double embryo transfer (DET). Materials and Methods: This retrospective cohort study included 233 women who conceived after DET at IVF center, Hacettepe University Faculty of Medicine. Patients with serum β-hCG concentration ≥ 25 IU/l assayed on day 14 after oocyte retrieval were included into the study. Results: Lower serum β-hCG concentrations were observed in non-viable pregnancy when compared to their viable counterparts. In addition, twins exhibited higher β-hCG concentrations than singletons did. Receiver operator characteristic (ROC) curve analysis showed a significant relationship between serum β-hCG concentrations and the occurrence of twin pregnancy (area under the curve = 0.85, 95% confidence interval = 0.79–0.91, p < 0.001). For twin pregnancy, when β-hCG ≥ 175 IU/l, sensitivity was 77.3%, specificity was 80.0%, positive predictive value (PPV) was 48.2%, and negative predictive value (NPV) was 93.8%. Conclusion: β-hCG ≥ 175 IU/l might be used as a new cut-off value for early prediction of viable dizygotic twins following IVF-DET treatment cycles.

Key words: Beta-human chorionic gonadotropin; In vitro fertilization; Double embryo transfer; Dizygotic twinning.

Introduction

Markers that have been sought to distinguish between viable and non-viable pregnancies before live intrauterine pregnancy can be verified by transvaginal ultrasonography. A single determination of serum human chorionic gonadotropin (hCG) concentration has been found to be predictive of pregnancy outcomes in several different studies [1-7]. These studies have postulated that hCG level is a reliable and highly predictive tool for various pregnancy outcomes. Few researchers have also investigated the predictive value of hCG for discriminating multiple pregnancies. However, they either used intact hCG instead of β-hCG or included single to four ET cycles in their analysis when interpreting the multiplicity [8, 9]. As worldwide single embryo transfer (SET) policy is now being seriously considered, transfer of three or more embryos has subsequently been restricted to double embryo transfer (DET) which should also be based on prognostic indicators of the patient. Therefore, the results of previous studies might not be suitable for the present authors’ current practice with DET, since the measures of β-hCG validity (sensitivity, specificity, positive and negative predictive values) would change by the prevalence of twinning in non-DET cycles.

The present authors’ primary objective was to use day-14 (after oocyte retrieval) β-hCG concentrations, to predict viable twin pregnancy following DET. Their secondary aim was to investigate in vitro fertilization (IVF) treatment characteristics in relation to dizygotic twinning.

Materials and Methods

The records of the subjects who conceived following assisted reproductive technology at the IVF unit of Hacettepe University Hospital, from January 2005 to July 2014, were analyzed for this study. A total of 236 ET cycles fulfilled the present inclusion criteria which were: (1) cycles arising from DET, (2) serum β-hCG concentration ≥ 25 IU/l assayed on day 14 after oocyte retrieval, and (3) data regarding the outcome was available. Furthermore, triplet pregnancies with mono-chorionic component (n=2) and fetuses with major fetal anomalies that resulted in termination of pregnancy (n=1) were excluded from the study.

This study was exempt from institutional review board review because of its retrospective, non-interventional nature: no patients were contacted and no identifying patient information was used for purposes of this study. Patients underwent IVF according to standard stimulation protocols, which involved pituitary downregulation with gonadotropin-releasing hormone (GnRH) agonist administered in the mid-luteal phase of the prior cycle (long protocol) or diluted GnRH agonist on days 2-4 of the cycle (micro-
dose protocol). Alternatively, GnRH antagonist short protocols started on the fifth day of stimulation. Controlled ovarian stimulation was achieved with hCG and/or recombinant follicle-stimulating hormone. The response to stimulation was monitored with serum E2 and transvaginal ultrasound. HCG was administered to stimulate the final stages of follicular development when follicles reached maturity, defined by two to four leading follicles reaching > 18 mm. Transvaginal follicle aspiration was performed 36 hours after hCG administration.

Embryos were transferred to the uterus either three days (cleavage stage) or five days (blastocyst stage) after oocyte retrieval. Embryo quality was assessed on the same day as transfer. The cleavage stage embryos were scored based on cell number and degree of fragmentation according to grading system of Hardarson (2001) [10]. The embryos with appropriate developmental stage, < 20% fragments and mild degree of uneven-sized blastomeres (grade I, grades II A and II B, and grade II AB) constituted the day-3 embryos suitable for transfer. In case of extended culture, all blastocyst stage embryos were evaluated using the grading system of Gardner and Schoolcraft (1994). The blastocysts were graded according to degree of expansion and quality of inner cell mass and trophoectoderm [11]. The total number of embryos suitable for ET (either at cleavage or blastocyst stage) was calculated for each patient.

On day 14 of oocyte retrieval, regardless of the day of ET, each patient had her β-hCG level assessed. The β-hCG levels were measured by chemiluminescence immunoassay technique on an autoanalyzer. The inter-assay coefficient of variation was determined as 4.5% which was obtained from the “measurement uncertainty” results of the present laboratory.

Pregnancy was defined by serially increasing serum β-hCG titers to at least 25 IU/l within 14 days after oocyte retrieval. All of the patients underwent transvaginal ultrasound at five to six weeks’ gestation or when β-hCG exceeded 2,000 IU/L, in order to determine the location and number of pregnancies. Biochemical pregnancy was defined as transient pregnancies that spontaneously resolved before sonographic confirmation. Observation of fetal cardiac activity was performed at six to seven weeks of gestation. The non-viable pregnancy included biochemical pregnancy, ectopic pregnancy, first-trimester abortions (an-embryonic pregnancy, missed abortion, and spontaneous abortion prior to 12 weeks of gestation in both singleton and twin pregnancies) and second trimester abortions (in both singleton and twin pregnancies). Viable pregnancy was defined as one resulting in delivery of at least one live fetus at ≥ 23 weeks of gestation.

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0. After determining whether the variables met the normality and homoscedasticity assumptions, non-parametric analyses were performed. Kruskal–Wallis tests were conducted to explore the impact of pregnancy outcome on serum β-hCG concentrations, patient, and IVF treatment characteristics. Mann-Whitney U tests were conducted to analyze continuous and discrete ordinal variables, and nominal data were analyzed with χ² tests. All Mann–Whitney U and χ² tests were two-tailed, and Bonferroni’s correction was used to adjust for multiple comparisons unless otherwise stated. Bonferroni’s correction involves dividing the alpha level of 0.05 by the number of tests that the researcher intends to perform and using the revised alpha level as the criterion for determining significance. In the present analyses, this process resulted in a stricter alpha level of 0.05/3 = 0.0166. Receiver operator characteristic (ROC) curve analysis was used to assess the predictive value of β-hCG and maternal age on occurrence of twin births. The percentage for area under the curve (AUC) and confidence intervals (CI 95%) were generated for the ROC curve. Discrimination threshold was chosen on the basis of optimal sensitivity and specificity. Diagnostic indices (sensitivity and specificity) and positive (PPV) and negative predictive values (NPV) were calculated. Multiple logistic regression analysis was used to investigate the relationship between occurrence of twin pregnancy and various explanatory variables. A p value < 0.05 was considered statistically significant.

Results

A total of 233 consecutive cycles (among 224 women) with DET were subject to analysis. The mean age at treatment was 33.8 (range 25–50) years. Viable pregnancies represented 61.8% and non-viable pregnancies 38.2% of all pregnancies, respectively. Table 1 shows the pregnancy outcomes of the studied patients.

A Kruskal–Wallis analysis was conducted to explore the impact of pregnancy outcome on serum β-hCG levels. There was a statistically significant difference in β-hCG

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-viable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy loss (n=89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>49</td>
<td>21.0</td>
</tr>
<tr>
<td>Spontaneous abortion (first trimester)</td>
<td>33</td>
<td>14.2</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>Spontaneous abortion (second trimester)</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>Viable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton (n=100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivered singleton</td>
<td>96</td>
<td>41.2</td>
</tr>
<tr>
<td>Spontaneously reduced twin</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>Twin pregnancy (n=44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivered Twin</td>
<td>44</td>
<td>18.9</td>
</tr>
</tbody>
</table>

| Total number of pregnancies | 233 | 100 |

Figure 1. — The median (minimum-maximum) β-hCG concentrations for each pregnancy outcome are presented. *significant, p < 0.001.
Revisiting serum beta-human chorionic gonadotropin concentrations as a predictor for dizygotic twinning after in vitro fertilization

concentrations between the groups: non-viable (pregnancy loss), singleton, and twin pregnancies ($p < 0.001$). A Mann–Whitney U test with Bonferroni’s correction was used to adjust for multiple comparisons between these groups, and the results indicated that the median score for each group was significantly different from the other ($p < 0.001$ for each). Lower serum β-hCG concentrations were observed in pregnancies that resulted in pregnancy loss than the singletons. In addition, twin pregnancies had higher β-hCG concentrations than the singletons did (Figure 1).

In terms of the impact of pregnancy outcome on the patient and IVF characteristics, there were no significant differences in body mass index (BMI), duration of infertility, number of previous unsuccessful attempts at IVF and type of infertility between the pregnancy outcomes ($p > 0.05$) (Table 2). Similarly, proportion of MII oocytes, proportion of oocytes fertilized, frozen-thaw cycles, day of ET, highest number of cells, and mean morphology score per ET were found to be comparable between the groups ($p > 0.05$). However, there were statistically significant differences in maternal age ($p = 0.001$), total gonadotropin dose ($p = 0.012$), number of retrieved oocytes ($p = 0.001$), MII oocytes ($p = 0.001$), oocytes fertilized ($p = 0.006$), and embryos suitable for transfer ($p = 0.002$). Subsequently, a

**Table 2.** — Patient and IVF cycle characteristics between the pregnancy outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Non-viable (n=89)</th>
<th>Singletons (n=100)</th>
<th>Twins (n=44)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>35.2 ± 5.9a</td>
<td>34.0 ± 5.7a</td>
<td>30.6 ± 5.2b</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of infertility (months)</td>
<td>81.9 ± 63.3</td>
<td>84.2 ± 70.2</td>
<td>75.9 ± 44.4</td>
<td>Ns</td>
</tr>
<tr>
<td>Previous unsuccessful attempts at IVF</td>
<td>25 (28.1%)</td>
<td>29 (29.0%)</td>
<td>13 (29.5%)</td>
<td>Ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 3.7</td>
<td>25.3 ± 3.9</td>
<td>24.8 ± 3.7</td>
<td>Ns</td>
</tr>
<tr>
<td>Male factor</td>
<td>34 (38.2%)</td>
<td>49 (49.0%)</td>
<td>23 (52.3%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Unexplained infertility</td>
<td>26 (29.2%)</td>
<td>29 (29.0%)</td>
<td>8 (18.2%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Anovulation</td>
<td>14 (15.7%)</td>
<td>9 (9.0%)</td>
<td>10 (22.7%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>11 (12.4%)</td>
<td>10 (10.0%)</td>
<td>2 (4.5%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Poor ovarian reserve</td>
<td>14 (15.7%)</td>
<td>9 (9.0%)</td>
<td>10 (22.7%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.3 ± 1.9</td>
<td>9.7 ± 1.8</td>
<td>9.0 ± 1.2</td>
<td>Ns</td>
</tr>
<tr>
<td>Total gonadotropin dose (IU)</td>
<td>2974 ± 1425a</td>
<td>2716 ± 1215ab</td>
<td>2170 ± 720b</td>
<td>0.012</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>7.9 ± 5.5a</td>
<td>9.9 ± 6.5a</td>
<td>13.0 ± 7.9b</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of MII oocytes</td>
<td>6.6 ± 4.4a</td>
<td>8.3 ± 5.7a</td>
<td>10.7 ± 6.5b</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of oocytes fertilized</td>
<td>87.1 ± 12.7</td>
<td>84.5 ± 16.9</td>
<td>84.2 ± 14.4</td>
<td>Ns</td>
</tr>
<tr>
<td>Oocytes fertilized (%)</td>
<td>5.4 ± 3.6a</td>
<td>6.4 ± 4.4a</td>
<td>8.5 ± 5.8b</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of embryos suitable for transfer</td>
<td>82.6 ± 16.4</td>
<td>79.7 ± 18.6</td>
<td>78.3 ± 18.3</td>
<td>Ns</td>
</tr>
<tr>
<td>Frozen-thawed cycles</td>
<td>22 (24.7)</td>
<td>14 (14.0)</td>
<td>6 (13.6)</td>
<td>Ns</td>
</tr>
<tr>
<td>Day of ET</td>
<td>Day 3 80 (89.9%)</td>
<td>84 (84.0%)</td>
<td>38 (86.4%)</td>
<td>Ns</td>
</tr>
<tr>
<td></td>
<td>Day 5 9 (10.1%)</td>
<td>16 (16.0%)</td>
<td>6 (13.6%)</td>
<td>Ns</td>
</tr>
<tr>
<td>Number of cells of the best embryo</td>
<td>7.8 ± 1.8</td>
<td>8.1 ± 1.6</td>
<td>8.4 ± 1.3</td>
<td>Ns</td>
</tr>
<tr>
<td>Mean Morphology score per ET</td>
<td>Day 3 1.9 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>Ns</td>
</tr>
<tr>
<td></td>
<td>Day 5 2.5 ± 1.1</td>
<td>3.0 ± 0.6</td>
<td>2.7 ± 1.0</td>
<td>Ns</td>
</tr>
</tbody>
</table>

IVF: in vitro fertilization; BMI: body mass index; MII: metaphase 2; ET: embryo transfer; Ns: non-significant.

Values across a row with different superscripts (a–b) indicate significant differences between pregnancy outcome categories ($p < 0.05$), and values across an individual row with matching superscripts (a–b) indicate no significant differences between pregnancy outcome categories.

Data are presented as mean ± standard deviation or n (%).
Multivariate analysis by logistic regression was performed that included β-hCG and maternal age. As shown in Table 3, the results indicated that serum β-hCG ≥ 175 IU/l (β = 13.33, p < 0.001), and maternal age ≥ 35 years (β = 0.28, p = 0.003) were still significant contributing factors to occurrence of viable twin pregnancy.

**Table 3. —Relationships between serum β-hCG and maternal age for occurrence of twin pregnancy analyzed by multivariate logistic regression.**

<table>
<thead>
<tr>
<th>Serum β-hCG ≥ 175 IU/l</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.33</td>
<td>5.92–30.02</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Maternal age ≥ 35 years 0.28 0.12–0.64 0.003

OR: odds ratio; CI: confidence interval.

A multivariate analysis by logistic regression was performed that included β-hCG and maternal age. As shown in Table 3, the results indicated that serum β-hCG ≥ 175 IU/l (β = 13.33, p < 0.001), and maternal age ≥ 35 years (β = 0.28, p = 0.003) were still significant contributing factors to occurrence of viable twin pregnancy.

**Discussion**

The maternal characteristics and IVF cycle parameters were found to be comparable, apart from the maternal age, between the singleton and twin pregnancies. The authors found that the occurrence of twin pregnancy decreases by 3.6-fold in women ≥ 35 years. Thus, the woman’s age affects occurrence of twin pregnancy, even when DET is specified. Similar to the present findings, it was shown that the female age was negatively correlated with the occurrence of multiple pregnancy [12-16]. Few studies have also reported that developmental stage and morphology score might be predictors of multiple pregnancy [14, 17]. Although number of retrieved oocytes / MII oocytes / fertilized oocytes / embryos suitable for transfer seems to be higher and developmental stage / morphological scores superior in twin pregnancies when compared with singletons, the differences did not reach statistical significance. In fact, the strict embryo selection criteria that the present authors used, might lead to transfer of only good quality embryos with appropriate stage of development. A larger sample size, however, might allow increasing the significance level of these findings, since the confidence of these results is likely to increase.

The present results indicated that there was an increasing β-hCG trend from non-viable toward singleton, and twin pregnancies. Similarly, in the series of Poikkeus et al. (included single to three ET cycles), intact hCG concentrations were about four-fold higher in viable pregnancies than in non-viable ones [8]. In another study, Bjercke et al. evaluated 417 IVF pregnancies and concluded that when intact hCG value was > 55 IU/l, the chance of having a vital pregnancy on day 12 after IVF-ET was 90% [1]. In the present study, however, the AUC values of β-hCG obtained from ROC curve analyses (data not shown) were found to be much lower for discriminating viable than twin pregnancies (0.77 vs. 0.85). It was likely due to a more prominent overlap in β-hCG ranges between non-viable and singleton pregnancies. Therefore, β-hCG might be a more efficient marker for discriminating twin than viable pregnancies after IVF-DET treatment cycles. In addition, Kathiresan et al. performed a study in order to assess the degree to which maternal characteristics and cycle parameters were predictive of higher β-hCG levels. They measured serum β-hCG concentrations on day-15 after oocyte fertilization and proposed that β-hCG concentration > 250 IU/l in cycles involving day-3 ET might be suggestive of multiple pregnancy [9]. However, the probability of predicting multiple pregnancy with this β-hCG value was found to be low (18%). On the other hand, it was observed in the present study that when β-hCG ≥ 175, sensitivity was 77.3%, specificity was 80.0%, PPV was 48.2%, and NPV was 93.8%.

A multivariate analysis by logistic regression was performed that included β-hCG and maternal age. As shown in Table 3, the results indicated that serum β-hCG ≥ 175 IU/l (β = 13.33, p < 0.001), and maternal age ≥ 35 years (β = 0.28, p = 0.003) were still significant contributing factors to occurrence of viable twin pregnancy.
hCG ≥ 5 IU/l with a sensitivity of 97%, specificity of 36%, PPV of 39%, and NPV of 97%. Finally, it is evident that the current literature lacks homogeneity when dealing with the markers measured (intact hCG vs. β-hCG), time of measurement, or characteristics of the populations studied.

The limitations of this study are mainly due to its retrospective nature, which prevented the authors from controlling for potential bias. Thus, further prospective studies investigating β-hCG concentrations in a subgroup of patients with only DET are still warranted. Moreover, each institution should analyze their own data to determine β-hCG cutoffs based on their own experience, as β-hCG concentrations might vary depending on the type of assay used.

Conclusion

β-hCG might be a useful marker for discriminating twin pregnancies following IVF-DET treatment cycles. The present data also demonstrates that younger women’s age is associated with higher the probability of achieving a twin pregnancy. As the individualized transfer policy is now being seriously considered, the present data might provide clues for dizygotic twinning when IVF-DET is still indicated.

References


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Uterine arteriovenous fistula after perforation during the placement of an intrauterine device – Minimally invasive treatment using uterine artery embolization

W. Kondo1, M. Tessmann Zomer1, F.L. Erzinger2

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2 Department of Vascular Surgery Instituto da Circulação, Curitiba, Paraná; Marcelino Champagnat Hospital, Curitiba, Paraná (Brazil)

Summary
Uterine arteriovenous fistula (AVF) is a rare, but potentially life-threatening condition. Acquired fistulae may occur as a result of trauma or instrumentation, endometrial carcinoma, gestational trophoblastic disease, and intrauterine devices (IUDs). Herein the authors present the case of a 33-year-old woman with a uterine AVF developing after uterine perforation during the placement of a levonorgestrel IUD. The fistula was diagnosed using color Doppler ultrasonography and angiography and the treatment was conducted by minimally invasive approach using uterine artery embolization.

Key words: Uterine arteriovenous malformation; Arteriovenous fistula; Intrauterine device; Uterine artery embolization.

Introduction
Uterine arteriovenous malformation (AVM) is considered a rare condition; however, it may lead to severe life-threatening situations as a result of profuse or irregular bleeding from the abnormal communication between artery and vein [1]. It may be classified as congenital and acquired [2]: (a) the former develops from failures in the embryological differentiation, leading to multiple abnormal vascular connections [3] and (b) the latter is typically a result of trauma or instrumentation, although it has also been associated with endometrial carcinoma, gestational trophoblastic disease, diethylstilbestrol exposure, and intrauterine devices (IUDs) [4-7].

Historically, the diagnosis of uterine AVM was only possible through exploration per laparotomy or upon pathological examination of the uterine specimen after hysterectomy had been performed. Subsequently, angiography became the gold standard for diagnosis. More recently, color Doppler ultrasonography has been suggested for obtaining a reliable diagnosis [8]. The ultrasonographic diagnosis of AVM is based on the presence of hypoechoic tortuous spaces in the myometrium demonstrating vascular flow as evidenced by color Doppler [1, 9, 10]. MRI and CT scan may be useful to assist the diagnosis in cases where angiography was not able to define adjacent organ involvement [11-13].

Traditionally, hysterectomy was the treatment of choice [1]. Other treatment options include ligation of major vessels, ligation of the iliac artery [14], ligation of AVM vessels [15], and ligation of uterine artery [16]. Recently, uterine artery embolization has been successfully used in more than 50% of the patients [10].

In this paper, the authors report one case of iatrogenic acquired uterine arteriovenous fistula (AVF) after uterine perforation during the placement of an IUD treated by means of uterine artery embolization. The paper was approved by the local Institutional Review Board.

Case Report
A 33-year-old woman, G3 A3, came to the present authors' service with sonographic findings indicating a uterine arteriovenous fistula. Four months prior, she had a uterine perforation during the placement of a levonorgestrel IUD. She presented persistent vaginal bleeding for the following four days and transvaginal ultrasound showed a normal-sized uterus with the IUD penetrating the myometrium at the uterine fundus in the right side. There was a pelvic hematoma measuring 37×24×36 mm at the distal end of the IUD and a moderate amount of free liquid in the pelvic cavity. She underwent a laparoscopic procedure for IUD removal and the uterine defect was closed using zero 25 poliglecaprone suture (Figure 1). She was discharged 12 hours after the procedure.

In the postoperative follow-up, the patient developed amenorrhea for the following four months. Transvaginal gray-scale ultrasound revealed cystic hypoechoic areas at the uterine wall;
Figure 1. — A: During laparoscopy, the levonorgestrel IUD was found inside the pelvic cavity in the middle of a pelvic hematoma. (B) The hematoma at the uterine fundus. C to F: Suturing the place of perforation at the uterine fundus. G to I: Aspiration of the clots and blood from the pelvic and abdominal cavities and final aspect after the procedure.

Figure 2. — A and B: Transvaginal ultrasound with color Doppler showing hypervascular areas with turbulent flow at the uterine fundus. C: Pelvic MRI showing the AVF at the uterine fundus.

Figure 3. — A: Pre-embolization CT angiography demonstrating the hypervascularization at the left side of the uterus and the uterine fundus, originating from the left uterine artery. (B) Hypervascularization reduction after embolization.
color Doppler showed turbulent flow in the myometrium at the uterine fundus, characteristic features of an AVF (Figures 2A and 2B). Serum level of beta-HCG was below 25 mIU/ml, thus excluding gestational trophoblastic disease.

Pelvic MRI showed periuterine vascular structures affecting the myometrium at the uterine fundus, originating from the superficial layer until the deep layer of the myometrium with loss of the junctional zone (Figure 2C).

CT angiography showed prominence of the uterine arteries, especially at the left side, associated with the presence of vascular structures with significant rise of the arterial phase at the left parametrium and within the myometrium, particularly at the uterine fundus (Figure 3A). These findings were consistent with the possibility of an AVF.

The right femoral artery was punctured under local anesthesia and sedation. After selective catheterization of the left uterine artery, an angiography was performed and confirmed the previous findings of the CT angiography (Figure 4A). Then, a microcatheter was placed inside the artery and the embolization of the left uterine artery was successfully performed using a non-adhesive liquid embolic agent (Figure 4A). The total time of the procedure was 85 minutes.

The patient was discharged on the second day after the procedure using oral analgesia with paracetamol. She was suggested to return to normal activities in 7 days. CT angiography was repeated six months after the embolization confirming the success of the procedure (Figure 3B).

Discussion

Uterine perforation during the placement of a levonorgestrel IUD occurs in 0.11% of patients and may be a potentially serious complication of its use [17]. In most cases it may be retrieved from the pelvic/abdominal cavity by laparoscopy but some patients require conversion to laparotomy to repair perforation of adjacent organs [18].

The development a uterine arteriovenous shunt after removal of an embedded IUD has already been described in the medical literature [19]. To the best of the present authors' knowledge, this is the first case reported in the literature of development of uterine AVF after uterine perforation during the placement of levonorgestrel IUD that was removed from the pelvic cavity by laparoscopy.

In the present case, the uterus seemed normal during the laparoscopic retrieval of the levonorgestrel IUD. Although the exact place of the perforation was sutured during the laparoscopic procedure, the AVF subsequently developed at the uterine fundus.

In the majority of the cases, uterine AVFs present as a life-threatening hemorrhage that does not respond to medical treatment [7, 10]. The present patient was asymptomatic, and the uterine AVF was diagnosed during transvaginal ultrasound once she presented amenorrhea, due to the formation of uterine synechiae after the laparoscopic retrieval of the levonorgestrel IUD.

Angiography is considered the gold standard for the definitive diagnosis of AVFs and its goals are to define the vascular anatomy, assess the extent of the vascular fistula, and identify the feeding vessels [1, 20, 21].

Since the first uterine artery embolization procedure reported by Forssman et al. [22], there have been numerous reports on the treatment of uterine AVM by this approach [9, 10, 23-25]. Nowadays, uterine artery embolization is the most common treatment option in 59% of the patients with uterine AVM [10]. Several embolic agents may be used: gelfoam, polyvinyl alcohol, steel coils, tris-acryl-gelatin, isobutyl-2-cyanoacrylate, acrylic polyamide, detachable balloons, thrombin, ethanol, and histoacryl [10, 26].

In the present patient, uterine artery embolization was conducted using a non-adhesive liquid embolic agent comprised of ethylene vinyl alcohol copolymer dissolved in dimethyl sulfoxide, and suspended micronized tantalum powder to provide contrast for visualization under fluoroscopy. Onyx® 18 It is safer than other liquid agents because it allows for a slow and controlled injection due to its high viscosity and longer time for polymerization.

Minor complications have been reported after uterine artery embolization, and these relate to minor pelvic pain and postoperative fever [9, 20]. Recurrence after embolization occurred...
in 17% of the cases in the review from Peitsidis et al. [10].

A tendency of uterine AVMs to proliferate during pregnancy has been reported. Some authors consider the presence of a uterine AVM an absolute contraindication for pregnancy [26]. In the systematic review of Peitsidis et al. [10], 17 pregnancies were reported after uterine artery embolizations (17%). All pregnancies resulted in viable healthy neonates, except in one case in which total abdominal hysterectomy was performed in a uterus at 7.4 weeks of gestation due to uncontrolled heavy bleeding [27]. The mean time of delay from treatment to subsequent pregnancy was 15.7 ± 11.7 months with range (2 to 36) months.

In conclusion, uterine AVF may be a potentially serious complication of uterine perforation during IUD placement. The diagnosis of such entity may be done by means of color Doppler ultrasonography and/or angiography and the minimally invasive approach using uterine artery embolization should be considered the first choice of treatment.

References


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Vasa previa rupture in velamentous insertion of the umbilical cord: an analysis and report of a case

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Maternal and Child Health Hospital, Pudong New Area, Shanghai (China)

Summary
Ruptured vasa previa in term pregnancy is rare but usually catastrophic if emergency delivery is not achieved. The authors present a case of ruptured vasa previa in velamentous cord insertion placenta. The fetus survived after intensive treatment immediately after delivery by cesarean section, but, unfortunately, died after the family gave him up. Defects in the vessel wall architecture were visualized and confirmed by histopathologic examination and might be responsible for the vessel rupture. Prenatal sonographic identification of cord insertion site into the placenta is encouraged as standard of practice to prevent this accident.

Key words: Vasa previa; Velamentous insertion; Umbilical cord.

Introduction
The present is an analysis and report of a case of ruptured vasa previa in velamentous cord insertion placenta. A 31-year-old woman, gravida 5 and para 1 (G5P1), at a gestational age of 39 weeks and six days, was admitted to the present hospital due to vaginal discharge for over one hour. The patient had no vaginal bleeding and no history of abdominal pain during her pregnancy. An ultrasound examination at 24 weeks of gestation indicated placenta previa in a breech presentation, which had not been followed up through re-examination. Reproductive history: 1-0-3-1 (last pregnancy: vaginal birth of a healthy infant at term). A physical examination upon hospitalization showed a heart rate of 118 bpm. The abdominal circumference was 100 cm, and the symphysis-fundal height was 35 cm. Estimated fetal weight was 3,500 grams. A gentle pelvic examination with genital disinfection indicated a cervical dilation of 1-cm, in a -3 position; the fetal membrane ruptured, the amniotic fluid was clear, and no vaginal bleeding was observed. Diagnosis upon admission: G5P1, 39 weeks and six days gestation, ROA position, premature rupture of fetal membrane, and suspected placenta previa.

The patient immediately underwent a lower uterine segment cesarean section under local and epidural anesthesia due to “antepartum hemorrhage (suspected vasa previa rupture and placenta previa) and fetal distress”. Intraoperative observation indicated clear amniotic fluid amounting to approximately 300 ml. At 7:15, a male infant was delivered in an left occiput transverse (LOT) position. The infant weighed 3,325 g with an Apgar score of 0-2-4. The umbilical cord was 50 cm long and pale in color. No nuchal cord was observed. The placenta was located in the posterior uterine wall and naturally peeled off intact. The placenta was observed with velamentous cord insertion and vasa previa. One blood vessel of the vasa previa had ruptured, showing broken ends (Figure 2).

The newborn appeared to not be breathing, with no heartbeat, pale skin, sluggish limbs, no reflection, and no vital signs. He died after the family gave him up. Defects in the vessel wall architecture were visualized and confirmed by histopathologic examination and might be responsible for the vessel rupture. Prenatal sonographic identification of cord insertion site into the placenta is encouraged as standard of practice to prevent this accident.

Case Report
A 31-year-old woman, gravida 5 and para 1 (G5P1), at a gestational age of 39 weeks and six days, was admitted to the present hospital due to vaginal discharge for over one hour. The patient had no vaginal bleeding and no history of abdominal pain during her pregnancy. An ultrasound examination at 24 weeks of gestation indicated placenta previa in a breech presentation, which had not been followed up through re-examination. Reproductive history: 1-0-3-1 (last pregnancy: vaginal birth of a healthy infant at term). A physical examination upon hospitalization showed a body temperature (T) of 36.7°C, a pulse rate (P) of 92 beats/minute, a respiratory rate of 20 breaths/minute, and a blood pressure (BP) of 118/80 mmHg. The patient was conscious and calm and was in normal general condition, without cardiovascular or pulmonary abnormalities. The abdomen was soft and nontender, without rebound tenderness or organomegaly. No shifting dullness (+) was detected, and no edema (-) was observed in either extremity. The abdomen was distended and consistent with pregnancy. Occasional uterine contractions were observed. One fetus was in the right occiput anterior (ROA) position. Fetal heart rate was 136 bpm. The abdominal circumference was 100 cm, and the symphysis-fundal height was 35 cm. Estimated fetal weight was 3,500 grams. A gentle pelvic examination with genital disinfection indicated a cervical dilation of one-cm, in a -3 position; the fetal membrane ruptured, the amniotic fluid was clear, and no vaginal bleeding was observed. Diagnosis upon admission: G5P1, 39 weeks and six days gestation, ROA position, premature rupture of fetal membrane, and suspected placenta previa.

The patient had abrupt vaginal bleeding during fetal monitoring, amounting to approximately 150 ml (mixed with amniotic fluid) and bright red in color. The fetal monitoring indicated a rapid drop in fetal heart rate to approximately 55 bpm (see fetal heart monitoring trace, Figure 1), and the fetal heartbeat subsequently became undetectable. After continuously repeated examination of the fetal heart rate, it was detected to be 50 bpm at 7:00. The patient immediately underwent a lower uterine segment cesarean section under local and epidural anesthesia due to “antepartum hemorrhage (suspected vasa previa rupture and placenta previa) and fetal distress”. Intraoperative observation indicated clear amniotic fluid amounting to approximately 300 ml. At 7:15, a male infant was delivered in an left occiput transverse (LOT) position. The infant weighed 3,325 g with an Apgar score of 0-2-4. The umbilical cord was 50 cm long and pale in color. No nuchal cord was observed. The placenta was located in the posterior uterine wall and naturally peeled off intact. The placenta was observed with velamentous cord insertion and vasa previa. One blood vessel of the vasa previa had ruptured, showing broken ends (Figure 2).
tinuously, and the heart rate increased to 120 bpm, with weak heart sounds. Shallow breathing was observed 20 minutes after birth, at a rate of ten bpm. The Apgar score was then 0-2-4-7. After a venous cannulation was performed, an intravenous injection of naloxone hydrochloride, cefotaxime sodium, penicillin, vitamin K1, etamsylate, and normal saline was administered. Pressurized oxygenation was continued through endotracheal intubation to maintain arterial hemoglobin oxygen saturation (SapO2) of 85-90%. Spontaneous breathing reached 30-40 breaths/minute at 45 minutes after birth, along with a heart rate of 120 beats/minute and SapO2 at 93%. One hour later, the patient and her family decided to give up the treatment of the newborn due to financial reasons and the newborn died. Routine blood test results from the newborn showed white blood cell count at 7.83×10^9/L, red blood cell count at 2.63×10^12/L, hemoglobin at 98 g/L, hematocrit at 35.6%, platelet count at 128×10^9/L, percentage of neutrophils at 51.9%, percentage of lymphocytes at 41.8%, CRP below 1 mg/L, an O blood type of Rh D+, and blood glucose at 2.6 mmol/L (using a glucose meter).

Placental pathological report (2013-07428): (1) Placenta with velamentous cord insertion in late gestation with one umbilical cord and three blood vessels (two arteries and one vein). The rupture of a vein traveling within the fetal membrane, accompanied by hemorrhage, was observed five cm from the site where the blood vessels in the umbilical cord connected to the placenta. (2) No abnormal development of the placental villi was observed. A small number of villi showed mild aging, with slightly narrowed intervillous spaces, mild increase in fibrinous exudates, and focal hemorrhage in the placental floor. (3) Focal hemorrhage was observed in the fetal membrane, accompanied by mild edema.

Discussion

Cause of vasa previa rupture

Placenta with velamentous cord insertion refers to the condition in which the umbilical cord attaches to the fetal membrane, and the umbilical vessels insert into the fetal membrane without a protective covering. The umbilical vessels travel between the amniotic and chorionic membranes before reaching the placenta. This condition is also called velamentous umbilical cord insertion. The fetal membrane vessels that lack the support of the umbilical cord or placenta and run through the lower uterine segment or across the internal orifice of the uterus in front of the fetal presentation are called vasa previa [1]. Although the cause of vasa previa remains unclear, most researchers believe the hypothesis proposed by Benirseake to be the most reasonable. In this hypothesis, the umbilical cord is normally attached to the placenta initially; however, during the subsequent placental developmental process, the umbilical cord is left behind due to the lateral growth of the chorionic villi, leading to malnutrition of the attached segment of the umbilical cord. As a result, the atrophic chorionic villus becomes smooth chorion, and velamentous umbilical cord insertion occurs, followed by the secondary formation

Figure 1. — Fetal monitoring indicated a rapid drop in fetal heart rate to approximately 55 bpm from 155 bpm.

Figure 2. — One blood vessel of the vasa previa has ruptured, showing the broken ends.
of the vasa previa [2, 3]. This patient was a multipara with a history of three instances of induced abortion and thus was very likely to have “endometrial barrenness” caused by endometrial infection and transformation. Consequently, malnutrition at the point where the umbilical cord attached could cause a lack of peripheral protection from the Wharton’s jelly of the blood vessels attached to the fetal membrane, leading to intrapartum rupture of the vessels during labor.

Incidence rate of vasa previa

Vasa previa rupture is very rare in clinical settings. However, it is life threatening to the fetus once it occurs because the rupture of blood vessels leads to acute blood loss and hypoxia, resulting in fetal suffocation and death within a short period of time [4]. Under normal circumstances, in approximately 90% of pregnancies, the umbilical cord attaches to the middle portion or slightly deviated from the middle on the fetal side of the placenta, while in approximately 10% of cases, the umbilical cord attaches to the edge of the placenta. Velamentous umbilical cord insertion occurs in only 1% of pregnancies [5]. The incidence rate of vasa previa rupture is even lower, only approximately 0.02% according to domestic reports. It has been reported that fetal distress occurred in 46.1% of vasa previa rupture cases, and the mortality rate was 75-100% [6, 7].

Characteristics of vasa previa rupture

(A) Vasa previa rupture and the resulting death usually occur in late gestation or in the intrapartum period, related to the changes in intrauterine pressure. (B) The rupture of blood vessels is often accompanied by the rupture of the fetal membrane. (C) The vaginal bleeding is painless, with a continuous fresh blood stream. (D) The rupture of the vasa previa is followed by fetal heart rate changes. Continuous fetal heart rate monitoring can detect fetal abnormalities in a timely manner. (E) The laboratory tests can distinguish between maternal and fetal blood through vaginal blood, amniotic fluid flow, and a sudden drop in the fetal heart rate from 155 beats/min to 55 beats/min (Figure 1) in a burst of contractions.

Impact on perinatal infants

Once vasa previa rupture is diagnosed, cesarean section should be performed immediately to terminate the gestation if there is no condition for vaginal operative delivery. The rupture of the vasa previa is clinically rare. Once it occurs, it threatens fetal life because the rupture of the blood vessels causes acute fetal ischemia and hypoxia that lead to perinatal sudden death. The amount of vaginal bleeding is a direct factor affecting the prognosis of the fetus because the lost blood comes directly from the fetus. The full-term fetal blood volume is approximately 240-300 ml. When acute blood loss reaches one-third of the fetal blood volume, the body loses its full compensatory capability and regulatory mechanisms, resulting in hemorrhagic shock and death within a short period of time [8]. Therefore, the umbilical cord should be compressed to push as much blood to the fetus as possible before severing the umbilical cord, or 20-30 ml of placental blood should be injected into the newborn through the umbilical vein after severing the umbilical cord to reduce the series of neonatal complications caused by anemia. The newborn in this report did not receive timely blood transfusion to correct anemia after birth, and the family gave up the rescue because of financial reasons, leading to neonatal death, from which lessons should be learned.

References


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A case of discordant monochorionic diamniotic twin with umbilical cord entanglement after spontaneous rupture of the dividing membrane

Kwan Young Oh, Sang Kyu Kang, Chan Hee Jin, Yun Suk Yang

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Summary
Spontaneous antepartum rupture of the dividing membrane in monochorionic diamniotic twins with discordancy is extremely rare. The authors report a case of cord entanglement after spontaneous rupture of the dividing membrane within discordant monochorionic diamniotic twins. The subject was a 30-year-old woman pregnant with discordant monochorionic diamniotic twin at 27+4 gestational weeks. The relatively thin dividing membrane was sound until it passed parallel to the two umbilical cords where it then became ill-defined. The patient presented with a monochorionic diamniotic placenta, a remnant of the disrupted dividing membrane, and entangled umbilical cords. The authors report this subject with literature review.

Key words: Spontaneous rupture; Dividing membrane; Discordant twin; Umbilical cord entanglement; Monochorionic diamniotic placenta.

Introduction

Discordant twins occur in monochorionic diamniotic (MCDA) placenta because of vascular anastomosis. The blood flow through the vascular connections becomes unbalanced, resulting in discordant twin such as twin-to-twin transfusion syndrome (TTTS) [1]. Monochorionic monoamniotic (MCMA) twin gestations have the highest perinatal mortality rate because of umbilical cord entanglement [2, 3]. Umbilical cord entanglement can occur in MCDA twins due to rupture of the dividing membrane (DM). Once the DM is disrupted, the pregnancy is similar to MCMA twins, with an increased risk of umbilical cord entanglement and an increased perinatal mortality rate [4]. Spontaneous rupture of the membrane in MCDA twins with discordancy is extremely rare. The authors report a case of rupture of the membrane in discordant MCDA twins with literature review.

Case Report

A 30-year-old nulliparous woman was transferred to the present hospital at 27+4 weeks gestation, because of discordant twin. The patient was diagnosed with MCDA twin pregnancy during the first trimester. On ultrasonography, a MCDA twin gestation with discordant 29+3 weeks and 26+0 weeks gestation size fetuses with amniotic fluid indexes (AFIs) of 25 and 7 cm was observed. The first fetus had cardiomegaly and an enlarged bladder. The second fetus displayed an absence of diastolic flow of the umbilical artery (Figure 1) and the bladder was not visible. In addition, a difference in the diameters of the umbilical cords was identified (Figure 2). Except for the obscure difference of amniotic volume, the discrepancy of fetal size, bladder appearance, and abnormal Doppler finding of the umbilical artery are appropriate for the diagnostic criteria of TTTS by Quintero et al. [1].

The relatively thin dividing membrane was sound until it passed parallel to the two umbilical cords where it then became ill-defined. The patient was admitted cautiously due to the possibility of spontaneous rupture of the dividing membrane and potential cord entanglement. Upon delivery at 29+3 weeks due to fetal compromise, the patient presented with a monochorionic diamniotic placenta, a remnant of the disrupted dividing membrane, and entangled umbilical cords. The authors report this subject with literature review.

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A case of discordant monochorionic diamniotic twin with umbilical cord entanglement after spontaneous rupture of the dividing membrane

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other twin weighed 1,130 grams and had Apgar scores of 4 at one minute and 7 at five minutes. However, the babies needed tracheal intubation and were admitted to the neonatal intensive care unit. The hemoglobin level of first baby was 17.6 mg/dL, and that of second baby was 15.7 mg/dL. An examination of the placenta disclosed an MCDA organ with a remnant of the disrupted dividing membrane. Histopathology confirmed the MCDA placentation. The patient’s postoperative course was uneventful and she was discharged on postoperative day 5.

Discussion

In monochorionic diamniotic placenta, discordant twins develop from vascular anastomosis. The unbalanced blood flow through the vascular anastomosis results in discordant twins such as TTTS [1]. The present case was appropriate for diagnosis of TTTS, except for discrepancy of amniotic volumes. Based on the criteria by Quiñentero et al., this case was stage III because of the absent bladder and abnormal Doppler finding of the umbilical artery [1]. However, the difference in amniotic volume was not greater, and there were abnormal findings of the umbilical vessels and DM, which were suggested to rupture the DM and cord entanglement. The present authors confirmed the discordant twins with ruptured DM and cord entanglement after delivery. TTTS was not diagnosed in the babies of the present case because of the difference in the hemoglobin level. The authors thought that this result might be attributed to the rupture of the DM. Actually, iatrogenic rupture of the DM, called septostomy, could be used as a modality of TTTS treatment. Through the procedure, the progression of the disease used to be delayed, and the babies’ condition improved, especially, that of the donor twin because of increased amniotic volume [6]. In this case, the spontaneous rupture of the DM was suggested to delay the progression to TTTS by the same effect as septostomy.

During pregnancy, spontaneous rupture of the dividing membrane of MCDA twins is extremely rare and difficult to diagnose prenatally. The major etiologic factors are suspected to include a traumatic or physical rupture of the membrane [5]. The other probable cause is septostomy, which is a potential therapeutic modality for TTTS [6]. In addition, iatrogenic septostomy following amniocentesis and other invasive procedures in MCDA twins have also been reported [7]. The present group also utilized amniocentesis as there was minimal risk of a secondary rupture from amnioreduction. The risk was determined very low due to the following reasons: 1) suggested evidence of rupture such as an obscure difference between the amniotic volume and the parallel running pattern of two umbilical cords were observed prior to the amnioreduction; 2) there was no observable difference in the ultrasound findings prior to and post procedure, ex-
A case of discordant monochorionic diamniotic twin with umbilical cord entanglement after spontaneous rupture of the dividing membrane

Except the difference of amniotic volume in the larger fetus; 3) the puncture site was specifically chosen as it was distant from the dividing membrane and near the larger twin, that showed marked polyhydramnios.

In the present case, the definite cause of the disrupted membrane is still uncertain. The authors speculate that the spontaneous rupture of the dividing membrane occurred due to a combination of thin membrane and active fetal movement [8]. The exact timing of the spontaneous rupture of the dividing membrane and the resulting umbilical cord entanglement is unknown. However, the diagnosis is very important for the prediction of umbilical cord entanglement and adverse fetal prognosis. Therefore the diagnosis of MCDA twins should be made cautiously, with special attention paid to the thickness of the membrane, the running pattern, and the insertion site of umbilical cords. Once the dividing membrane is ruptured, the MCDA twin gestations have high perinatal mortality rates of up to 70%, and a greater than 50% chance of umbilical cord entanglement [9]. Umbilical cord entanglement may be initially loose but it still has the potential to tighten and compromise fetal circulation later in pregnancy. In the present case, suspicious signs of fetal compromise were detected and a timely cesarean delivery was performed. Therefore, the authors recommend a thorough assessment of the DM at ultrasound examination in severe discordant MCDA twin which show obscured discordant amniotic volume. In addition, the umbilical cords should be assessed for evidence of entanglement.

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A case of Cantrell syndrome diagnosed in the first trimester

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Summary
Here, the authors report a case of Cantrell’s syndrome which was diagnosed by ultrasound at 12th week of pregnancy and confirmed by autopsy. Cantrell syndrome/pentalogy is defined as congenital combination of five main distinctive components: defects at the lower part of the sternum, anterior diaphragm, midline supraumbilical abdominal wall defect, diaphragmatic pericardium, and ectopia cordis. In the present case, in addition to these anomalies, there was cleft palate and cleft lip at the midline. Association of cleft lip and palate with Cantrell's syndrome may be due to the extension of defective migration of mesodermal primordial structures, which is mainly in abdomen and thorax, towards facial structures. Therefore, in prenatal diagnosis facial anomalies should be examined carefully in all cases with Cantrell syndrome.

Key words: Cantrell syndrome; Congenital defects; First trimester.

Introduction
In 1958, Cantrell et al. described a syndrome characterized by the association of ectopia cordis and omphalocele. They called this rare syndrome of midline developmental defects as Cantrell pentalogy, the full spectrum of which consists of an anterior diaphragmatic defect (I), a midline supraumbilical (thoracoabdominal) abdominal wall defect (II), a defect at the diaphragmatic pericardium (III), congenital cardiac anomalies (IV), and a defect of the lower sternum (V) [1]. Many variants of this syndrome has been described in the literature. It is not necessary to diagnose all of the components, and in fact very few cases meet the complete pentalogy spectrum among about 100 case reports of Cantrell syndrome in the literature [2]. In this case report, the authors present a case of incomplete Cantrell syndrome, accompanied by cleft lip and palate anomalies, in which the diagnosis was established by the 12th gestational week ultrasound and confirmed by autopsy.

Case Report
After her first pregnancy ultrasound at the sixth gestational week, a 21-year-old, gravida 2, para 1 woman was admitted for first trimester screening. In prenatal ultrasonography, ectopia cordis in which the fetal heart was seen as protruding from the defect at the anterior chest wall and fetal midline facial defect was observed. As these findings supported the diagnosis of Cantrell syndrome, the prognosis of pregnancy was discussed with the parents. Patient was referred to a tertiary center. As family requested termination of pregnancy because of the poor prognosis, induction was began with vaginal administration of misoprostol 200 mcg every four hours. Six hours after the first dose of misoprostol, patient underwent a complete abortion. On postabortive gross examination, extensive anterior chest wall defect, partial ectopia cordis, cleft palate, cleft lip, and nasal dysplasia were observed. The patient was discharged the morning after abortion without complications. Autopsy examination revealed an anterior diaphragmatic defect, a diaphragmatic pericardial defect, lower sternum defects, ectopia cordis including large ventricular septal defect, cleft palate, cleft lip, and nasal dysplasia. There was no evidence of a supraumbilical abdominal wall defect.

Discussion
Cantrell pentalogy is a rare syndrome of congenital midline developmental failure, the full spectrum of which consists an anterior diaphragmatic defect, a midline supraumbilical abdominal wall defect, a diaphragmatic pericardial defect, congenital cardiac anomalies, and a defect of the lower sternum. The incidence was reported from 1: 65,000 to 1: 200,000 [3]. Defined as the displacement of heart outside the thorax, ectopia cordis is an anomaly usually fatal in the first few days after birth. Complete ectopia cordis is defined if the heart has no pericardial cover outside the thorax while, partial ectopia cordis is diagnosed if pericardium and skin covers the heart outside the thorax [4]. There is accompanying ventricular septal defect (VSD) in all cases of ectopia cordis, while atrial septal defect (ASD) is present in 53% [5].

The pathogenesis of Cantrell syndrome is not fully understood. Cantrell et al. proposed an embryologic developmental failure of a segment of the lateral mesoderm.
around 14-18th days of gestation [1]. As a result, the development of transverse septum of the diaphragm fails and a pair of mesodermal folds located in the upper abdomen do not migrate to the ventromedial. Organs eviscerate through sternal and abdominal wall defects.

The most common abdominal wall defect in the Cantrell syndrome is omphalocele, which was absent in the present case [6]. Anterior diaphragmatic and pericardial defects are also common. The most common cardiac anomaly reported is thoracoabdominal ectopia cordis [5]. However, ectopia cordis is not a main component of pentalogy of Cantrell, the syndrome can be diagnosed without ectopia cordis [5]. In the present case, ectopia cordis was present in association with a large VSD. Other anomalies that can accompany the five main components of the syndrome have been reported as: craniofacial malformations (cleft lip and palate, anophthalmia, microphthalmia, hypertelorism), cystic hygroma, meningocele, anencephaly, pulmonary hypoplasia, vertebral anomalies, finger anomalies, pes equinovarus, adrenal agenesis, renal agenesis, intestinal malrotation, and hypospadias [1-8].

Diagnosis of Cantrell syndrome can usually be made by prenatal ultrasonography in the first trimester [9]. While presence of ectopia cordis and omphalocele facilitates early prenatal diagnosis, paucity of the defect may delay the diagnosis to the second trimester [7]. Pentalogy of Cantrell should be ruled out in a fetus with omphalocele. If pericardial effusion is seen, anterior diaphragmatic hernia and diaphragmatic pericardial defects should be suspected, and in the presence of them, a detailed ultrasound should be performed for the aforementioned findings of pentalogy of Cantrell [8]. Prenatal magnetic resonance imaging, which is used more often in the second trimester, may facilitate the diagnosis of fetal anomalies [10]. Because of the infrequency and variations in the clinical spectrum, both the diagnosis and gaining experience of the surgeons become more difficult.

Cantrell pentalogy has been described in many varieties in the literature since components of this syndrome can occur in different clinical severity and combinations. It is not essential to diagnose all of the findings, and very few cases meet the original pentalogy spectrum among about 100 case reports described in the literature so. In the present case, supraumbilical midline abdominal wall defect was not observed. In addition to the other four anomalies of the spectrum, midline cleft palate and cleft lip, and nasal dysplasia was present. Association of cleft lip, cleft palate, and nasal anomalies with Cantrell’s syndrome may be due to the extension of defective migration of mesodermal primordial structures, which mainly leads to midline fusion defects in abdomen and thorax, towards facial structures. Consequently, in the present authors’ opinion, facial anomalies should be carefully examined in all cases with Cantrell syndrome.

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Appendectomy for asymptomatic appendicitis during caesarean section - an interesting case report

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Summary

Background: The authors present an interesting case report of an appendectomy during caesarean section in an asymptomatic pregnant woman, which highlights the need of peritoneal cavity check during every caesarean section. Materials and Methods: A 32-year-old para 0 woman at 34 weeks of gestation attended to the present clinic because of feeling reduced fetal movements in the last 24 hours. She underwent a non-stress test (NST), that was non-reassuring and no contractions were recorded. The woman underwent a caesarean section, which revealed a large phlegmonic appendix. Appendectomy was decided after the closure of the uterine cavity. Results: The woman was treated with appendectomy. Histology came back as an appendicitis three days later. Conclusions: Acute appendicitis during pregnancy may be associated with serious maternal and fetal complications. It is also associated with a high risk of premature delivery. In the absence of lower abdominal pain and inflammatory changes, the incidence of acute appendicitis is low, but exists. In every caesarean section at any week of gestation, we should check the peritoneal cavity and especially the appendix, as appendicitis is the most frequent non-obstetric surgical situation in pregnancy. Asymptomatic appendicitis should be considered as a cause in every pregnant woman who mentions preterm contractions or/and reduced fetal movements.

Key words: Pregnancy; Caesarean section; Asymptomatic appendicitis; Appendectomy.

Introduction

Acute appendicitis is the most common general surgical problem encountered during pregnancy [1]. The diagnosis is particularly challenging during pregnancy because of the relatively high prevalence of abdominal/gastrointestinal discomfort, anatomic changes related to the enlarged uterus, and the physiologic leukocytosis of pregnancy [2].

Case Report

A 32-year-old para 0 woman at 34 weeks and two days of gestation, attended the present clinic because of feeling reduced fetal movements the last 24 hours. She was a non-smoker and did not consume any alcohol. Her gynecological history was free and she had no other health problems. Her pregnancy was uneventful up to that point, with normal ultrasound findings on the first, second, and third trimester studies. On admission she was offered an ultrasound investigation and non-stress test (NST). Ultrasound examination revealed an estimated fetal weight (EFW) of about 2,320 grams and the measurement of pulsatility index (PI) of umbilical artery (UMA) was normal. NST did not record any contractions but it was non-reassuring with a baseline fetal heart rate (FHR) of 105 bpm and decreased variability. Blood test revealed WBC: 11,460/mcL, NE: 72%, HCT: 34.2%, and PLT: 182,000/mcL. Treatment options were discussed, she underwent an emergency caesarean section and a 2,280-gram male infant with an Apgar score of 9 was born. After the closure of the uterine cavity, a checkpoint of peritoneal cavity revealed a large and rubber appendix of about five cm long. (Figures 1-2). A general surgeon's advice was asked and he suggested appendectomy due to acute appendicitis and high risk of rupture in the next days. Histological examination the next day came back as an acute appendicitis. The woman remained in the hospital for three days. Paracetamol was used for analgesia and metronidazole and cefoxitin postoperatively. She was discharged from hospital on the fourth day.

Discussion

Pregnant women are less likely to have a classic presentation of appendicitis than nonpregnant women, especially in late pregnancy [3, 4]. The most common symptom is, right lower quadrant pain, close to McBurney’s point in the majority of pregnant women, regardless of the stage of pregnancy [5]. In the present case there were no signs of acute appendicitis. The woman mentioned no abdominal pain, there was no leucocytosis, and no fever or anorexia. The only symptom was decreased fetal movements. However, the examination revealed a non-reassuring NST and she underwent caesarean section, where the appendicitis was revealed as an accidental finding and led to appendectomy. It is possible that the reduced fetal movements, was a complication of the appendicitis and was the only sign for a diagnosis, or it was too early in order to have symptoms. It is very important to check the peritoneal cavity and other abdominal organs during a caesarean section. In the present case, the...
risk of rupture of appendix the next days after delivery would have been very high, if appendicitis was misdiagnosed intraoperatively and that could have cost another surgery a few days after delivery.

Conclusion

Too many situations can lead to pregnancy complications in any gestational age. Appendicitis does not occur often, but is one of them. A high index of suspicious is necessary, when no other pathological findings can be seen. Checking the peritoneal cavity during a caesarean section, should be performed by every obstetrician.

References


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A laparoscopic surgery for deep infiltrating endometriosis and the review of literature

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Summary
Deep infiltrating endometriosis (DIE) is a complex disorder that affects 6% to 12% of all women in the reproductive age. In these cases, treatment is more difficult with possible incomplete pain relief and a considerable possibility of recurrence. Here, the authors report a case of a 41-year-old woman with a history of severe dysmenorrhea, dyspareunia, and chronic pelvic pain because of deep infiltrating pelvic and peritoneal endometriosis, who underwent segmental colorectal resection three years ago for large bowel obstruction due to colonic endometriosis. To ensure complete removal of the disease, the authors injected gonadotropin-releasing hormone agonist (GnRHa) in three periodic cycles before laparoscopic surgery. We performed laparoscopic hysterectomy and deep pelvic nodule resection and pelvic adhesion releasing. After five days of hospitalization, the patient recovered totally and was not experiencing any pain at three months’ follow-up. Laparoscopic treatment has more become the standard of treatment for DIE. A review of the literature regarding pathology and physiology of DIE and surgical aspects of its management is undertaken. The authors would like to renew the current laparoscopic surgery in curing the DIE, as they believe that this is also a useful addition to the literature.

Key words: Deep infiltrating endometriosis; Gonadotropin-releasing hormone agonist; Laparoscopic surgery.

Introduction
Deep infiltrating endometriosis (DIE) mostly infiltrates the uterosacral ligaments, peritoneum, vagina, bladder, rectum, and rectosigmoid colon and can cause a complete distortion of the pelvic anatomy [1, 2]. Reported cases that received segmental colorectal resection and had recurrence have been seldom reported [3]. Here, the authors report a case of a 41-year-old woman with a history of severe dysmenorrhea, dyspareunia, and chronic pelvic pain due to deep infiltrating pelvic and peritoneal endometriosis, who received segmental colorectal resection three years ago for large bowel obstruction owing to colonic endometriosis. A review of the literature regarding pathology and physiology of DIE and surgical aspects of its management is undertaken.

Case Report
A 41-year-old woman, with a more than ten years of severe dysmenorrhea, dyspareunia and chronic pelvic pain, had received segmental colorectal resection three years ago for large bowel obstruction owing to colonic endometriosis. Gynecological examination showed multiple solid nodules in the vaginal posterior fornix and the patient felt severe pain upon palpation. The size of the uterus was approximately 8×6×6 cm. The left appendix area was thickened. Transvaginal ultrasound image showed adenomyosis and a 2.0×1.0-cm encapsulated mass in the left appendix area (Figures 1A, B). MRI showed that the shape of the uterus was unclear because of deep infiltrating endometriosis (DIE) and the deep pelvic and peritoneal endometriosis which had destroyed the normal anatomy of Douglas (Figure 1C). Laboratory tests revealed an elevated cancer antigen 125 (CA 125) of 109 U/ml in the serum. Laparoscopic view of the bowel and peritoneal endometriosis invaded the sigmoid colon, and enlarged uterus and anatomical distortion of the Douglas were revealed (Figure 2A). The revised American Fertility Society (rAFS) score was 112. The present authors performed laparoscopic hysterectomy and deep pelvic nodule resection and pelvic adhesion releasing. The view of the pelvic after the operation and the ultimate specimens are shown in Figures 2B, C. Pathologic diagnosis confirmed DIE (Figures 3A, B, C). After five days of hospitalization, the patient recovered totally and was not experiencing any pain at three months’ follow-up. The level of CA125 had reduced almost to a normal level.

Discussion
Endometriosis has been defined as a chronic disease which is characterized by the presence of endometrial glands and stroma outside the uterus. As a consequence, endometriosis can cause different symptoms such as chronic pelvic pain, infertility, dysmenorrhea, deep dyspareunia, cyclical bowel or bladder symptoms (eg, bloating, constipation, diarrhea, rectal bleeding, and hematuria), and abnormal menstrual bleeding according to the location of ectopic endometrium [4]. The disease is histologically categorized into three types: peritoneal superficial endometriosis (SUP), ovarian endometrioma (OMA), and deep infiltrating endometriosis (DIE). DIE is defined as en-
dometriosis located more than five mm beneath the peritoneal surface [3]. From 6% to 12% of women in their reproductive period are affected by DIE [5].

DIE mostly infiltrates the uterosacral ligaments, peritoneum, vagina, bladder, rectum, and rectosigmoid colon and can cause a complete distortion of the pelvic anatomy [1, 2].

Figure 1. — A: Transvaginal ultrasound image, sagittal view. The enlarged uterus and adenomyosis and a 2.0×1.0-cm encapsulated effusion in the left appendix area (white arrow). No gap in the pouch of Douglas (red arrow). B and C: MRI showing the shape of uterus is unclear because of DIE (black arrows) and the deep pelvic and peritoneal endometriosis which destroyed the normal anatomy of Douglas and sigmoid colon and utero-vesical fold (white arrow).

Figure 2. — A: Laparoscopic view after the superficial pelvic adhesion releasing. The deep peritoneal endometriosis which destroyed the normal anatomy of Douglas and the enlarged uterus (U) and the serious adhesion of Sigmoid colon. The authors performed hysterectomy and deep pelvic node resection and pelvic adhesion releasing. The ectopic endometriosis has been basically removed . B shows the pelvic structure after the operation. C shows the resected uterus and the deep pelvic and peritoneal nodule.

Figure 3. — Final pathologic results which show a deep infiltrating endometriosis. A: immunohistochemical staining for adenomyosis (×400), B: hematoxylin-eosin stain (× 50), and C: immunohistochemical staining (×400) for pelvic and peritoneal nodule.
semination of cells to non-gynecologic sites. These sites include lungs and pleural cavity, skin, lymph glands, and brain [8]. Pelvic endometriosis may infiltrate the pelvic wall and somatic nerves causing severe neuropathic symptoms. However, the severity of the symptoms of endometriosis do not always correlate with the anatomic severity of the disease [9]

Although the etiology of DIE remains unclear, the therapy of DIE is of great urgency to alleviate the suffering of the patient. GnRHa administration remains the best therapeutic treatment for affected patients to reduce the tumor and pathologic nodes in size before surgery [10]. After gonadotropin therapy, an accurate preoperative evaluation of the extension of endometriotic lesions is essential for a successful outcome [11]. This case suggested that preoperative gonadotropin therapy could be effective for locally advanced DIE. It seems that it has played an important role on surgical success when the surgeon is able to completely separate DIE. Laparoscopic management for DIE is a safe and feasible procedure [12], which can show the pathologic location of DIE easily and reduced the operational time [13]. Laparoscopic hysterectomy, therefore, should be the preferred surgical option for women with severe pelvic endometriosis who require a hysterectomy. Also the deep pelvic and peritoneal nodule and the pelvic adhesion are also excised. Just as this case, after resection of deep infiltrating endometriosis (DIE) vs. incomplete surgical treatment with or without GnRHa administration after surgery”.

This case implies that GnRHa therapy plays an important role on surgical success when the surgeon is able to completely separate DIE. Laparoscopic management for DIE is a safe and feasible procedure.

References


Postpartum splenic rupture: a possible iatrogenic event, but finalized with successful conservation of the spleen

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Summary
The authors present the case of a postpartum splenic rupture induced probably by iatrogenic injury (recent vaginal delivery with a prolonged expulsion with uterine fundus compression) including the left hypochondria region costal grid. The case was solved with splenic preservation and achieving hemostasis only by local plugging and Gelaspon. The case raised also other problems regarding the etiology of splenic rupture, in establishing a causal relationship between an intrapartum splenic injury, and the three episodes of inferior genital tract hemorrhaging, in establishing the cause of the infectious syndrome from the 24th postpartum day, (parietal infection or splenic abscess requiring splenectomy).

Key words: Postpartum splenic rupture; Iatrogenic complication; Ruptured spleen preservation.

Introduction
Intra- or postpartum splenic rupture is a rare event usually not immediately recognized and solved, but it represents an extreme surgical emergency with consequences on the vital maternal and fetal prognosis.

The authors present a case of a postpartum splenic rupture that they presumed to be induced by obstetrical trauma at vaginal birth: prolonged expulsion with uterine fundus and costal grid compression.

Case Report
A 23-year-old patient, nullipara, accomplished a vaginal delivery in a second level obstetrical unit, resulting in a live newborn of 3,550 grams. She was discharged three days post-delivery, without clinical problems. The 7th day post delivery, she returned with significant vaginal bleeding. After instrumental uterine revision, oxytocics, prostaglandins, correction of volemia, and coagulation, the patient was discharged again. On the 14th day postpartum, she returned with another episode of vaginal bleeding, and a subtotal inter-adnexal hysterectomy was performed in order to achieve complete hemostasis. The pathology result was: acute postpartum metritis. On the 21st day postpartum, she returned with significant repeated vaginal bleeding. The patient was transferred in the present tertiary obstetrical unit. Here, the authors performed a totalisation of the hysterectomy and bilateral hypogastric artery ligature. Surgical hemostasis was achieved, and a Douglas drain was installed. During the first 12 hours, the patient’s condition was stable, but immediately after, the patient was mobilized, her general condition deteriorated, with signs of hemorrhagic shock, and drainage though the Douglas tube that increased up to 500 ml of bloody fluid in one hour. Re-intervention was decided upon to verify the hemostasis. The vaginal stump was normal; persistent bleeding was originating from the right upper quadrant of the abdomen. The general surgeon was called and found a one-cm superficial capsular laceration, two cm above the splenic pedicle. He decided that bleeding could be stopped by local pressure and Gelaspon, and opted for splenic preservation, Douglas drainage, and left and right hypochondria drainage. Four units of blood and eight units of fresh frozen plasma were given during these last two operations. An abdominal ultrasound performed after the discovery of splenic rupture, indicated the presence of a splenic hematoma of 73.05 cm3 volume, but it remained stable during serial ultrasound evaluations.

Postoperatively, the evolution was hemodynamically stable, but a parietal infection with E. coli was diagnosed, resulting in a differential diagnosis of a splenic hematoma becoming infected in a splenic abscess. The surgeon decided again to preserve the spleen, and after 14 days of poli-antibiotherapy, the infectious symptomatology disappeared. Laboratory values at patient admission in the present hospital were significant for: anaemia, thrombocytosis, increased APTT, and increased PDW (see Table 1). The patient returned three months later and her clinical status was normal.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.8 g/L</td>
<td>13.5-18 g/L</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>29.0%</td>
<td>32-48</td>
</tr>
<tr>
<td>Platelet count</td>
<td>494x10³</td>
<td>100-450x10³</td>
</tr>
<tr>
<td>APTT</td>
<td>54.6/sec</td>
<td>25-43/sec</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>225 mg/dL</td>
<td>200-400 mg/dL</td>
</tr>
<tr>
<td>PDW</td>
<td>16.9%</td>
<td>10-15%</td>
</tr>
</tbody>
</table>

APTT: activated partial thromboplastine time; PDW: platelet distribution width.
Discussion

The etiology of a splenic rupture is frequently unclear. Most post-partum splenic ruptures are produced by the rupture of an aneurysm of the splenic artery [1-3], but in the present case, the vascular pedicle of the spleen was intact.

Ultrasound examination in the present case described a heterogeneous collection of 66x45x31.7 mm (73.05 cm³) volume near the hilum, with hypoechoic areas, suggestive of hematoma. The authors could not identify at colour and spectral Doppler sonography, a transonic pulsatile structure with turbulent arterial flow, in contact with the hematoma, to sustain the hypothesis of intrasplenic arterial true aneurysm or pseudo-aneurysm rupture. Unfortunately, a limitation of this case was the impossibility of performing an abdomino-pelvic CT, MRI or angiography immediately after surgery or later.

The patient denied any abdominal trauma caused by an involuntary fall or family violence, but she declared during her recent delivery, a prolonged expulsion with uterine fundus compression, including compression in the left hypochondria region costal grid. No systemic disease that could affect the spleen was identified.

Cheung et al. [4] described a case of splenic rupture four days after a cesarean delivery in a patient who was on anticoagulation treatment with Warfarin. The bleeding was controlled by argon beam coagulation and tachocomb. There are two other published cases of splenic rupture after low molecular weight heparin taken during the third trimester of pregnancy; both cases resolved with splenectomy [5, 6]. In the present case, after the subtotal hysterectomy, the patient received a prophylactic dose of low molecular weight heparin, but she interrupted it two days before the next episode of hemorrhaging.

Another possible cause of rupture of the spleen discussed was a possible direct trauma of the spleen caused by the application of abdominal retractors at the moment of hysterectomy totalisation and hypogastric artery ligation. However retractors were protected with swabs, and the authors believe that the pubo-subumbilical incision did not allow the retractor to arrive in direct contact with the spleen.

The second issue raised by the case was the decision to preserve the spleen versus performing an emergency splenectomy. The surgeon opted for splenic conservation, because the patient was young. The bleeding was controlled only by local compression and hemostatic Gelaspon, she became hemodynamically stable immediately after bleeding cessation, there was no other intra-abdominal lesion, and she needed only four units of blood for intra- and post-operative transfusion.

The third problem raised by the case was the existence of a parietal infection with E. coli, clinically manifested with septic syndrome, imposing a differential diagnosis of over-infected splenic hematoma, which would have required splenectomy. The patient received antibiotics according to the antibiogram of the abdominal wound and septic symptoms resolved.

The fourth question about the case is whether the bleeding could have been amplified by platelet changes secondary to the splenic trauma, because the authors considered that the main etiology was genital infection (acute post partum metritis), coupled with the use of heparin. The authors do not have sufficient arguments to sustain or to reject this hypothesis, because the patient presented all the time a higher than normal number of platelets due to the genital infection and they did not perform a peripheral blood smear.

Conclusion

The authors consider the presentation of this case useful, because, to their knowledge, it is the first case of postpartum splenic rupture resolved with splenic preservation and achieving hemostasis only by local plugging and Gelaspon. The case raised also other problems regarding the etiology of splenic rupture, in establishing a causal relationship between an intrapartum splenic injury and the three episodes of inferior genital tract hemorrhaging, in establishing the cause of the infectious syndrome from the 24th postpartum day, (parietal infection or splenic abscess requiring splenectomy).

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Uterine rupture during labor in women with twice successful vaginal births after cesarean delivery


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Summary

Uterine rupture during labor is a serious complication resulting in maternal and neonatal morbidity and mortality. We present the extremely rare case of a 38-year-old gravid woman admitted with labor pain at term, about to experience a uterine rupture during labor. She had previously twice delivered vaginally, and during her third pregnancy had a low transverse Cesarean section. Prior to arriving at the hospital with labor pains, she had routine prenatal care with normal prenatal laboratory tests. One day the woman reported having sudden epigastric pain, and 40 minutes after her admission a pelvic exam was completed. The unborn baby had a persistent fetal bradycardia of 60 beats/min and after 14 minutes, an emergency Cesarean section was performed. A complete uterine rupture was revealed, and a live neonate was promptly delivered with an Apgar score of 1 at one minute and 5 at five minutes. On the fifth postoperative day the woman and her baby were discharged home with no maternal and neonatal complications.

Key words: Uterine rupture; Labor; Vaginal birth after cesarean section (VBAC).

Introduction

Rupture of the uterus during labor is a serious complication resulting in maternal and neonatal morbidity and mortality [1]. However, successful trials of labor following vaginal birth after a cesarean section (VBAC) results in decreased maternal morbidity in terms of blood transfusion, hysterectomy, and febrile morbidity, when compared with repeated cesarean sections [2]. For this reason, the American College of Obstetricians and Gynecologists has recommended that a woman with one previous transverse low-segment cesarean section should be offered a trial of labor after an appropriate thorough explanation of maternal and perinatal risks and benefits [3]. However, currently there is no guideline regarding trials of labor in women with more than one previous successful VBAC. Furthermore, uterine rupture during labor has been rarely reported in women with multiple successful VBACs. Hence, the authors present an extremely rare case of a uterine rupture during labor in a gravid woman who had two successful vaginal deliveries after low transverse cesarean section.

Case Report

A 38-year-old, gravida 4, para 3 was admitted at 40 weeks + 1 days with spontaneous labor. Nine years earlier, she had delivered her first baby, a male, weighing 2,970 grams by low-transverse cesarean section due to breech presentation. Two years later, she had her second baby, female, weighing 2,680 grams through the first successful trial of VBAC. Three years later, her third baby was born by a second trial of VBAC, male, weighing 3,070 grams. During the current pregnancy, she had routine prenatal care with normal prenatal laboratory tests including ultrasound, fetal echo, and glucose challenge test. On admission to the hospital at 11:00 a.m., the fetal position was vertex and its heart rate was normal (147 beats/minute) with variability moderately detected. Sonographic examination showed a singleton fetus in the uterine cavity with no abnormalities. The patient was hemodynamically stable. Vaginal examination revealed a cervix of four cm dilated, at 50% effacement, and there was no bleeding or amniotic fluid leakage. However, 40 minutes later, she had sudden epigastric pain associated with persistent fetal bradycardia at 60 beats/minute (Figure 1). The fetal heart rate failed to show recovery on left lateral positioning and hydration with normal saline fluids, the woman was given 15 L/minute of oxygen with a reservoir bag mask. Upon pelvic examination the fetal head was impalpable and the woman complained of constant epigastric pain. A uterine scar rupture was suspected, and an emergency cesarean section was carried out in less than 14 minutes after fetal distress. Upon entering the abdominal cavity, a large amount of bloody fluid was encountered. A large defect in the lower uterine segment was also observed at the site of the previous cesarean scar. The fetal head was expelled via the ruptured uterine wall. Within 14 minutes of the situation the decision to delivery by cesarean section was made, and a live male neonate was promptly delivered and handed to the neonatal invasive care unit team. Initially the neonate had no respiratory effort and maintained a heart rate of less than 100 beats/minute. He required immediate intubation and Ambu-bagging. Apgar scores were 1 at one minute and 5 at five minutes. The birth weight was 3,200 grams. After three hours, intubation was removed and the baby’s oxygen saturation level was higher than 95 percent during spontaneous respiration. For evaluation of birth asphyxia, brain ultrasonography was done after five days from birth, showing normal neurosonography. The mother was discharged home with her baby on the fifth postoperative day and no maternal and neonatal complications were observed within 3 months after discharge.
In this case, the authors report a uterine rupture during labor in a woman with two successful VBACs. The case presented here emphasizes the possibility of uterine rupture, even in women who successfully delivered vaginally after cesarean section. To the best of the authors’ knowledge, this is the first report of uterine rupture during labor in a Korean woman with two successful VBACs.

Uterine rupture is defined as a complete separation of the myometrium, with or without extrusion of the fetal parts, into the maternal peritoneal cavity and requires emergency cesarean section or postpartum laparotomy. This is the ultimate complication that can occur during a trial of labor. The risk of uterine rupture during delivery is known to be slightly higher than in elective repeated cesarean section (7% vs. 1%; \( p = 0.034 \)) [4]. Abnormalities in fetal cardiotocography are associated with 55-87% of uterine ruptures. Other recognized signs of uterine rupture include loss of station of presenting part and new inefficient contractility [5]. In this case, the authors also found that a uterine rupture during labor was accompanied with loss of presenting part and persistent fetal bradycardia to an average of 60 beats/minute.

The most serious complications of uterine rupture include neonatal death and fetal hypoxic brain injury. Guise et al. [5] calculated a rate of 0.14 additional neonatal deaths per 1,000 trials of labor were related to uterine rupture. This figure is similar to that in the National Institute of Child Health and Human Development-Maternal and Fetal Medicine Unit Network study, in which two neonatal deaths occurred among every 124 ruptures, for an overall rate of rupture-related perinatal death of 0.11 per 1,000 trials of labor [1]. However, the authors did not encounter neonatal mortality and morbidity in this case. They attribute this fortunate outcome to the relatively short decision time of less than 14 minutes. According to a study by Holmgren et al., there is a correlation between the decision-to-delivery time and neonatal outcome in women who experience uterine rupture during trial of labor following VBAC [6]. Neonates delivered within 18 minutes after a suspected uterine rupture had normal umbilical pH levels or five-minute Apgar scores greater than 7. Poor long-term outcome occurred in three neonates with a decision-to-delivery time longer than 30 minutes [6].

In this present case, the patient was a multiparous woman consisting of one cesarean section, followed by two vaginal deliveries. It is possible that multiparity in itself resulted in a uterine rupture during labor, and found this was consistent with previous studies. Zeteroglu et al. reported that the occurrence of a uterine rupture was significantly associated with grand multiparity, scarred uterus, lack of antenatal care, unsupervised labor at home, and low socioeconomic status of the patients [7]. Ofir et al. also reported that multiparity was one of the major risk factors for uterine rupture during labor [8].
In conclusion, rupture of the uterus after multiple VBACs is rare, but still may be a life-threatening event. Women who have a past history of two successful VBACs seem to choose trial of labor instead of an elective repeated cesarean section due to successful previous VBACs. However, because multiparity is one of the major risk factors for uterine rupture during labor, physicians should inform the risk of uterine rupture to women with previous multiple VBAC who choose a trial of labor. Prompt decision-making and careful surveillance during labor is the key for reducing maternal and neonatal morbidity and mortality in these women.

References


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