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Introduction

Males with sperm with low hypo-osmotic swelling (HOS) test scores < 50% rarely achieve pregnancies with either intercourse or intrauterine insemination (IUI) [1]. Interestingly, sperm with low HOS test scores provide normal fertilization rates with conventional oocyte insemination during in vitro fertilization-embryo transfer (IVF-ET), but these embryos rarely implant [2].

There are ways to achieve pregnancies with low HOS test scores [3]. One method is to pre-treat the sperm before IUI or IVF with the protein digestive enzyme chymotrypsin and galactose [4, 5]. The most effective method is to bypass the contact of the sperm with a hypothesized associated toxic protein with the zona pellucida by performing intracytoplasmic sperm injection (ICSI) [6].

The present study evaluated whether poor motility reduces the effectiveness of ICSI in achieving pregnancies with sperm with low HOS test scores.

Materials and Methods

A retrospective evaluation of IVF-ET cycles was performed requiring the male partner to have a semen concentration of ≥ 20 x 10^6/ml and the HOS test scores were < 50%. Pregnancy and implantation rates were compared according to the percentage of motile sperm. ICSI was performed in all cycles. Data were based on the couple’s first IVF-ET cycle. Female partners were aged ≤ 39 years.

Results

Fertilization and pregnancy rates according to the percentage of motility of the sperm in female partners aged ≤ 39 years whose male partner was also ≤ 39 years of age with a normal sperm concentration but low HOS test score (< 50%) (≥ 20 x 10^6/ml) are seen in Table 1. Table 1 shows no trend for lower pregnancy or implantation rates with poor motility vs. normal motility following IVF-ET using sperm with HOS scores < 50%.

Evaluating sperm with < 20% motility, the clinical and delivered pregnancy rates/transfer were 57.9% (11/19) and 52.6% (10/19), respectively vs 50.0% (18/36) and 41.7% (16/36) for those with motility ≥ 50%. The < 10% motility group was insufficiently powered to make conclusions as to whether very low motility percentage may show slightly lower fertilization rates, but the results did not show any trend to be lower than the other groups.

Discussion

The added burden of low percentage motility does not adversely affect pregnancy rates following IVF with ICSI using sperm with low HOS test scores. Despite previous vivid demonstration of very poor (approaching zero) pregnancy rates following transfer of embryos formed from conventional oocyte insemination using sperm with low HOS test scores, the recorded pregnancy rates following ICSI are no lower than the normal pregnancy rates for this institution using sperm with normal HOS test scores.

Summary

Purpose: To determine the confounding effect, if any, of poor motility of sperm that are already compromised by an abnormal hypo-osmotic swelling (HOS) test on pregnancy outcome following in vitro fertilization-embryo transfer (IVF-ET) and intracytoplasmic sperm injection (ICSI). Materials and Methods: Clinical and live-delivered pregnancy and implantation rates were retrospectively evaluated in first cycles of couples undergoing IVF-ET with ICSI where the HOS test was < 50% according to deciles of subnormal percentage motility (< 50%) and compared to those with normal motility ≥ 50%. Results: The combination of very poor motility and low HOS test scores did diminish pregnancy rates following IVF with ICSI. Conclusions: The only part of fertilization of the oocyte that ICSI does not overcome is phase 2 of oocyte activation. Based on these data, the combination of very low percentage motility and low HOS test scores do not adversely affect pregnancy outcome following IVF with ICSI.

Key words: Hypo-osmotic swelling test; Intracytoplasmic sperm injection; Motility; In vitro fertilization-embryo transfer.
In normal conception, oocyte activation occurs by a two-signal process. The acrosome reaction stimulation – the initial signal of \( \text{Ca}^{2+} \) (signal 2) – occurs in an oscillatory fashion [7]. Though ICSI almost always stimulates signal 1, it does not always result in stimulating a signal 2. Achievement of oocyte fertilization despite failure with ICSI can be achieved by artificial oocyte activation of signal 2, e.g., with calcium ionophore [8].

These data not only confirm previous studies showing that ICSI corrects the embryo implantation defect that occurs with low HOS scores, but also show that very poor motility does not negate the beneficial effect of ICSI for the HOS defect [8]. Sperm morphology was not assessed in this study since a previous study found that poor morphology did not diminish the efficacy of ICSI for low HOS tests either [9].

**References**


A comparison of three types of therapies for three different ovulation disorders in establishing pregnancies and evaluation of laboratory parameters that could influence the outcome

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Summary

Purpose: To evaluate the empirical use of progesterone (P) in the luteal phase for unexplained infertility. Methods: Clinical and live-delivered pregnancy rates in three treatment cycles were compared in women with unexplained infertility vs women taking follicle-maturing drugs for women completely anovulatory or those who release the oocyte before the follicle is mature. Results: There was insufficient power to show a significant difference in the 19.5% live-delivered pregnancy rate found in women with a mean length of infertility duration of 2.1 years who just used P in the luteal phase vs the 30.1% rate seen in women with clear-cut ovulatory defects treated with follicle-maturing drugs in the follicular phase and P in the luteal phase. Conclusions: Though a larger study would possibly show a lower pregnancy rate in those women with unexplained infertility empirically treated with P vs the women with ovulation defects, the empirical use of P allows easy treatment without the side-effects of follicle-maturing drugs, e.g., hostile cervical mucus, vasomotor symptoms or ovarian cysts. The study was not designed to determine if empirical use of follicle-maturing drugs with P support for unexplained infertility would be more effective than P supplementation alone.

Key words: Progesterone; Luteal phase; Unexplained infertility; Natural cycles.

Introduction

A previous study from the 1980’s found that 58 of 100 women who had at least one year of infertility that was not related to tubal, male or cervical problem issues, but who had an endometrial biopsy that was obtained in the late luteal phase and was more than two days out-of-phase, appeared to attain a mature dominant follicle (as defined as making at least an 18 mm diameter dominant follicle and a serum estradiol (E2) ≥ 200 pg/ml) [1]. Randomly treating these 58 women with either progesterone (P) vaginal suppositories in the luteal phase vs a follicle-maturing drug (clomiphene or human menopausal gonadotropin (hMG)) in the follicular phase without luteal phase support, found that out of 31 women treated with P vaginal suppositories, there were 24 clinical pregnancies (77%) in six months and only one spontaneous abortion (4.1%). In contrast 27 women were given follicle-maturing drugs, there were only three clinical pregnancies (11%) in six months and two spontaneous abortions (66.7%) [1]. Interestingly, 25 failures on follicle-maturing drugs during the first six months were now treated during the second six months with P vaginal suppositories and 16 (64%) conceived with only one spontaneous abortion (6.2%) [1].

For the 42 women who did not attain a mature follicle, there was a higher pregnancy rate with follicle-maturing drugs than with luteal phase P. The data showed that the 12 women treated with luteal phase P, there were three (25%) pregnancies and no spontaneous abortions. For the ten women treated with follicle-maturing drugs but with no luteal phase P support, seven (70%) became pregnant but four (57.1%) had spontaneous abortions. Interestingly of the 20 women treated with both follicle-maturing drugs in follicular phase and P in the luteal phase, 14 of them conceived (70% same percentage as without luteal phase support) but only one spontaneous abortion (7.1%) compared to 57.1% without P supplementation [1].

There has been great debate regarding the accuracy of the endometrial biopsy. Of more concern, some insurances refuse payment and thus there could be quite an expense for a test of debatable value. Thus at our institution a decision was made to abandon the endometrial biopsy as a diagnostic tool.

Alternatively, because it was our hypothesis that the main function of P in allowing embryos to implant and in preventing miscarriage is to induce a 34 kDa protein which inhibits natural killer cell activity at the maternal-fetal interface [2-4] (and detection of low levels of this factor is not possible as yet on a wide scale commercial basis), we decided that if we cannot detect a follicular maturation defect, the luteinized unruptured follicle syndrome, a subtle
nancy rates were determined within three treatment cycles. The median number of follicles for group 1 was 1.5 vs one for groups 2 and 3, respectively. Dosages of follicle-maturing drugs were used. The median number vaginally.

However, one could consider this group as unexplained infertility. Actually group 3 was just assumed to have a pure luteal phase defect based on failure to detect any other abnormality. Combining groups 1 and 2 together, the ongoing pregnancy rate was 30.1% (25/83) with P definitely helped in the establishment of the pregnancy. Nevertheless this study has stimulated us to set up a randomized controlled study to compare the efficacy of P therapy alone in the luteal phase vs P alone. For groups 1 and 2 there was a clear-cut ovulatory problem as a contributing cause to the infertility problem, whereas P therapy alone may not have been effective in some women in group 3 who simply did not have a problem with inadequate P secretion in the luteal phase but some other occult problem. Nevertheless, this study has stimulated us to set up a randomized controlled study to compare the efficacy of P therapy alone in the luteal phase vs mild use of FSH in the follicular phase with P in the luteal phase in women with unexplained infertility the results would be somewhat improved by the combined use of low-dose follicle-stimulating drugs and P luteal support vs P alone. For groups 1 and 2 there was a clear-cut ovulatory problem as a contributing cause to the infertility problem, whereas P therapy alone may not have been effective in some women in group 3 who simply did not have a problem with inadequate P secretion in the luteal phase but some other occult problem.

The main objective of this study was to determine the efficacy of empirical luteal phase P therapy over a three-month treatment period for unexplained infertility. As a basis of comparison, group 3 would be compared to women who did not have regular cycles and were clearly anovulatory (group 1) and/or to women with regular menses but who did not attain a mature follicle in the cycle of investigation (group 2).

Table 1. — Pregnancy outcome according to empirical use of luteal phase P support for unexplained infertility vs follicle maturing drugs plus P in women with clear-cut ovulatory defects.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Group 1 (anovulatory)</th>
<th>Group 2 (follicle maturation defect)</th>
<th>Group 3 (mature follicle unexplained infertility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of treatment cycles</td>
<td>60</td>
<td>23</td>
<td>41</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>20</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>(positive ultrasound at 8 weeks)</td>
<td>(33.3%)</td>
<td>(39.1%)</td>
<td>(24.4%)</td>
</tr>
<tr>
<td>Live fetus 12 weeks</td>
<td>18 (30.0%)</td>
<td>7 (30.4%)</td>
<td>8 (19.5%)</td>
</tr>
</tbody>
</table>

Materials and Methods

Consecutive couples limited to females aged ≤ 39.9 years with a minimum of one year of infertility who did not have a problem that required IVF-ET, were selected for evaluation. They had to demonstrate one of the three types of ovulation disorders described above. Actually group 3 was just assumed to have a pure luteal phase defect based on failure to detect any other abnormality. However, one could consider this group as unexplained infertility. No exclusions were made for day 3 serum follicle stimulating hormone (FSH).

Group 1 was treated with either clomiphene citrate or low-dose FSH. Group 2 was treated with low-dose FSH beginning at day 8 or later. Group 3 was treated with luteal phase P supplementation vaginally. No women were purposely hyperstimulated. The lowest dosages of follicle-maturing drugs were used. The median number of follicles for group 1 was 1.5 vs one for groups 2 and 3, respectively. Groups 1 and 2 also received P supplementation. Pregnancy rates were determined within three treatment cycles.

Results

There were 55 couples that were evaluated. The median age was 33 years. The mean length of infertility was 2.1 ± 0.9 years. Primary infertility was present in 44% of the women and secondary infertility in 56%. One hundred twenty-four treatment cycles were evaluated.

The pregnancy outcome according to the type of fertility defect is seen in Table 1. Combining groups 1 and 2 together, the ongoing pregnancy rate was 30.1% (25/83) which possibly related to insignificant power, was not significantly higher than group 3 receiving empirical P therapy (19.5%).

Discussion

Since the women in the group with unexplained infertility (group 3) were all treated with P without placebo controls, it cannot be stated with certainty that the treatment with P definitely helped in the establishment of the pregnancies. Nevertheless there is reason to believe that it did help since the results in clinical and ongoing pregnancies were about 70% as good as anovulatory women (group 1) where treatment was definitely necessary. Furthermore, the mean length of infertility for group 3 was 2.3 ± 1.0 years. Previous studies have suggested that the chance for spontaneous conception in a 33-year-old group of infertile women with this length of infertility in three months would be less than three percent.

These data provide certain treatment strategies. Based on these data, a treating gynecologist without proper monitoring facilities to perform IUI if hostile cervical mucus develops from treatment with clomiphene citrate should probably recommend empirical use of luteal phase P rather than empirical use of follicle-maturing drugs. Such a strategy could reduce the risk of hostile cervical mucus, ovarian cysts, side-effects from the anti-estrogen effect of clomiphene citrate, or the risk of multiple births.

These data should not be interpreted that for unexplained infertility the results would be somewhat improved by the combined use of low-dose follicle-stimulating drugs and P luteal support vs P alone. For groups 1 and 2 there was a clear-cut ovulatory problem as a contributing cause to the infertility problem, whereas P therapy alone may not have been effective in some women in group 3 who simply did not have a problem with inadequate P secretion in the luteal phase but some other occult problem.

References


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Effects of early-cleavage embryo transfer on in vitro fertilization-embryo transfer pregnancy outcomes

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Summary

Purpose: To observe the effects of early-cleavage embryo transfer (ET) on pregnancy outcomes in vitro fertilization-embryo transfer (IVF-ET). Materials and Methods: The data of 6,548 two pro-nucleate (2PN) embryos and 968 patients who underwent IVF or intracytoplasmic sperm injection (ICSI) were analyzed. Of the 968 cycles, early-cleavage embryos were used in 432 cycles (early-cleavage group), late-cleavage embryos were used in 246 cycles (late-cleavage group), and both early and late-cleavage embryos were used in 290 cycles (mixed group). Results: High-quality embryo rate was significantly higher in early-cleavage group than in late-cleavage group (82.74% vs 59.83%; p < 0.01). Both clinical pregnancy and implantation rates in IVF or ICSI were significantly higher in early-cleavage group than in late-cleavage group (all p < 0.01). In ICSI, both clinical pregnancy and implantation rates were significantly higher in mixed group than in late-cleavage group (all p < 0.05). Conclusion: Early-cleavage ET can improve pregnancy outcomes in IVF or ICSI.

Key words: Clinical pregnancy rate; Early cleavage; High-quality embryo; Implantation rate.

Introduction

How to choose a high-quality embryo with development potential to improve clinical pregnancy rate has become a focal point in vitro fertilization-embryo transfer (IVF-ET). Embryo morphology score has occupied a leading place in the choice of embryos. In recent years, a great deal of attention has been paid to early cleavage. It has been reported that early cleavage is an indicator of embryo quality and development potential [1]. It has been described that pregnancy outcomes are strongly associated with embryo morphology and high-quality ET has similar pregnancy outcomes, but pregnancy outcomes are not significantly correlated with early cleavage [2]. It has been believed that early cleavage has predictive value for the pregnancy outcomes in intracytoplasmic sperm injection (ICSI), but has no effects on the pregnancy outcomes of IVF [3]. At present, most studies are about that the effects of early cleavage on pregnancy outcomes are evaluated in day two or day three ET, or different stimulation protocols. Little research has been done regarding the effects of early cleavage on pregnancy outcomes in ICSI or IVF. The purpose of this study was to observe the effects of early cleavage on pregnancy outcomes in ICSI or IVF. The purpose of this study was to observe the effects of early cleavage on pregnancy outcomes in ICSI or IVF, and further confirm that early cleavage possesses better embryo development potential. This study has certain significance for improvement in IVF-ET pregnancy outcomes.

Materials and Methods

All study methods were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects enrolled into the study gave written formal consent to participate.

Early cleavage was observed in all IVF and ICSI cycles performed in the present Center between March and August 2011. The data of 968 cycles were retrospectively analyzed. Inclusion criteria included (1) patients were less than 40-years-old; (2) day three high-quality embryos were transferred; (3) IVF was mainly due to unilateral or bilateral oviduct obstruction, chronic pelvic inflammatory disease, endometriosis, polycystic ovary syndrome (PCOS), the frequency of failure of artificial insemination by husband ≥ three, and unexplained infertility; (4) ICSI was mainly due to severe oligo-astheno-teratospermia, obstructive azoospermia, and low fertilization rate after conventional IVF.

Exclusion criteria were (1) donor oocytes or sperm used in IVF; (2) rescue ICSI. Of the 968 patients, 546 patients had primary infertility (56.40%), and 422 patients had secondary infertility (44.60%).

Superovulation protocol

Superovulation was performed according to long-protocol or ultralong-protocol [4]. When down-regulation reached the standard, gonadotropin (Gn) which was intramuscularly given. Gn, highly-purified recombinant follicle-stimulating hormone (r-FSH), was gonal-F or puregon (100 IU/ampoule). The dose of Gn was adjusted according to follicular development and serous endocrine. Highly-purified menotropin for injection (75 IU/ampoule) was or was not given in the late follicular phase. When the dominant follicle ≥ 18 mm, 2,000 IU of human chorionic gonadotropin (HCG) and 250 ug of ovidrel were intramuscularly given; 34 to 36 hours later, oocytes were collected by transvaginal ultrasound-guided puncture.

Short-term insemination

The retrieved oocytes were incubated for two to three hours, then placed in sperm (1.0 x 10^6/ml/1.2 x 10^6/ml). Four to six hours later, most cumulus cells were removed. After fertilization was determined according to second polar body, the embryos were incubated for 16-18 hours followed by observing pronuclei.
ICSI insemination

The retrieved oocytes were incubated for two to three hours, then were digested with hyaluronidase to remove cumulus cells. Mature oocytes were used in ICSI, 16-18 hours later, pronuclei were observed. Pronuclei were observed according Scott and Smith scoring system [5]. Two polar bodies or two pronuclei, or both two polar bodies and two pronuclei were regarded as normal fertilization. One pronucleus or multiple pronuclei (≥ three) were regarded as abnormal fertilization. No pronucleus and cleavage were regarded as fertilization failure.

Early cleavage was observed 27-28 hours after IVF or 24-25 hours after ICSI, respectively. Cleavage into two cells or more was regarded as early-cleavage embryo.

Embryo quality

Embryo quality was evaluated according to Peter scoring system 48 and 72 hours after ovum pick-up [6]. Embryo grading criteria were (1) grade I: uniform blastomere with intact zona pellucida and moderate refractivity; (2) grade II: slightly nonuniform blastomere and less than ten percent fragment; (3) grade III: blastomere as that in grade II with intact zona pellucida and less than 50% fragment; (4) grade IV: blastomere being viable and more than 50% fragment; (5) grade V: 2PN in day two, or delayed fertilization; (6) grade VI: inviable embryo, blastomere lysis. High-quality embryo includes grades I and II embryos.

Choice and transplantation of high-quality embryos

Grade I or II normal zygotes with six cells or more were transferred 72 hours after ovum pick-up. Two embryos or less were transferred in the patients with the first cycle, three embryos were transferred in the patients with the age ≥ 35 years or more than two cycles. Progesterone was intramuscularly injected and duphaston was orally given for luteal support. HCG in urine and blood were determined 14 days and 18 days after ET, respectively. It was diagnosed as clinical pregnancy that B-mode ultrasound showed embryo sac and fetal heart beat 35 days after ET.

Grouping

In this study, there were 968 cycles. Of the 968 cycles, early-cleavage embryo was used in 432 cycles (early-cleavage group), late-cleavage embryo was used in 246 cycles (late-cleavage group), and both early and late-cleavage embryos were used in 290 cycles (mixed group).

Statistical analysis

Statistical analysis was performed with SPSS 16.0 software. Measurement data were expressed as mean (x ± s) and were analyzed with t test. Numeration data were expressed as rate and were analyzed with Chi-squared (χ²) test. Test criterion was set at alpha (α) = 0.05 and statistical significance was established at p < 0.05.

Results

In 968 patients with the age of 30.93 ± 4.47 years, fertility rate was 80.80% (7,819 / 9,676) and cleavage rate was 96.11% (7,515 / 7,819). High-quality embryo rate was significantly higher in early-cleavage embryos (82.74%) than in late-cleavage embryos (59.89%) (p < 0.01, Table 1).

Pregnancy outcomes in IVF

This study included 694 IVF cycles. There were no statistical differences in age, duration of infertility, dose of Gn, basal FSH, levels of estradiol (E2), and progesterone (P) on HCG day and endometrial thickness on the day of ET between the three groups (all p > 0.05). The number of retrieved oocytes was significantly more in early-cleavage group than in mixed group (p < 0.05). Cleavage rate was significantly higher in early-cleavage group (97.26%) than in other two groups, and high-quality embryo rate was significantly lower in late-cleavage group (52.56%) than in other two groups (all p < 0.01). There was no significant difference in the number of high-quality ETs between the three groups. Clinical pregnancy and implantation rates were significantly higher in early-cleavage group (61.92% and 38.47%) than in other two groups (all p < 0.05). Clinical pregnancy and implantation rates were similar in late-cleavage group and mixed group. There were no significant differences in high-order birth rates and spontaneous abortion rates between the three groups (Table 2).

Pregnancy outcomes in ICSI

This study included 274 ICSI cycles. There were no statistical differences in general status between the three groups (all p > 0.05). Fertility rate was significantly higher in late-cleavage group (76.11%) than in mixed group (p < 0.05), but cleavage rate was significantly lower in late-cleavage group (93.5%) than in other two groups (all p < 0.01), and high-quality embryo rate was lower in late-cleavage group compared with other groups but without significant difference. Clinical pregnancy and implantation rates were significantly higher in early-cleavage group (73.13% and 45.21%) and mixed group (65.43% and 39.76%) than late-cleavage group (all p < 0.05). Clinical pregnancy rate (73.13%) and implantation rate (45.21%) in early-cleavage group were higher compared with mixed group but without statistical significance (all p > 0.05). There were no significant differences in high-order birth rates and spontaneous abortion rates between the three groups (Table 2).

Table 1. — Relation between early cleavage and high-quality embryos.

<table>
<thead>
<tr>
<th></th>
<th>Early-cleavage embryos</th>
<th>Late-cleavage embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of normal fertilized ovum (n)</td>
<td>3,384</td>
<td>3,164</td>
</tr>
<tr>
<td>No. of day 3 high-quality embryos (n)</td>
<td>2,800</td>
<td>1,895</td>
</tr>
<tr>
<td>High-quality embryos in IVF (%)</td>
<td>(82.74)*</td>
<td>(59.89)</td>
</tr>
<tr>
<td>High-quality embryos in ICSI (%)</td>
<td>(84.78)</td>
<td>(60.31)</td>
</tr>
<tr>
<td></td>
<td>(440 / 519)*</td>
<td>(781 / 1,295)</td>
</tr>
</tbody>
</table>

IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; * indicates p < 0.01, compared with late-cleavage embryos.
Effects of early-cleavage embryo transfer on in vitro fertilization-embryo transfer pregnancy outcomes

Table 2. — Comparison of general status and pregnant outcomes in IVF between the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Early-cleavage group</th>
<th>Late-cleavage group</th>
<th>Mixed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of IVF cycles (n)</td>
<td>365</td>
<td>120</td>
<td>209</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.8 ± 4.0</td>
<td>31.6 ± 4.0</td>
<td>30.9 ± 3.9</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>4.1 ± 2.8</td>
<td>4.3 ± 3.0</td>
<td>4.1 ± 3.1</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>7.8 ± 2.0</td>
<td>7.8 ± 2.4</td>
<td>7.3 ± 2.2</td>
</tr>
<tr>
<td>Dose of Gn (IU)</td>
<td>2.094 ± 837.2</td>
<td>2.377 ± 809.3</td>
<td>2.277 ± 838.0</td>
</tr>
<tr>
<td>E2 on HCG day (pg/ml)</td>
<td>4.427 ± 2.1566</td>
<td>4.409 ± 2.2188</td>
<td>4.043 ± 2.0852</td>
</tr>
<tr>
<td>P on HCG day (ng/ml)</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.4</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>Endometrial thickness on ET day (mm)</td>
<td>12.4 ± 2.6</td>
<td>12.3 ± 2.3</td>
<td>12.5 ± 2.6</td>
</tr>
<tr>
<td>No. of retrieved oocytes (n)</td>
<td>10.5 ± 4.9</td>
<td>9.9 ± 5.1</td>
<td>9.5 ± 4.8</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>84.78 (1243/1525)</td>
<td>83.04 (990/1193)</td>
<td>83.14 (1657/1993)</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>97.54 (1335/1384) **</td>
<td>92.92 (910/989)</td>
<td>94.93 (1573/1675)</td>
</tr>
<tr>
<td>High-quality embryo rate (%)</td>
<td>63.82 (2103/3254)</td>
<td>52.56 (483/919)</td>
<td>62.17 (978/1573)</td>
</tr>
<tr>
<td>No. of transferred embryos (n)</td>
<td>2.0 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>61.92 (226/365)  **</td>
<td>40.43 (94/240)</td>
<td>44.98 (94/209)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>38.47 (287/746) **</td>
<td>28.57 (70/245)</td>
<td>31.85 (143/449)</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>9.29 (21/226)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-order birth rate (%)</td>
<td>32.74 (74/226)</td>
<td>38.78 (19/49)</td>
<td>41.49 (39/94)</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of general status and pregnant outcomes in ICSI between the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Early-cleavage group</th>
<th>Late-cleavage group</th>
<th>Mixed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ICSI cycles (n)</td>
<td>67</td>
<td>126</td>
<td>81</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.8 ± 4.5</td>
<td>30.2 ± 4.8</td>
<td>30.2 ± 5.2</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.9 ± 2.5</td>
<td>4.7 ± 3.1</td>
<td>4.6 ± 3.1</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>7.3 ± 1.7</td>
<td>7.7 ± 3.4</td>
<td>7.6 ± 2.2</td>
</tr>
<tr>
<td>Dose of Gn (IU)</td>
<td>2.064 ± 673.5</td>
<td>2.117 ± 794.1</td>
<td>2.137 ± 736.1</td>
</tr>
<tr>
<td>E2 on HCG day (pg/ml)</td>
<td>4.750 ± 2.1944</td>
<td>4.058 ± 2.2003</td>
<td>4.269 ± 2.037.7</td>
</tr>
<tr>
<td>P on HCG day (ng/ml)</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.5</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>Endometrial thickness on ET day (mm)</td>
<td>12.8 ± 2.1</td>
<td>12.8 ± 2.6</td>
<td>12.5 ± 2.2</td>
</tr>
<tr>
<td>No. of retrieved oocytes (n)</td>
<td>11.0 ± 4.5</td>
<td>10.4 ± 4.7</td>
<td>10.3 ± 3.1</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>73.57 (496/674)</td>
<td>74.11 (496/674)</td>
<td>70.94 (542/764)</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>99.76 (1345/1346) **</td>
<td>92.92 (910/989)</td>
<td>94.93 (1573/1675)</td>
</tr>
<tr>
<td>High-quality embryo rate (%)</td>
<td>63.82 (2103/3254)</td>
<td>52.56 (483/919)</td>
<td>62.17 (978/1573)</td>
</tr>
<tr>
<td>No. of transferred embryos (n)</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>73.13 (496/674) **</td>
<td>46.03 (58/126)</td>
<td>65.43 (53/81)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>45.21 (60/1346)</td>
<td>29.82 (82/275)</td>
<td>39.76 (66/166)</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>6.12 (4/69)</td>
<td>8.62 (5/58)</td>
<td>7.55 (4/53)</td>
</tr>
<tr>
<td>High-order birth rate (%)</td>
<td>32.65 (16/49)</td>
<td>43.10 (25/58)</td>
<td>45.33 (13/29)</td>
</tr>
</tbody>
</table>

ICSI: intracytoplasmic sperm injection; FSH: basal follicle-stimulating hormone; Gn: gonadotropin; HCG: human chorionic gonadotropin; ET: embryo transfer; ** indicates p < 0.05, compared with early-cleavage group and mixed group; * indicates p < 0.05, compared with early-cleavage group and mixed group.

Discussion

The final goal of assisted-reproductive technology is to improve clinical pregnancy rate and reduce high-order birth rate, so the choice of high-quality embryos with development potential is crucial to ET. At present, there are two kinds of selection criteria for cleavage-stage embryos. One kind is pronucleus morphology scoring system. The evaluation of cleavage-stage embryos by observing the size, number, and arrangement of nucleoli is readily affected by subjective factors of observers because pronucleus development has some characteristics, such as time sequence and three-dimensional spatial distribution, and the developmental outcomes of the embryos with high pronucleus morphology scoring are not necessarily good. Another kind is based on the number, size, and uniformity coefficient of blastomeres, and the proportion of fragmentation, which is most closely related to embryo quality and is one of the most important evaluation methods. However, blastomere morphology scoring system is not ideal for the evaluation of embryo development potential. In order to explore the best method for evaluation of embryo developmental potential, a great deal of attention has been paid to early-cleavage embryos.

Fertilized eggs may divide into two-cell embryos 20 hours after ICSI or 24 hours after IVF, which is the first mitosis of fertilized eggs and is called early cleavage. The time to the first zygotic cleavage varies by eight hours in humans (22-30 hours) [7]. Lundin et al. [3] observed early cleavage in mature oocytes 25-27 hours after insemination, followed by day two ET, and found that embryo quality, pregnancy rate, implantation rate, and live birth rate were significantly improved in early cleavage embryos; therefore it might serve as an independent predictor of live birth rate in ICSI. If the time to observe early cleavage after insemination is extended, early cleavage may be a parameter to select ET in IVF. Fenwick et al. [8] observed the development potential of early-cleavage embryos from the blastocyst stage and found that blastocyst formation and implantation rates were high in early-cleavage embryos, which further confirms that early cleavage is an important biological marker to predict embryo development potential. Since March 2011, the authors have observed early-cleavage 24 hours after ICSI and 27-28 hours after IVF, and found an early-cleavage rate of 51.67%. They also found that high-quality embryo rate was significantly higher in early-cleavage embryos than in late-cleavage embryos, which is consistent with the results from the literature [9]. This may be related to that early-cleavage embryos derive from the oocytes with higher maturity which have strong metabolic adaptation, also may be related to the calcium oscillations caused by sperm entry into oocytes because sperm quality is one of important factors to affect early cleavage. In brief, early cleavage reflects embryo development potential, but whether it affects pregnancy outcomes remains to be determined by large-sample retrospective analysis.

It is reported that early cleavage is only related to embryo development potential but not to pregnancy outcomes [10]; clinical pregnancy and implantation rates are associated with high-quality embryos, but are not associated with early cleavage [2, 11]; early cleavage as a reference standard of day three ET is not necessary [12]. In this study, sample size was large, only day three high-quality embryos were used for ET, and all patients were aged less than 40 years. The present results indicated that in either IVF or ICSI, early-cleavage embryos significantly improved clinical pregnancy and implantation differences in high-order birth rates and spontaneous abortion rates between the three groups (Table 3).
tion rates, which is similar to the results reported by Jing et al. [13]. The authors conclude that there are significant differences in pregnancy and implantation rates between high-quality embryos with early cleavage and high-quality embryos without early-cleavage; compared with late-cleavage high-quality embryos, early-cleavage high-quality embryos can significantly improve pregnancy outcomes. The present results demonstrate that early cleavage is not only related to embryo development potential but is also strongly-associated with pregnancy outcomes. The authors also can see from their results that the effects of early cleavage on ICSI pregnancy outcomes are marked, both partial early-cleavage ET and all early-cleavage ET can significantly increase pregnancy and implantation rates, namely that as long as there is early-cleavage ET, pregnancy outcomes will be improved. High-order birth rate was increased in early-cleavage group, but there was no significant difference compared with late-cleavage group. Based on this, the authors infer that early cleavage may not affect high-order birth rate, which remains to be demonstrated by large-sample statistical analysis. Different from ICSI, partial early-cleavage ET could increase clinical pregnancy and implantation rates in IVF, but there was no statistical significance compared with late-cleavage ET. This may be related to the differences in infertile factors between ICSI and IVF; because infertile factors are mainly from the male in ICSI, so improvement in embryo quality readily produces good effects on pregnancy outcomes, while in IVF, infertile factors are complex, so mixed ET can increase embryo quality to some extent, but does not necessarily improve pregnancy outcomes.

In summary, early cleavage has an important significance. Observation of early cleavage is a good evaluation method for embryos because it is simple, objective, rapid, and the time of embryo exposure to external environment is brief. Early cleavage may be served as an effective indicator of embryo development potential. In day three ET, the preferred choice of early-cleavage ET in ICSI and only early-cleavage ET in IVF, can effectively improve pregnancy outcomes.

References


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**Introduction**

Despite marked progress in assisted reproductive technology (ART), the pregnancy rate per in vitro fertilization-embryo transfer (IVF-ET) cycle stagnates at about one-third over the last decade [1]. One of the major unsolved problems in human reproduction is embryo implantation failure (EIF), which is recognized as negative conception following transfer of morphologically good embryos and/or blastocysts. Given that the blastocyst euploidy rate obtained in ART are 50%-60% [2, 3], development of efficient therapeutic approaches to overcome EIF has a potential to improve IVF-ET cycle. Using a three-mm wide curette, EIF was performed once between days 6 and 12 of the spontaneous cycle. Their IVF-ET outcomes in the subsequent cycle were compared with those in 49 patients who did not opt for EBI. Results: The clinical pregnancy rate (37.5% vs 12.2%), embryo implantation rate (23.6% vs 6.3%), and ongoing pregnancy rate (25.0% vs 8.2%) were significantly higher in the EBI group than in the non-EBI group. No serious complaints and complications were noted. Conclusion: Single curettage EBI in the proliferative phase of the preceding cycle significantly improved IVF-ET outcome in infertile patients with repeated EIF.

**Materials and Methods**

**IVF protocols**

Gonadotropin-releasing hormone agonist (GnRHa) short protocol or antagonist protocol was used for controlled ovarian stimulation. In the former protocol, intranasal spray of buserelin acetate (Buserexcure, 600 µg/day) was initiated on day 1 of the menstrual cycle, whereas intramuscular injection of 300 IU human menopausal gonadotropin (HMG) was started on day 3. In the latter protocol, intramuscular injection of 300 IU HMG was initiated on day 3, while cetrorelix acetate was injected subcutaneously when one or more leading follicles reached a...
maximal diameter of 15 mm. On the day that at least two leading follicles reached a maximal diameter of 18 mm, 5,000 IU human chorionic gonadotropin was administrated intramuscularly. Transvaginal ultrasound-guided (TVUS-guided) oocyte pickup was performed 35 to 36 hours following hCG administration. After being preincubated for three to four hours, the oocytes were subjected to conventional insemination or intracytoplasmic sperm injection (ICSI). On the following day, fertilization was confirmed by the presence of two pronuclei. The embryos were subjected to daily morphological evaluation. According to Veeck’s classification [14], good embryo was defined as grade 1 or 2 embryo (equally cleaved blastomeres). On day 3 following insemination, one of the good embryos was transferred transvaginally into the uterine cavity using ET catheter under the guidance of transabdominal ultrasound. The remaining embryos were further cultivated in blastocyst medium. The embryos that developed to the stage of blastocysts [15] were vitrificated and frozen on day 5 following insemination. Assisted hatching using zona drilling technique was introduced to the patients and performed by the patients’ preference.

Hormone replacement therapy was used in cryopreserved-thawed ET cycles. Oral conjugated equine estrogen, 1.25 mg twice daily, was introduced on day 2 of the menstrual cycle, and increased to 2.5 mg, twice daily, on day 6. Patients returned regularly for TVUS measurement of endometrial thickness on day 12 onwards. Progestogen, two mg twice daily, was introduced if endometrial thickness measured eight mm or greater. On day 5 following progestogen initiation, the blastocysts were thawed and transferred as described above.

Serum hCG concentration was measured on day 11 following ET or on day 9 following blastocyst transfer using an automated enzyme immunoassay. According to the manufacturer’s guidance, the value with 2 IU/l or more was regarded as a positive pregnancy test. Luteal support with progesterone was continued until nine weeks of gestation. Clinical pregnancy was considered as the presence of intrauterine gestational sac at five weeks of gestation. Embryo implantation rate was calculated as the proportion of the ETs with a documented fetal heartbeat at seven to eight weeks of gestation. Ongoing pregnancy was defined as a viable pregnancy at 12 weeks of gestation. EIF was defined as a negative pregnancy test following transfer of high-grade early cleavage embryos and/or blastocysts.

**EBI for repeated EIF**

The study was approved by the local ethical committee of the Institutional Review Board. The infertile patients with a history of three EIFs were enrolled in the study under informed consent. Based upon the patient’s treatment preferences, single EBI was or was not performed once between days 6 and 12 in the spontaneous cycle prior to the subsequent IVF-ET cycle. A thin metal curette (three-mm width) was inserted through the cervical os and advanced gradually into the uterine cavity until resistance was felt. After single scratch in the uterine cavity, curette was removed to confirm endometrial sampling. If endometrial tissue was absent on the curette, an additional scratch was performed. All patients were given a prophylactic two-day oral administration of clarithromycin (400 mg/day).

**Statistics**

Datasets were compared using two-tailed Student’s t-test, non-parametric Mann-Whitney U-test, Fisher’s exact test, or two-by-two contingency table in combination with Pearson’s χ² test. A p value less than 0.05 was considered significantly different.

**Results**

From January 2010 to December 2011, 91 infertile outpatients had three consecutive negative serum pregnancy
Discussion

Based on the findings in rodents that EBI is most effective under the influence of progesterone [16], early clinical trials adopted multiple timed biopsies in the secretory phase (on day 21 and 26) of the preceding cycle [12, 13]. Intrauterine intervention during the secretory phase, however, is fraught with the risk of iatrogenic abortion. The present authors here demonstrated that the proliferative phase of the preceding cycle is an effective and safe period to perform EBI.

One recent preliminary report showed that 45% of infertile patients with a history of repeated EIF conceived clinically in controlled ovarian stimulation/fresh IVF-ET cycles following single EBI in the mid-secretory phase of the preceding cycle [17], but this study lacked a control group. The present authors confirmed that single EBI significantly increases clinical pregnancy, embryo implantation, and ongoing pregnancy rates. On the contrary to the EBI in the preceding cycles, single EBI performed on the day of oocyte pickup had a negative impact on endometrial receptivity and reproductive outcome in fresh ET cycles [18, 19]. The current findings suggest that the preceding cycle of the IVF-ET cycle is a good option for single EBI.

As for the devices, many of the previous studies employed disposable flexible suction catheters to injure the uterine lining [5, 17]. These types of EBI devices are currently unauthorized and officially inaccessible in Japan. Although there are apparently no published trials that directly compared the effects between the different types of EBI devices, the current authors obtained satisfactory reproductive outcomes using a conventional curettage. No serious complaints and complications were seen following curettage biopsy. One obvious benefit of curettage EBI is cost-effectiveness. The current results implicate that curettage EBI is available to infertile patients suffering from repeated EIF.

In conclusion, single curettage EBI in the proliferative phase of the preceding cycle is a safe and effective method to improve IVF-ET outcome in infertile patients with repeated EIF. The authors suggest the availability of this method in infertility treatment, although further studies are required to optimize the conditions for EBI.

References


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Idiopathic premature ovarian failure: what is the most suitable ovarian stimulation protocol?

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Summary

Objective: To evaluate the ovarian response to ovarian stimulation in women with idiopathic premature ovarian failure (POF) in a prospective, controlled, and sequential crossover pilot study. Materials and Methods: Ten women with idiopathic premature ovarian failure and normal karyotype were included in the study. Phase I was comprised of three consecutive control cycles consisting each of estrogen progestin sequential therapy. Phase II was comprised of three consecutive treatment cycles combining the use of gonadotropin-releasing hormone agonist (GnRHa) in the background of estrogen priming, followed by gonadotropin ovarian stimulation and corticosteroid immunosuppression. Results: Ovulation rates in the treatment cycles (0/10; 0%) did not differ from control cycles (0/10; 0%). Conclusions: The findings of this pilot study showed that the combination of estrogen priming, corticosteroid immune-suppression, GnRHa pituitary desensitization, and followed by gonadotropin ovarian stimulation is ineffective in restoring ovarian function in women with idiopathic POF.

Key words: Premature ovarian failure; Ovarian stimulation; Gonadotropins; Corticosteroids; Gonadotropin-releasing hormone agonist; Estrogen replacement.

Introduction

Premature ovarian failure (POF) is the loss of normal ovarian function before the age of 40 years. While affected women suffer mostly from amenorrhea and climacteric symptoms, infertility remains the most significant concern. Although oocyte donation is considered the treatment of choice, it is an unacceptable option for many women and societies. Many forms of follicular stimulation protocols have been suggested to enhance reproductive outcome, but remain highly controversial. Such treatments include estrogen replacement therapy for ovarian sensitization [1, 2], gonadotropin-releasing hormone agonists (GnRHa) for pituitary down-regulation [3-5], corticosteroid administration for immune suppression [6-10], and exogenous gonadotropins for ovarian stimulation. Research on the effect of such therapies among women with POF is limited. In addition, results are often flawed by the fact that women have the potential to ovulate on their own even if they are not on therapy.

The aim of this pilot study was to examine the clinical benefits of a treatment protocol designed to address all hypotheses proposed for ovulation induction in POF (estrogen priming, GnRHa pituitary desensitization, corticosteroid immunosuppression, and gonadotropin ovarian stimulation) on the improvement of ovarian function in women with the idiopathic spontaneous type of this disorder.

Materials and Methods

Patient population

The authors approached a sample of thirty-one women with idiopathic POF with an age range of 18-35 years who presented to a hospital-affiliated private fertility clinic seeking pregnancy. All refused oocyte donation and were offered to be enrolled in the clinical trial, which extended over a period of three years. This study was approved by the Institutional Review Board. All patients were informed about the investigational nature of the treatment, its uncertain outcome, and related psychological consequence; only ten (32.3%) agreed to take part in the trial and signed informed consents.

Women were diagnosed with POF if they had a baseline serum follicle-stimulating hormone (FSH) levels > 40 mIU/ml and estradiol (E2) levels < 20 pg/ml obtained on two separate instances with clinical amenorrhea exceeding six months. Inclusion criteria were: age range between 18 and 35 years, normal 46 XX karyotype, normal autoimmune profile (antinuclear antibodies, thyroglobulin antibodies, thyroid microsomal antibodies, antiperoxidase antibodies), normal hysterosalpingographic evaluation, and normal semen parameters. Excluded were patients who received chemotherapy or radiotherapy.

Study protocol

Three months prior to the trial, all hormonal medications were stopped. The clinical trial which consisted of two phases had a prospective, controlled, and sequential crossover design.

Phase I: Women received cyclical hormonal replacement therapy for three consecutive cycles: conjugated oral estrogens and daily continuously (1.25 mg) and oral medroxyprogesterone acetate daily for 12 days cyclically (10 mg).

Phase II: Women underwent three consecutive treatment cycles. Pituitary desensitization using the GnRHa buserelin acetate (600 µg intra-nasally daily) was started seven days prior...
to the end of the 12-day progestin administration period. Corticosteroid immunosuppression therapy (40 mg orally daily) was also initiated at the same time. Both medications were continued until the end of each treatment cycle in parallel with the daily dose of conjugated estrogens. Controlled ovarian stimulation using human menopausal gonadotropins (HMG) (225 IU intra-muscularly daily) was started on the second day of menstruation and for at least ten days thereafter. Each treatment cycle was preceded by a washout cycle during which cyclical hormonal replacement therapy was administered as described above in control cycles.

Transvaginal ultrasound (TVUS) examinations were performed at baseline and at five-day intervals. Follicular development was considered significant if the follicle largest diameter was 12 mm or above, time at which HMG therapy was continued beyond ten days. Human chorionic gonadotropins (hCG) (10,000 IU single intramuscular injection) were proposed when a follicle exceeding 16 mm in diameter was observed.

Serum E2 measurements were obtained on baseline and at five-day intervals. A rising serum E2 level ≥ 50 pg/ml was considered as a marker of ovarian steroidogenic activity. Serum progesterone (P) was obtained after seven days of hCG administration, and a serum level of ≥ 3.0 ng/ml was considered as an indicator of ovulation. Pregnancy was confirmed by a positive serum β-hCG titer.

**Measures**

The primary clinical outcome measures were the occurrence of ovulation (follicle largest diameter > 16 mm and serum luteal P level of ≥ 3.0 ng/ml). The secondary clinical outcome measures included: follicular development (follicle largest diameter ≥ 12 mm), ovarian steroidogenic activity (rising serum E2 level ≥ 50 pg/ml), and successful pregnancy (serum β-hCG titer ≥ 5 mIU/ml).

**Statistical analysis**

The Fisher exact and chi-squared tests were utilized for data analyses. In order to confirm the success of the proposed treatment protocol over expectant management, a 20% increase in analyses. In order to confirm the success of the proposed treatment protocol over expectant management, a 20% increase in

**Results**

A preliminary analysis of collected data on the first ten treated women demonstrated the failure of the proposed treatment protocol to achieve ovulation in any woman over twenty-five treatment cycles in Phase II and thirty control cycles in Phase I.

Women’s characteristics are shown in Table 1. The mean age was 26 years (range, 18 - 32). All women were nulliparous and only three had primary amenorrhea (30%). The mean duration of amenorrhea among women with secondary POF was 21 months (range, 6 - 36). None of the patients had menstruation during the previous six months. One woman had a positive family history of POF, and none had a history of autoimmune disorders. All women underwent a total of three control cycles in phase I, but only five completed the third treatment cycle in phase II (an attrition rate of 50%).

Table 2 shows the stimulation characteristics of two women found to have active ovarian steroidogenesis in response to the treatment protocol (patients 7 and 8). Ultrasound examinations in the sample population showed the presence of small-sized follicles in six women (20%) on baseline at the start of Phase I and in four (16%) at the onset of Phase II. Detected baseline follicles underwent progressive development beyond 12 mm in diameter in two (6.7%) control and one (4.0%) treatment cycle. None however matured beyond 16 mm to meet the criteria for hCG administration. Limited steroidogenic activity was also detected in all three cycles (Table 3). No women had ovulation and consequently none got pregnant.

**Discussion**

The findings indicate that the use of the combination treatment protocol described, administered in the dosage and for the duration proposed, did not enhance ovarian and/or reproductive functions in estrogen-primed women with idiopathic spontaneous POF.

The hypothesis that POF causes an irreversible ovarian process was rejected by many investigators who reported a 5% to 10% spontaneous conception rates following initial diagnosis [11]. This fact led several researches to seek the use of medical therapies to improve the follicular response in affected women [1, 5-7] with many controversial findings [2-4, 8, 12, 13]. The present study differs from all previously reported ones in that it evaluated a treatment approach that combined all the therapies previously described in the literature in a single protocol. Estrogen supplementation is believed to enhance follicular activity in an estrogen-depleted system by increasing gonadotropin receptor sensitivity [14]. While Tartagni et al. [13], using a double-blind randomized placebo-controlled design, showed that pre-treatment with estrogens significantly improved successful ovulation rates (32%) in women with POF, Taylor et al. [1], using a randomized controlled cross-over design failed to demonstrate similar effects. In an attempt to suppress a presumed putative gonadotropin-driven antigen, Check et al. [2] in an uncontrolled prospective study, demonstrated successful ovulations and pregnancies, following GnRH-a pituitary suppression prior to gonadotropin ovarian stimulation. These findings were not supported by Nelson et al. [3]. As an autoimmune oophoritis was suggested in women with POF [11], Badawy et al. [15] found a significant improvement in ovulation rates (20.7%) when dexamethasone was randomly added to GnRHa plus gonadotropin therapy in women with idiopathic POF. These findings however could not be confirmed by another randomized placebo-controlled study [8].

Because of the protean nature of POF and its many etiologies, a combination treatment approach addressing multiple biological hypotheses, was considered reasonable. Such a strategy was adopted previously by two groups of investigators who reported beneficial results in women with POF [12, 15]. Blumenfeld et al. [12]...
demonstrated high ovulation (87%) and conception (40%) rates with corticosteroid therapy and GnRH a pituitary suppression followed by HMG ovarian stimulation in POF of autoimmune origin over three consecutive treatment cycles. Similarly, Badawy et al. [15] found a significant improvement in ovulation (20.7%) and conception (6.9%) rates in women with idiopathic karyotypically normal POF utilizing a similar combination approach. The present findings failed to demonstrate any measurable benefit in terms of follicle development, ovulation or conception rates in women with idiopathic spontaneous POF treated with the proposed combination stimulation protocol. Despite minor differences in the types, dosages, and time duration of the different agents utilized in these three studies, the most plausible explanation for differences in clinical response rates observed, remains linked to the protean nature of the ovarian disorder and to variations in population genetics and ethnic characteristics.

Ultrasonography was correlated with a better response to ovulation induction therapy. Such an observation, however, was not supported by other investigators [17]. Nelson et al. [17] found a poor correlation between serum E2 concentration and follicular diameter, and concluded that baseline follicles seen on ultrasonography are more likely to be dysfunctional in nature. The present findings concur with the latter view. All three cycles that expressed some follicular activity on stimulation as evidenced by ultrasound and biochemical findings, had originally small follicle-like structures on baseline ultrasound examination prior to initiation of therapy. However, in none did this activity lead to advanced folliculogenesis and ovulation. It follows that the presence of baseline ovarian follicles on ultrasound examination in women with POF is probably of little predictive value and is unlikely to yield any useful follicle response to ovarian stimulation.

One of the major limitations of this study is that a high proportion of women with POF initially approached declined enrollment into the study, yielding a low response rate of 32.3%. This has significantly narrowed the general applicability of these results. In addition, the high “drop-out” rate after the second treatment cycle (Phase II) resulted in an incomplete data set. It also showed that the only two women who experienced positive ovarian follicle-like activity during Phase II belonged to this group, which introduced bias by depriving the study from a number of treatment-response observations in a potentially more responsive group of women. The authors also employed conjugated estrogens for supplementation, which unlike ethinyl-E2, express some cross-reactivity with the serum 17-β E2 assay, potentially interfering with the interpretation of serum E2 data.

In conclusion, estrogen-primed women with idiopathic spontaneous POF did not appear to benefit from a multi-disciplinary approach that combined pituitary down-regu-

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<th>Table 1. — Individual characteristics of women with idiopathic POF.</th>
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(+) indicates a positive finding; (-) indicates the absence of any positive finding.

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<th>Table 3. — The stimulation characteristics of two women with idiopathic POF who had evidence of ovarian follicle-like activity.</th>
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lation, immune suppression, and gonadotropin ovarian stimulation. Infertile women with POF who reject oocyte donation and seek ovarian stimulation, need to be counseled extensively a priori on the low response rates of any investigational treatment strategy proposed. Justice is made by enhancing patient autonomy, which is best-served by explicitly explaining the doubtful beneficence of such investigational treatment strategies.

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Lipid peroxidation and antioxidant status in vagina microenvironment of patients with several common vaginitis


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Summary

Objective: Oxidative stress has been suggested to play an important role in many diseases, including vaginitis. To evaluate oxidative biomarkers in the secretion of cervix samples of vaginitis, this study will illustrate the status of lipid peroxidation and antioxidant status in vaginal microenvironment. Materials and Methods: A total of 257 patients with vaginitis, including candida vaginitis, bacterial vaginosis, and trichomonas vaginitis were involved in this study. Cervico-vaginal fluid was collected from these patients before and after treatment, and the malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), hydrogen peroxide (H$_2$O$_2$), and vitamin C levels were measured by enzyme-linked immunosorbent assay (ELISA). Results: The results revealed that the MDA and H$_2$O$_2$ levels were increased in the vaginitis patients, while there was no significant difference in MDA level among different kinds of vaginitis before treatment. The CAT and vitamin C levels in vaginitis were decreased before treatment. Moreover, the data also showed that the MDA and H$_2$O$_2$ levels were decreased, while the CAT, SOD, and vitamin C levels were increased after received treatment, respectively, and there was no significant difference between controls and vaginitis. Conclusion: This study indicated that oxidative stress played an important role in vaginitis.

Key words: Vaginitis; Oxidative stress; Microenvironment; Biomarker.

Introduction

Vaginitis is a common disease caused by an infection or by non-infectious causes. In women of childbearing age, the incidence of bacterial vaginosis was 40%-50%; while the incidence colpitis mycotica and trichomonas vaginitis were 20%-25% and 10%-15%, respectively [1-3]. Vaginitis is prone to relapse because its mechanism is still unknown [4-5]. Previous studies had demonstrated that the alteration, exudation, tissue damage, oxidative stress, etc might be involved in the infection and inflammation.

Oxidative stress is defined in general as an excess formation and insufficient removal of highly-reactive molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [6, 7]. Free radicals are continually produced in the body as a result of normal metabolic process and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protect against the reduced activity of antioxidant defenses or both of these phenomena result in oxidative stress. Some major antioxidant enzymes are superoxide dismutase (SOD) and catalase (CAT). Malondialdehyde (MDA), which plays important roles in antioxidization damage, helps the animal to cope with oxidative damage [8, 9]. Free radicals under the function of SOD would produce oxygen molecule and hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ would be converted to water under the function of catalase, so as to clean free radicals to reduce lipidic superoxide damage. Excess stress response would inhibit immunity function, causing physiological dysfunction, increasing susceptibility to infection, and even death. The origin of stress includes variation in temperature, infection, shock, etc. Therefore, how to prevent stress response and alleviate the harm caused by stress is currently one of the key subjects of research in this field. However, oxidative stress is not an easily definable condition, and none of the indices used for its evaluation could be defined as the most appropriate criteria in universal terms.

As one kind of infection, vaginitis may be related to oxidative stress, and the ROS and antioxidant enzymes participate in the progress of vaginitis. However, the exact biological role of oxidative stress and antioxidants in patients with vaginitis remains so far equivocal. Besides, the level of oxidative stress in the vagina of the patients with local vaginitis is still unclear.

Mougeot et al. found that oxidative stress in the pathology of many women plays an important role in reproductive and gynecological inflammations [10]. Vaginitis is an opportunistic mucosal infection that affects three out of four women at least once during their reproductive years [11, 12]. It has been suggested that oxidative stress plays an important role in some physiological conditions and in many diseases, including vaginitis [13, 14]. Vaginal infection can be evaluated as a local mucosal infection similar to urinary tract infections, and likewise it is found to be effective on the oxidative stress. One study in rats had proved that vaginal candidiasis caused oxidative stress by damaging antioxidant enzymes, which revealed that the MDA plasma level decreased [15]. In diabetic patients with vaginitis, the down-regulated MDA level was also valid. Another study had shown that a main avenue of defense against fungal infection uses oxidative killing of microorganisms [16], but it remained unknown whether...
the oxidative stress was involved in bacterial vaginitis. In addition, there was no study on the level of oxidative stress in human vaginitis sample. Therefore, the aim of this study was to evaluate the effect of vaginal infection on the oxidative stress by using some oxidative biomarkers in the secretion of cervical samples, and elucidate the effect of oxidative stress on vaginitis in the vaginal microenvironment.

Materials and Methods

Patients

This was a prospective, clinical, and comparative study which was performed between April and August, 2010 in a single centre (Second Hospital of Lanzhou University, Lanzhou, GanSu, China) and involved in 257 patients who had gynecological evaluation within routine checkups or for vulvovaginal symptoms (increased vaginal discharge, genital itching, etc.). The criterion of inclusion and exclusion were the following: all the subjects who were between 20 and 49 years of age and were in follicular phase. Individuals with pre-existing systemic diseases or chronic conditions, such as diabetes or an immunological disease (human immunodeficiency virus or systemic lupus erythematosus), were excluded. The subjects in pregnancy were also ruled out. All subjects enrolled in the study required regular menstrual cycles, refrained from douching, vaginal medications, and no sexual intercourse in last three days before the examination. Furthermore, all patients were refrained from systemic antimicrobial, antifungal drugs, and undergoing chemotherapy and immunosuppressive agent within the previous 30 days.

Based on diagnosis and microbiological examination [17] standardized by vulvovaginal symptoms, all the subjects were divided into four groups: 1) patients with candida vaginitis; 2) patients with bacterial vaginosis; 3) patients with trichomonas vaginitis; and 4) 60 healthy females were also involved in this study as the normal controls. The study protocol and informed consent document were reviewed and approved by the University of Lanzhou Institutional Review Board. Documented informed consent was obtained from all subjects prior to participation in this study.

All the patients received the following therapy against the different vaginitis, respectively: the patients with trichomonas vaginitis were treated with dispersible 500 mg oral ornidazole twice a day for seven days; the patients with candida vaginitis were treated with 0.15 g oral fluconazole twice a day, and miconazole nitrate vaginal suppositories (0.4 g) was inserted vaginally once a day for three days; the bacterial vaginosis patients vaginally received 0.2 g metronidazole vaginal effervescent tablets once a day for seven consecutive days. The therapeutic efficacy was evaluated at 21-35 days after the beginning of treatment, and all the patients had been cured after therapy.

Samples collection

A speculum was used to collect cervico-vaginal fluid from posterior fornix of the vaginal canal. All samples were collected and used the same test system which contained two swabs. Using these swabs, two vaginal samples were taken from each individual. One cotton swab was placed in a tube containing one ml of sterile saline and sent to be evaluated microscopically for the presence of candida sp., trichomoniasis, and “clue” cells. The other cotton swabs (pre-weighed) were placed in a tube containing two ml of sterile saline and stored at –70°C for further MDA, SOD, CAT, H2O2, and vitamin C detection.

Assay of oxidative stress biomarkers

About 100 ul of 8.1% sodium dodecyl sulfate (SDS) was added to dissolve the secretion in the swab, then vortexed, and incubated for 10 min at room temperature. Then 375 ul of 20% acetic acid (pH 3.5) and 375 ul of thiobarbituric acid (0.6%)
Lipid peroxidation and antioxidant status in vagina microenvironment of patients with several common vaginitis

were added. Placing the sample in a boiling water bath for 60 min and cooled at room temperature. Finally 1.25 ml of butanol: pyridine (15:1) was added, and after vortexed and centrifuged at 1,000 rpm for five min, and 750 ul of the organic pink layer was measured at 532 nm. 1, 1, 3, 3-tetraethoxypropane was used as a standard.

Evaluation of SOD activity was determined. In brief, the assay mixture in a final volume of three ml contained sodium pyrophosphate buffer (0082 M, pH 8.3), phenazine methosulphate (186 mM), nitro blue tetrazolium (300 mM), nicotinamide adenine dinucleotide (NADH) (780 mM), diluted enzyme preparation, and distilled water. The reaction was initiated by the addition of NADH, following incubation at 37.8°C for 90 s. The reaction was stopped by adding one ml glacial acetic acid and the reaction mixture was vigorously shaken with four ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged, and butanol layer was separated. The colour intensity of the chromogen in butanol was measured at 560 nm against butanol over a spectrophotometer. A mixture without enzyme preparation was run in parallel to serve as control. The SOD activity was expressed in units/mg protein. One unit of the enzyme was the amount required to inhibit the rate of chromogen formation by 50%.

CAT activity was measured according to the Aebi method. Using a molar extinction coefficient of 43.6 M⁻¹/cm, the rate of the first 30 seconds was used to calculate the activity. Catalase activity was expressed as U mg⁻¹/Hb. The H₂O₂ concentration and the vitamin C level in the secretion of the vagina were both determined. To investigate the level of vitamin C, trichloroacetic acid was added to dissolve the secretion in the swab, and 100 ul assay mixture was dissolved in the three ml solution containing xylene (2.56 mM) and ammonium ferrous sulfate (250 mM) at a 9:1 dilution. The mixture was shaken for 5 s, let stand for 30 min at room temperature, then centrifuged at 2,000 g, for 10 min. Supernatant of optical density was measured at 560 nm to evaluate the level of H₂O₂.

Statistics

The descriptive statistics for each of the variables were calculated. Before analysis, each variable was examined for its distributional characteristics. All the data in the figures and tables are shown as means (± SD). Variation of the oxidative stress markers levels in the results obtained between two groups was calculated by Bonferroni test. The difference of the level of markers before treatment and post-treatment was calculated by paired t-test.

All the above hypothesis tests were two-sided, and a two-tailed p value of 0.05 or less was considered to be statistical significance.

Results

A total of 257 female patients (aged 20-49 years) with vaginitis were involved in this study. Meanwhile, a total of 60 healthy females aged 24-47 years served as the control group. Moreover, there was no significant difference in ages among the four groups (p > 0.05).

MDA

The results revealed that the MDA level in all the vaginitis patients including candida vaginitis, bacterial vaginosis, and trichomonas vaginitis was much higher than the normal controls, respectively (Table 1), while there was no significant difference in MDA level among...
In this study, the authors observed increased MDA and H$_2$O$_2$ levels in vaginitis compared to controls. This was associated with the corresponding decrease in CAT, SOD, and vitamin C levels. The authors therefore speculated that the oxidative stress would play an important role in vaginitis. These results are generally in agreement with some other studies. One study discovered that SOD activity could be a compensatory mechanism of cells that can suppress the superoxide radicals to combat the oxidative stress after Japanese encephalitis virus infection, and they concluded that SOD is involved in scavenging free radicals. Another study found that SOD and CAT together take part in stepwise oxygen reduction [21, 22]. In some other studies addressing liver injury [23], vivax malaria [24], peritonitis [25], the increased MDA level, and decreased SOD level were observed. Hence the increased MDA and H$_2$O$_2$ concentrations and the changes in the activities of the antioxidant SOD, vitamin C, and CAT showed the presence of oxidative stress in vaginitis, due to the infection, via an imbalance between antioxidants and pro-oxidants. In a state of oxidative stress, biological systems are not protected against the oxidative radical challenge that could result in toxic damage or death of the tissues and cells [26].

The elevated MDA and H$_2$O$_2$ concentrations in vaginitis in the present study could be attributed to increased ROS production, resulting in lipid peroxidation. The authors speculated that the infection would induce oxidative stress, such as excessive production free radicals, then destroy the mucosa, and lead to the necrosis of epithelium. Also, the excessive oxidative excess would influence neutrophil migration and healing of mucosa. The results obtained testify that the high levels of MDA and H$_2$O$_2$ in pre-treatment vaginitis may be the result of mucosal cell destruction by endotoxins. Actually, MDA level, a secondary product of lipid peroxidation, was frequently used as a biomarker of oxidative damage to lipids [13]. MDA and lipid peroxides are themselves free radicals with large reaction constants, which lead to oxidative damage, as modifications of proteins such as protein carbonylation. Thus it is possible that the increase in MDA level might have enhanced a system for the detoxification of lipid hydroperoxides [24, 25].

The authors found evidence of oxidative damage as indicated by increased H$_2$O$_2$ and decreased SOD and CAT in vaginitis. Hence, SOD is beneficial only in the presence of sufficient H$_2$O$_2$-detoxifying enzymes, such as catalase [27]. SOD and CAT are set to maintain the lowest possible levels of ROS in the cell, and is recognized as an essential component of an organism’s self-maintenance [28]. Thus Sheng R. et al. reported a concomitant increase in lipid peroxidation and a drop in antioxidant enzyme activities of SOD and CAT in the rat’s cardiomyocyte [29]. These data demonstrate that enhanced activities of SOD, CAT, and vitamin C can lead to the elimination of ROS. In particular, as other authors have shown, SOD may eliminate organic hydroperoxide from cells and defend cells from potential damage from the products of lipid peroxidation. In the
present study, the decrease in SOD, CAT, and vitamin C activities in vaginitis was observed simultaneously with the increased concentration of MDA. The lower SOD, CAT, and vitamin C activities were probably due to enhanced ROS production [30]. This finding may prove that anti-oxidant material is involved in the inactivation of toxic lipid peroxidation products accumulated during destructive processes in the early stage of infection. The authors assumed that the increased activity of SOD would result in an increased H_2O_2 concentration and consequently in a further increase in CAT activity. CAT is known to be inhibited by the accumulation of superoxide anion during destruction processes in the gut [31]. When vaginitis was cured, there was no statistical difference in the levels of MDA, H_2O_2, SOD, CAT, and vitamin C between the patients and healthy controls, which supports the study’s hypothesis. The authors speculate that with the inflammation that vanished, necrosis of mucosa cells and macrophages would not generate; then the level of oxidative stress and antioxidant decreased accordingly. Oxidative stress, resulting from increased oxidant production and reduced antioxidant levels, appears to be a basic mechanism in vaginitis processes and progressive inflammation in patients [32]. The present study indicated that pathogens induce vaginitis and cause oxidative damage.

Vaginitis treatment has been difficult, because its etiology, pathology, microbiology, and transmissions are not clear; therefore, its pathogenesis is particularly important. The data demonstrated that the oxidative stress was involved in the inflammation response of vaginitis. Some studies found that trichomoniasis can up-regulate the expression of nitric oxide (NO) and inducible nitric oxide synthase (iNOS) expression in monocyte macrophages [33, 34], which were both the pivotal molecule of the oxidative stress. One random clinical trial completed by Khajehi [35] confirmed that vitamin C supplement would enhance the curative effect of vaginitis by inhabitation of the oxidative stress, so the anti-oxidation therapy would benefit vaginitis. Besides, leucorrea microscopy is always the important method for the diagnosis of vaginitis, but the diagnostic method had a low specificity and sensitivity, impacted by the smear results. However, the oxidative stress biomarkers including MDA, CAT, SOD, etc. were the potential markers for the diagnosis of vaginitis. The authors have found the biomarkers varied significantly with vaginitis, compared to the controls, and with the progression of treatment, these biomarkers changed accordingly. Then the markers all were quantitative, and changed with the medical treatment. So compared to leucorrea microscopy, the oxidative stress biomarkers may be a more effective method for vaginitis diagnosis, and could monitor the treatment effectiveness more accurately.

There were several limitations in this study. One of them is that the influence of drug on the oxidative stress could not be excluded in vaginitis. In addition, the effect of the oxidative stress needs further study. This study demonstrated the oxidative stress biomarkers were involved in the infection process, but the effect of free radicals on the epithelial cells or the pathogen was still unclear.

In conclusion, the changes in MDA and H_2O_2 levels as well as the altered activities of the antioxidant enzymes SOD and CAT and vitamin C in vaginitis may be useful evidence for impaired antioxidant status and the occurrence of oxidative stress in vaginitis.

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Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile

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Summary

Purpose of investigation: The aim of this prospective randomized study was to evaluate a red clover based isoflavones supplementation in the treatment of climacteric syndrome and its effects on cardiovascular risk serum profile. Materials and Methods: The study included 150 healthy postmenopausal women that were randomly assigned to receive phytoestrogens tablets, amounting in a total daily intake of 60.8 mg red clover isoflavones plus 19.2 mg soy isoflavones (n = 75), or placebo (n = 75). The authors evaluated the following: daily number of hot flushes and Kupperman Index at baseline and after one and three months; serum total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen, and antithrombin III (ATIII) at baseline and after three and six months. Results: One hundred twenty-eight patients completed the study: 67 in the active group and 61 in the placebo group. The treatment led to a progressive significant reduction (p < 0.05) of the number of hot flushes in the active group compared to placebo already after one month, while Kupperman Index was statistically reduced after three months. No significant variation in total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, PT, PTT, fibrinogen, and ATIII were found. Conclusion: The present findings suggest that a red clover based isoflavones supplementation in healthy postmenopausal women is promptly effective on climacteric syndrome, improves neurovegetative symptoms, safe on cardiovascular risk serum profile, and does not modify lipids and coagulation.

Key words: Menopause; Phytoestrogens; Isoflavones; Red clover; Soy; Climacteric syndrome; Serum lipid; Clotting profile.

Introduction

Isoflavones are the main class of phytoestrogens, compounds naturally found in soy and in a wide variety of other plants, and provided with chemical and biological similarity to estradiol, with great affinity for estrogen receptors and both agonist and antagonist properties [1,2]. Also, red clover (Trifolium pratense), a wild plant by legume family, contains a high amount of the four most important isoflavones (genistein, daidzein, biochanin A, and formononetin) as opposed to soy which only contains the first two [3] and, moreover, their isoflavones are fast- and long-acting with high bioavailability and high receptorial affinity [4-6].

Isoflavones are mainly present as inactive glycosides (glycone isoflavones) and become active compounds (aglycone isoflavones) after removal of the sugar residue by gut bacterial beta-glycosidases [7], therefore, their association with milk ferments with glycosidase activity showed useful [8] and fructo-oligosaccharides showed to modify intestinal bioavailability of isoflavones [9]. Red clover extracts contains aglycone (active) isoflavones, as opposed to soy that contains isoflavones in glycoside (inactive) form requiring intestinal cleavage / activation [10].

Phytoestrogens intake and supplementation, despite very reduced effect compared to endogenous estrogens (in the order of 1,000 times less than estradiol), seem to have a role in the support of postmenopausal women by improving climacteric syndrome [11]. In particular, isoflavones may reduce the incidence and severity of neurovegetative symptoms, specially hot flushes and whole vasomotor disorders [12-18]. Consumption of as little as 30 mg of soy isoflavones, in soy protein or as an extract, reduces vasomotor menopausal symptoms by 30% - 50% (10% - 20% including the placebo effect), even if nowadays 40 - 80 mg are suggested, with larger efficacy for severe symptomatology and fractionate daily dose [19]. In fact, other evidences suggest that isoflavones do not significantly relieve menopausal vasomotor symptoms any better than placebo [20].

Moreover, postmenopausal women are a population at increased risk for coronary artery disease due to the serum changes in lipoprotein metabolism that accompany the loss of endogenous estrogen secretion [21], including elevated total and low-density lipoprotein (LDL)-cholesterol and decreased high-density lipoprotein (HDL)-cholesterol [22]. Phytoestrogens / isoflavones effects on cardiovascular risk serum profile are currently unclear because of conflicting data concerning the effects on serum...
lipids and clotting profile [23-26]: some studies showed a beneficial effect lowering total and LDL-cholesterol and increasing HDL-cholesterol [27-29]; other studies should show a positive effect on blood pressure and coagulation pathways [30], even if not significant modifications on clotting profile are reported, contrary to what is demonstrated with estrogen replacement therapy [31].

The aim of this prospective randomized study in a healthy postmenopausal population was to evaluate a red clover based isoflavones oral supplementation in the treatment of climacteric syndrome, by improving the neurovegetative symptoms, and its safety on cardiovascular risk serum profile, and its impact on serum lipids and clotting profile.

Materials and Methods

One hundred fifty healthy postmenopausal women were enrolled in this prospective randomized study between May and September 2012. Subjects were randomly assigned to two groups: active group (n = 75), daily receiving oral isoflavones (80 mg); placebo group (n = 75), daily receiving oral calcium (500 mg) and vitamin D3 (400 IU).

Investigational patients received two tablets each containing: 8% red clover (Trifolium pratense) extract isoflavones (30.4 mg) plus 40% soy (Glycine soy) extract isoflavones (9.6 mg), amounting in a total daily intake of isoflavones of 80 mg. In particular, red clover isoflavones contained were: genistein 1.0%, daidzein 2.0%, biochanin A 0.7%, formononetin 8.5%, ononin 0.8%, and sissotrin 0.4%.

The patients were selected according to the following inclusion criteria: age ≥ 45 years; clinical (≥ 12 months amenorrhea) and hormonal (serum estradiol < 110 pmol/l, serum follicle stimulating hormone (FSH) > 30 IU/l) diagnosis of postmenopausal condition; climacteric syndrome with neurovegetative symptoms and vasomotor disorders (≥ 20 hot flushes per week). The exclusion criteria were: early menopause (< 45 years); body mass index (BMI) > 28 kg/m²; use of hormone replacement therapy (HRT) less than six months before enrolment; dyslipidaemia and use of interfering drugs; coagulation pathways disorders, use of interfering drugs; high dietary soy intake.

The authors assessed neurovegetative symptoms by daily hot flushes frequency and Kupperman Index at baseline (T0), and after one (T1) and three months (T2). The Kupperman Index covers 11 menopausal symptoms: hot flashes, paresthesia, insomnia, nervousness, melancholia, vertigo, weakness, arthralgia or myalgia, headache, palpitations, and formication (each symptom rated 0-3 and weighted 4 for hot flashes and 2 for paresthesias, insomnia, and nervousness) [32].

Serum lipids evaluated were total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides, at baseline and after three (T2) and six months (T3); clotting profile was defined by prothrombin time (PT) (sec, %, INR), partial thromboplastin time (PTT) (sec, ratio), fibrinogen, and antithrombin III (ATIII) at baseline and after three (T2) and six months (T3).

All values are presented as the mean ± standard deviation (SD). The statistical analysis was used by paired and non-paired Student t test. The level of statistical significance was set at p < 0.05.

Results

One hundred twenty-eight patients completed the one-year study: 67 in the active group and 61 in the placebo group (drop-out active group 10.7%, drop-out placebo group 18.7%: total drop-out 14.7%). Eight patients [two in active group (2.7%) and six in placebo group (8.0%)] dropped out of the study for persistent symptomatology, one patient in active group (1.3%) deferred therapy for gastric intolerance and 13 patients as a whole (8.7%) left follow-up.

The women enrolled had mean age of 54.6 ± 5.0 years and BMI of 25.9 ± 1.7 kg/m²; menopause mean age was 49.7 ± 4.5 years and mean duration of menopause 5.5 ± 5.4 years. The clinic baseline characteristics of the subjects of two groups did not significantly differ (p > 0.05).

The results of the study are detailed in Tables 1-3 and Figures 1 and 2. In substance, the isoflavones treatment led to a progressive significant reduction (p < 0.05) of the number of hot flushes already after one month (from 9.2 ± 9.7 to 5.4 ± 7.0), while Kupperman Index was statistically reduced after three months (from 11.5 ± 7.2 to 7.5 ± 5.6) (Table 1). An identical superiority trend is reg-

| Table 1 – Effects of isoflavones 80 mg daily supplementation on climacteric syndrome (mean value ± SD) |
| - | T0 (baseline) | T1 (1 month of treatment) | T2 (3 months of treatment) |
| Hot flashes (per day) | 9.2 ± 9.7 | 5.4 ± 7.0* | 3.4 ± 5.9* |
| Kupperman Index | 11.5 ± 7.2 | 8.8 ± 5.7* | 7.5 ± 5.6* |

p<0.05 vs. baseline (*)

| Table 2 – Effects of isoflavones 80 mg daily supplementation on serum lipids (mean value ± SD) |
| - | T0 (baseline) | T2 (3 months of treatment) | T3 (6 months of treatment) |
| Total cholesterol | 218.7 ± 40.6 | 211.6 ± 36.5 | 216.1 ± 33.9 |
| LDL-cholesterol | 139.7 ± 40.2 | 133.6 ± 37.1 | 132.6 ± 34.7 |
| HDL-cholesterol | 62.7 ± 17.5 | 65.9 ± 14.1 | 66.2 ± 16.2 |
| Triglycerides | 90.7 ± 39.9 | 96.2 ± 39.3 | 94.5 ± 37.1 |

p>0.05 vs. baseline at T2 and T3
istered comparing these results to those of placebo group (Figures 1 and 2).

On the contrary, no significant variation in total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, PT, PTT, fibrinogen, and ATIII were found either at three (T2) or six months (T3) of treatment (Tables 2,3).

Discussion

Isoflavones (genistein, daidzein, glycitein, biochanin A, and formononetin), phytoestrogens naturally found in soy and a wide variety of other plants, have an ever more important and definite role in postmenopausal care for their chemical-structural similarity (phenolic ring and distance between hydroxyl groups) and biological-receptorial affinity with estradiol, greater for beta (ER-beta) than for alpha estrogens receptors (ER-alpha), overall with both agonist and antagonist properties [1,2]. Isoflavones, therefore, could be a valid choice in postmenopausal women with mild-moderate climacteric syndrome and/or contraindications or decline of classic HRT and, currently, numerous isoflavone preparations derived from soy or red clover are available as dietary supplement to treat menopausal disorders.

The North American Menopause Society (NAMS) recently performed an interesting review of randomized controlled trials (RCTs) on soy isoflavones treatment of postmenopausal vasomotor symptoms (14 RCTs; n=1422; dose range 40-160 mg/day) showing a significant improvement in the isoflavone arms compared to placebo in 11/14 trials (decrease in daily frequency of hot flashes 24% - 60%), with a dose of at least 50 - 60 mg/day for at least 12 weeks for a significant symptom improvement (but without linear dose/duration response relationship) [2]. Similarly, a meta-analysis favored soy isoflavones over placebo, even if the marked heterogeneity of the studies led to conclude that the efficacy on hot flushes could not be established with certainty [33]. On the contrary, some papers did not show comparable significant improvement on postmenopausal symptoms: a review of 17 soy isoflavone RCTs with conflicting results [34]; a meta-analysis barely favorable to isoflavones over placebo [35].

Red clover also, a wild plant by legume family whose flowers are usually dried for therapeutic use, contains a high amount of the four most important isoflavones: genistein and daidzein plus biochanin A and formononetin (methylated precursors of genistein and daidzein), as opposed to soy which only contains the first two [3]. This high content of isoflavones, as well as the shorter time for maximal plasmatic concentration (T-max) and longer plasma half-live (T-1/2) than soy extracts [5], plus the higher transactivational potency for ER-beta than ER-alpha [4,6], suggest red clover as a source of “high-quality” isoflavones.

Table 3 – Effects of isoflavones 80 mg daily supplementation on clotting profile (mean value ± SD).

<table>
<thead>
<tr>
<th></th>
<th>T0 (baseline)</th>
<th>T2 (3 months of treatment)</th>
<th>T3 (6 months of treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>11.5 ± 1.0</td>
<td>11.7 ± 1.1</td>
<td>11.5 ± 1.2</td>
</tr>
<tr>
<td>PT (%)</td>
<td>99.6 ± 11.4</td>
<td>97.8 ± 11.5</td>
<td>99.5 ± 13.3</td>
</tr>
<tr>
<td>PT (INR)</td>
<td>1.03 ± 0.09</td>
<td>1.03 ± 0.08</td>
<td>1.02 ± 0.09</td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>24.2 ± 4.3</td>
<td>27.0 ± 5.2</td>
<td>25.9 ± 4.8</td>
</tr>
<tr>
<td>PTT (ratio)</td>
<td>0.87 ± 0.10</td>
<td>0.90 ± 0.10</td>
<td>0.89 ± 0.11</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>317.3 ± 89.6</td>
<td>307.8 ± 71.8</td>
<td>296.9 ± 62.9</td>
</tr>
<tr>
<td>ATIII (mg/dl)</td>
<td>102.6 ± 18.7</td>
<td>101.9 ± 18.1</td>
<td>101.2 ± 19.5</td>
</tr>
</tbody>
</table>

p > 0.05 vs. baseline at T2 and T3

Figure 1 – Effects of isoflavones 80 mg daily supplementation on climacteric syndrome: number (per day) of hot flashes (mean value)

Figure 2 – Effects of isoflavones 80 mg daily supplementation on climacteric syndrome: Kupperman Index (mean value)

p < 0.05 vs. baseline and placebo at one and three months

p < 0.05 vs. baseline and placebo at three months
A systematic literature review (1951-2006) searching all RCTs of monosupplementation with red clover isoflavones accounted five trials suitable for meta-analysis; this analysis indicates a marginally significant reduction ($p = 0.05$) in the active treatment group (40 - 82 mg daily) compared with the placebo group [36]. On the contrary, a randomized double-blind clinical trial showed a reduction of vasomotor symptoms after 12-month intervention for red clover (57%) not significantly different from placebo (63%) [37], and a meta-analysis failed to show efficacy for red clover extracts over placebo [33].

Moreover, isoflavones impact on cardiovascular risk serum profile is still not fully clear because of conflicting data concerning the effects on serum lipids and clotting profile [23-26]. A meta-analysis of 38 studies concluded that consumption of 31 - 47 g soy protein/day could reduce plasma concentrations of LDL-cholesterol by 19.2 mg of soy isoflavones (plus 19.2 mg) leads to isoflavones supplementation for the treatment of post-menopausal women whose intestinal bacteria able to convert daidzein to equol (aglycone values). Finally, equol is a crucial isoflavone metabolite produced from daidzein by intestinal bacteria with a high affinity for ER-alpha [40], therefore, the clinical (added) value of equol production also requires further study, also comparing women whose intestinal bacteria able to convert daidzein to equol (equal producers) with those without that ability (equal non-producers) [2].

### References


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Outcome in single and twin pregnancies at 20 to 24 weeks gestation: ten years experience in one perinatal center

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E. Herrmann2, J. Yuan1, J. Reinhard1

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Summary

Objective: The aim of this investigation was to evaluate the outcome at 20 to 24 weeks gestation of twin and singleton extremely low birth weight infants. Study Design: The authors conducted a retrospective cohort study of live newborns at 20 to 24 weeks gestation admitted to one neonatal intensive care unit (NICU) from 2000 to 2009. Outcome mortality and predictors of outcome were evaluated. Results were compared for twin and singleton infants. Results: The cohort of infants consisted of 60 singleton infants and 17 twins. The results suggest an increased risk of death for twins when compared with singletons. A correlation between neonatal C-reactive protein (CRP) and bacterial culture positive results on admission to NICU might be a predictor of neonatal outcome. Conclusion: In extremely low birth weight infants, twin delivery is associated with an independent increased risk of death. Both first- and second-born twins are at increased risk.

Key words: Twins; Extremely low birth weight infants; Mortality; Predictors.

Introduction

Current guidelines are restrictive about life-supporting management at 20 to 24 weeks gestation [1], however this is based on limited data.

In twins, a five-fold higher risk of cerebral palsy has been described compared with singletons [2]. Term twins similarly showed to have a higher morbidity and mortality [3]. Controversy exists regarding more adverse outcomes among second-born twins [4-7]. In the same birth weight category, very low birth weight twins (birth weight < 1,500 g) had similar short-term outcomes compared to singleton infants [8, 9]. Growth-restricted twins and singletons were similar in the two groups, although worse compared with non-growth-restricted infants [10].

At 18 to 22 months, corrected age, extremely low birth weight twins were associated with an independent increased risk of death or neurodevelopmental impairment compared with singleton infants [11]. First- and second-born twins were at increased risk [10].

To the authors’ knowledge, there has been no report on the outcome of infants at the border of life (20 to 24 weeks gestation). This retrospective cohort study was designed to compare the short-term neonatal outcomes of twin in comparison with singleton infants for whom life support is controversial.

Materials and Methods

This is a retrospective cohort study of all live newborns at 20 to 24 weeks gestation at the Frankfurt University Hospital from January 1, 2000 to December 31, 2009.

Statistical analysis

For statistical analyses, the Mann-Whitney test and Mantel-
Haenszel test were used. The analyses were carried out using the SPSS Statistics 17.0 software. The means and standard deviation (SD) were processed. A $p < 0.05$ for a two-tailed test was considered statistically significant.

**Results**

In the study group, there was no difference in gestation age between singleton and twin pregnancies (Table 1). In 33.3% of singletons ($n = 60$), they were discharged from hospital, however twins were only discharged in 17.6% ($n = 17$). This difference did not reach statistical significance difference ($p > 0.05$; Table 2). In singletons, 48.3% of newborns’ deaths occurred before 12 hours of life, whereas 52.9% of twins died before 12 hours of life (twin A = 35.3%, twin B = 64.7%) ($p > 0.05$).

Between these two groups, there was no difference in age, body mass index (BMI), fetal birth weight, and length, however in the singleton study group, there were more multiparas ($p = 0.001$; Table 1). There was a trend of higher numbers of in-vitro fertilization (IVF) in the twin cohort, however statistical significance was not reached ($p = 0.08$). Since both groups had similar clinical and socio-demographic risk factors, no logistic regression adjustment was needed.

Between singleton and twin births, there was no significant difference with regards to: fetal presentation, mode of delivery, meconium staining, amniotic fluid, gender, ventilation, PVL, IVH, severe IVH (grade 3 or 4), necrotizing enterocolitis (NEC), bacterial culture positive on admission to NICU, nosocomial infections, histopathological chorionamnionitis, contractions, premature rupture of membranes, anhydramnion, vaginal bleeding, antepartum betamethasone administration, tocolysis, or no cerclage ($p > 0.05$; Table 2).

With the singletons, there was a correlation of survival with betamethasone (0.55, $p = 0.001$), birth weight (0.52, $p = 0.002$), mode of delivery (0.37, $p = 0.03$), and nosocomial infection (0.88, $p < 0.001$), as well as the number of nosocomial infections (0.79, $p < 0.001$). Similarly, there was a negative correlation with positive bacterial culture on admission to NICU, nosocomial infections, histopathological chorionamnionitis, contractions, premature rupture of membranes, anhydramnion, vaginal bleeding, antepartum betamethasone administration, tocolysis, or no cerclage ($p > 0.05$; Table 2).

When adjusted for birth weight, very low birth weight births [12], with an increasing concern that twin gestation may be associated with a higher mortality rate [13, 14]. No differences of neonatal birth weight and gestation age were detected in the two study cohorts, although neonatal mortality was higher in the twin cohort.

When adjusted for birth weight, very low birth weight twin and singleton infants controversy still exists whether in twins there is a higher adverse outcome rate [11] or not [9]. In the present study, in agreement with the study of Wadhawan et al. [11], the authors did find a higher mortality rate in twins compared with singletons. Similarly, twin B had slightly higher mortality rate, which adds to the con-

### Table 1. — Maternal and fetal demographics (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Singleton (n = 60)</th>
<th>Twin (n = 17)</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.5 ± 5.7</td>
<td>31.0 ± 5.2</td>
<td>n. s.</td>
</tr>
<tr>
<td>Parity</td>
<td>1.2 ± 1.1</td>
<td>0.3 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>24.7 ± 5.6</td>
<td>24.7 ± 5.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gestation age (weeks)</td>
<td>23.5 ± 1.1</td>
<td>23.9 ± 1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>603.5 ± 126.3</td>
<td>584.4 ± 152.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fetal length (cm)</td>
<td>30.6 ± 2.3</td>
<td>30.7 ± 1.9</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

### Table 2. — Parameters assessed to predict neonatal outcome (percentage).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Singleton (n = 60)</th>
<th>Twin (n = 17)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge</td>
<td>33.3%</td>
<td>17.6%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Presentation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic? (1)</td>
<td>17.9%</td>
<td>43.7%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Breech? (2)</td>
<td>53.5%</td>
<td>31.3%</td>
<td></td>
</tr>
<tr>
<td>Other? (3)</td>
<td>25.0%</td>
<td>25.0%</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>72.4%</td>
<td>76.5%</td>
<td></td>
</tr>
<tr>
<td>1° C-Section</td>
<td>8.6%</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td>2° C-Section</td>
<td>19.0%</td>
<td>17.6%</td>
<td></td>
</tr>
<tr>
<td>Meconium-stained amniotic fluid</td>
<td>29.8%</td>
<td>11.8%</td>
<td></td>
</tr>
<tr>
<td>Infant Male</td>
<td>37.1%</td>
<td>44.4%</td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>5.7%</td>
<td>44.4%</td>
<td></td>
</tr>
<tr>
<td>Ventilation</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>11.8%</td>
<td>11.1%</td>
<td></td>
</tr>
<tr>
<td>IVH</td>
<td>50.5%</td>
<td>55.6%</td>
<td></td>
</tr>
<tr>
<td>Grade 3 or 4</td>
<td>27.3%</td>
<td>55.5%</td>
<td></td>
</tr>
<tr>
<td>NEC</td>
<td>11.8%</td>
<td>28.6%</td>
<td></td>
</tr>
<tr>
<td>Bacterial culture positive on admission</td>
<td>47.1%</td>
<td>57.1%</td>
<td></td>
</tr>
<tr>
<td>Nosocomial infection</td>
<td>67.6%</td>
<td>44.4%</td>
<td></td>
</tr>
<tr>
<td>Histopathological chorionamnionitis</td>
<td>34.2%</td>
<td>18.8%</td>
<td></td>
</tr>
<tr>
<td>Contractions</td>
<td>46.3%</td>
<td>47.1%</td>
<td></td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
<td>31.5%</td>
<td>41.2%</td>
<td></td>
</tr>
<tr>
<td>Amnion prolapse</td>
<td>40.7%</td>
<td>23.5%</td>
<td></td>
</tr>
<tr>
<td>Anhydramnion</td>
<td>7.4%</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>22.2%</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>57.9%</td>
<td>58.8%</td>
<td></td>
</tr>
<tr>
<td>Tocolysis</td>
<td>65.5%</td>
<td>76.5%</td>
<td></td>
</tr>
<tr>
<td>No cerclage</td>
<td>65.5%</td>
<td>47.1%</td>
<td></td>
</tr>
</tbody>
</table>

A and twin B had a discharge rate of 17.6% and 17.6%, respectively.

### Discussion

In the last decade there has been an increase in multiple births [12], with an increasing concern that twin gestation may be associated with a higher mortality rate [13, 14]. No differences of neonatal birth weight and gestation age were detected in the two study cohorts, although neonatal mortality was higher in the twin cohort.

When adjusted for birth weight, very low birth weight twin and singleton infants controversy still exists whether in twins there is a higher adverse outcome rate [11] or not [9]. In the present study, in agreement with the study of Wadhawan et al. [11], the authors did find a higher mortality rate in twins compared with singletons. Similarly, twin B had slightly higher mortality rate, which adds to the con-
troversy regarding the outcomes of twins with reference to birth order [4-7, 11].

To achieve a more homogenous group of study infants and to exclude the varying resuscitation practices in referring institutions, the authors only evaluated inborn newborns.

In singletons, the mode of delivery was correlated with survival rate, i.e. secondary lower segment Cesarean section had the highest survival rate, however the study numbers were very small. A current larger study described no effect of the mode of delivery on survival, however lower morbidity and better prognosis for neurodevelopment outcome by Cesarean section [15].

Limitations of this study are: retrospective analysis, no long-term follow up data, and important variables that might affect outcomes, such as zygosity and twin-twin transfusion syndrome [16, 17], which were not available.

The present data indicate a higher risk of death in extremely low birth weight infants, independent of the influence of prematurity and birth weight. Further prospective studies, which also examine long-term outcome, are required.

References


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Effects of combined zidovudine/lopinavir/ritonavir therapy during rat pregnancy: morphological aspects

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Summary

Purpose: To evaluate the morphological aspects in rats subjected to an association of the antiretroviral drugs zidovudine/lopinavir/ritonavir in different doses administered throughout the gestational period. Materials and Methods: Forty pregnant rats were randomly allocated into four groups: control (Ctrl) and experimental (Exp1, Exp2, and Exp3), which received zidovudine/lopinavir/ritonavir in the doses of 10/13.3/3.3, 30/39.9/9.9, and 90/119.7/29.7 mg/kg per day from the first to the 20th day of pregnancy, respectively. At term, the animals were euthanized and maternal and fetal organ samples were removed for morphological analysis. Results: No major changes were identified in the group treated with the lowest dosing compared with the control. In group Exp2, the authors found hepatocytes with eosinophilic cytoplasm, pyknotic nuclei, and vasodilation. The proximal convoluted tubules of maternal kidneys showed eosinophilic areas and hyperchromatic nuclei, as well as signs of vasodilation. In the group treated with the highest dose (Exp3), the morphological changes in the maternal kidneys and livers were similar and more pronounced than those found in Exp2. The maternal pancreas of groups Exp2 and Exp3 evidenced moderate and progressive signs of tissue damage. The morphological features of all fetal livers, kidneys, and pancreases were normal. Conclusion: High doses of zidovudine/lopinavir/ritonavir association during the entire rat pregnancy period can cause definite morphological changes in maternal liver, kidneys, and pancreas. On the other hand, the corresponding fetal organs were not affected.

Key words: Zidovudine; Lopinavir; Ritonavir; Toxicology; Pregnancy; Rats.

Introduction

Due to the worldwide situation of human immunodeficiency virus (HIV) infection, taking into consideration epidemiological and clinical aspects, there is an urgent need to deepen our knowledge about the obstetrical challenges related to HIV-infected women [1, 2].

In 1994, under a grant from the National Institute of Allergy and Infectious Diseases of the United States of America, the Aids Clinical Trial Group (ACTG) performed studies and found that zidovudine (AZT) reduced the perinatal transmission of HIV to newborns of infected women by a two-third rate [3, 4]. Based on this outcome, zidovudine became the leading monotherapic drug for prevention of perinatal HIV transmission. The use of this substance in different countries has resulted in lower levels of vertical transmission of the virus. Later, experiments (ACTG 175) showed a desirable effect of zidovudine on pregnant women with cell counts of CD4 < 200 cells/mm³ (i.e., strongly immunodepressed), as well as on women taking AZT before pregnancy [5]. Between 1994 and 1997, other blockers of protease activity were discovered, such as ritonavir and lopinavir. Though side-effects of associations of these drugs can be worse than those due to each drug alone, their antiviral effects are significantly superior. The association results in a significant reduction of viral load in plasma, sometimes to undetectable levels of the virus [6].

One of the several treatment protocols of highly active antiretroviral therapy (HAART) usually includes one nucleoside analog (DNA chain terminator), one protease inhibitor, and either a second nucleoside analog (“nuke”) or a non-nucleoside reverse transcription inhibitor [7]. Though the association of antiretroviral drugs has been shown to be largely safe for the concept [8], the concomitant use of multiple drugs can cause alterations in their pharmacokinetics [9], with unpredictable results on the maternal and/or fetal compartments.

Recently, the present laboratory showed that ritonavir administered to pregnant rats during the entire period of gestation causes definite deleterious effects on fertility and a high maternal mortality rate coexisting with a 100% fetal survival rate, thus suggesting an important compartmentation of the drug [10]. Cunha et al. [11] showed that the administration of a combination of lopinavir plus ritonavir to pregnant rats can cause morphological as well as functional changes in maternal and fetal livers and kidneys, and supratherapeutic doses can be toxic to the animals. Although one cannot extrapolate animal drug effects to human beings, an acceptable first approach to the understanding of human drug effects is their study in animal models.

In the present laboratory, the authors aimed to search for some pharmacokinetic changes of the antiretroviral drugs...
that could provoke adverse effects on the liver, pancreas, and kidneys of pregnant rats and their fetuses. Since few trials have been conducted on the effect of antiretroviral drugs on rat pregnancy, the authors decided to test doses of zidovudine/lopinavir/ritonavir in association, corresponding to one, three, and nine times the human therapeutic doses. These same doses have previously been investigated in an experimental model study that appraised the effects on mothers and litters.

Materials and Methods

Female Wistar rats (rattus norvegicus albinus) of the EPM-1 variant, weighing approximately 200 g each, were provided by the Center for Development of Experimental Models of São Paulo Federal University (UNIFESP). The study was approved by the local animal care committee (Report 0402/09) and followed the guidelines proposed by the Canadian Council on Animal Care [12].

The animals were kept in plastic cages under controlled room temperature (22°C) and artificial light by fluorescent lamps with a constant day/night cycle (lights on 07:00-19:00), with free access to pelleted rat food and tap water. After a seven-day period of adaptation, the animals were allowed to mate in the proportion of one healthy male to three females during two hours. The immediate 24-hour period after mating was taken as day (0) of pregnancy if spermatozoids were detected in vaginal smears [13]. Forty pregnant rats were randomly divided into four groups. The control group (Ctrl) received distilled water and the experimental groups (Exp1, Exp2, and Exp3) received zidovudine/lopinavir/ritonavir in the corresponding doses of 10/13.3/3.3, 30/39.9/9.9, and 90/119.7/29.7 mg/kg/day by gavage from the first to the 20th day of pregnancy [14, 15].

At term, the animals were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) by intraperitoneal route. Upon laparotomy, four ml of maternal blood was taken directly from the ventricular chambers for further biochemical determinations: aspartate transaminase (AST) and alanine aminotransferase (ALT), blood urea nitrogen (BUN), lipase and creatinine, cholesterol activity, and glucose. Fetuses were extracted upon a longitudinal uterine incision. Maternal and fetal samples of livers and kidneys and maternal pancreas were taken and fixed in buffered ten percent formaldehyde for further routine processing, hematoxylin-eosin (H&E) staining, and light microscopy study.

Statistical analyses

The results were analyzed by one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test. Contingency tables were prepared and the Chi-square test was performed to analyze the death rate among the groups. The 2.01 GraphPad InStat software was used for this purpose. The differences were considered statistically significant when $p < 0.05$.

Results and Discussion

The light-microscopic appearance of the maternal livers in the Ctrl and Exp1 groups was essentially undistinguishable. Structures in samples from group Exp2 appeared very similar to those from the Ctrl and Exp1 groups, but congestion could be seen. The same but even more marked alterations were seen in the Exp3 group. There were areas containing a great number of hepatocytes with eosinophilic cytoplasm and heterochromatic nuclei, and extensive congestion (Figure 1).

Figure 1. — Photomicrographs of typical histological sections of rat livers from the control groups (Ctrl) and treated with zidovudine/lopinavir/ritonavir (Exp3, 90/119.7/29.9 mg/kg) throughout gestation. Hepatocytes with eosinophilic cytoplasm and heterochromatic nuclei (arrows) can be observed. x 200, H&E staining.
zidovudine, bind to mitochondrial DNA polymerase gamma, leading to mitochondrial dysfunction and consequently myopathy, myelosuppression, pancreatitis, peripheral neuropathy, and hepatic steatosis [16].

The authors found no significant differences in cholesterol and triglycerides in the four groups. It should be mentioned that a study of lopinavir/ritonavir for 24 weeks showed a rise in triglycerides and total cholesterol, and body fat gain, but these changes were not related to the plasma level of lopinavir [17]. Another study evaluating 22 patients who received lopinavir-ritonavir showed severe dyslipidemia when lopinavir plasma concentrations were above eight mg/ml [18]. It should be mentioned that some lipid droplets were found in the maternal pancreas in group Exp3 (Figure 2).

In the present experiment, the authors noticed reduced serum glucose levels in the Exp3 group compared to the other groups. This could be explained by the fact that combination therapy (HAART) can lead to glucose intolerance, similar to that seen in type I diabetes, suggesting a deleterious effect on the pancreas whereby the conversion of proinsulin into insulin is inhibited due to the activity of protease inhibitors [19]. The facilitated transport of glucose is an energy-independent system which has the primary function of mediating the exchange of glucose between blood and cell cytoplasm, forming a selective pattern among the three major pools of glucose, i.e., the brain, extracellular fluid, and cytoplasm. There are at least seven glucose transporters in mammals. GLUT 1, also called glucose transporter type HepG2, expressed in adult hepatocytes and fetal tissue, is present in erythrocytes. The reduction in blood glucose evokes an increase in GLUT 1, resulting in an increased flow of glucose through the blood-brain barrier, as well as being responsible for the supply of glucose to the placenta. GLUT 3 is the largest carrier in the placenta of rats. It is located in

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**Figure 2.** — Photomicrographs of typical histological sections of a rat pancreas from the control group (Ctrl) and treated with zidovudine/lopinavir/ritonavir (Exp3, 90/119.7/29.9 mg/kg) throughout gestation. The Exp3 group shows fat infiltration (arrows). x 200, H&E staining.

**Figure 3.** — Photomicrographs of typical histological section of a maternal kidney from the control group (Ctrl) and treated with zidovudine/lopinavir/ritonavir (Exp3, 90/119.7/29.9 mg/kg) throughout gestation. Observe glomerulus (GL) and proximal convoluted tubules show eosinophilic areas (*) and hyperchromatic nuclei (arrows). H&E staining, x 200.
the syncytiotrophoblast and is involved in the transfer of glucose to the embryo; it has a high affinity for glucose [20]. A study conducted in 15 HIV-positive patients on the use of lopinavir/ritonavir with hyperinsulinemia and/or dyslipidemia, showed that substitution of lopinavir/ritonavir with atazanavir/ritonavir increased uptake of glucose by muscles in vivo and decreased fasting glucose [21]. These findings are consistent with inhibition of GLUT 4 by lopinavir and ritonavir [22]. It is believed that this finding is due to malnutrition caused by gastrointestinal problems (nausea, poor appetite, and diarrhea), which are adverse effects of lopinavir/ritonavir. A study evaluating the effects of protease inhibitors on the metabolism of glucose found that long-term exposure to protease inhibitors not only induced peripheral insulin resistance, but also affected insulin secretion stimulated by glucose in pancreatic β cells, resulting hyperglycemia [23]. The authors found the opposite in the Exp3 group.

Significant differences were observed regarding the maternal kidneys of the Ctrl group and the other groups under microscopic examination. The gross structural appearance of the kidneys of groups Exp1 and Exp2 were very similar to those and the proximal convoluted tubules showed eosinophilic areas and hyperchromatic nuclei; some vasodilation could also be seen. The kidneys from group Exp3 were structurally similar to those from the other groups. However, more severe alterations of the proximal tubules were seen and there were conspicuous glomerular damages and more extensive vasodilation.

These morphological changes suggest that, although three to four percent of the elimination of the tested drugs occurs through the kidneys, there were just mild deleterious renal effects.

Since the circulating plasma levels of creatinine did not increase and BUN increased in the Exp3 group (Table 1), it is conceivable that the functional capacity of the maternal kidney was able to overcome the effects of the drugs in the doses studied here. The mean values of these metabolites (BUN) were significantly higher in the group Exp3 than those observed in the control animals (Table 1). A possible explanation for this result is drug-induced alteration of the morphology and intrarenal hemodynamics. In fact, since the vasodilation seen in the treated groups (Figure 3) could also occur at the afferent arteriole level, increased serum concentrations of creatinine would have been eliminated through the urine, thus resulting in reduced circulating concentrations of creatinine, but no BUN. The concentration of urea has a higher positivity rate than creatinine, which often can be indicative of some potential renal adverse effects.

Regarding the fetal organs, the morphological appearance in the control group showed proper development of the organs for age. The experimental groups did not present any developmental abnormalities. The passage of various compounds through the placental barrier is blocked or significantly impaired by the placental p-glycoprotein [24]. Other barriers in which P-gps are importantly involved are the blood-brain, the blood-nerve, the blood-testis, and the intestinal barriers [25].

### Conclusion

In conclusion, pregnant rats treated during the entire gestation period with doses of zidovudine plus lopinavir plus ritonavir up to nine times those indicated for humans caused dose-dependent focal morphological changes in maternal liver and kidneys. However, the morphological alterations were not paralleled by systemic biochemical changes, which could be interpreted as specific damages to these organs. Fetal livers and kidneys from animals which had been treated with the highest drug doses did not show morphological alterations.

### References

Effects of combined zidovudine/lopinavir/ritonavir therapy during rat pregnancy: morphological aspects


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De novo symptoms and their impact on life quality in patients following transvaginal reconstructive pelvic surgery with polypropylene mesh

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Summary

Objective: To study the de novo symptoms and their impact on life quality in patients that underwent transvaginal reconstructive pelvic surgery (RPS) with polypropylene mesh. Materials and Methods: From May 2004 to March 2011, 114 severe pelvic organ prolapse (POP) patients with Stage III-IV by POP-Q system underwent RPS with polypropylene mesh. Patients completed pelvic floor distress inventory short form (PFDI-20) and pelvic floor impact questionnaire short form (PFIQ-7) preoperatively and repeated them at two and six months, and one year postoperatively. Results: Ninety-six (84%, 96/114), 85 (75%, 85/114), and 77 (68%, 77/114) patients, respectively, completed the questionnaires at two months, six months, and one year postoperatively. All patients had POP-Q staging scoring ≤ I at one year after surgery. Nineteen (19.8%, 19/96) patients had mesh exposure at two and six months (7.8%, 6/77) at one year follow-up. Most vaginal and pelvic symptoms, urinary and obstructive defecation bothersome symptoms improved significantly at two months postoperatively and this improvement was maintained at the one year follow-up. Mean score of PFDI-20 and PFIQ-7 all improved significantly postoperatively at two and six months and at one year follow-up (p < 0.01). Fifty percent (48/96) of patients had postoperative de novo symptoms at the two months follow-up predominantly presented with bothersome vaginal discharge (35.4%, 34/96) and pelvic muscle symptoms (20.8%, 20/96). Patients with de novo symptoms had higher postoperative POPDI-6 and POPIQ-7 scores (p < 0.05) than those without at the two month follow-up, but no significant difference was seen at the six month and at one year follow-ups. Patients with bothersome vaginal discharge had higher vaginal mesh exposure rate (41.2%, 14/34) than patients without (8.1%, 5/62) (p = 0.0003). One year after operation, 77 (68%) patients completed the non-validated satisfaction questionnaire. Seventy-four (96%, 74/77) patients said that they were either ‘very satisfied’ or ‘satisfied’ with the outcome of their surgery, while three (4%, 3/77) reported unsatisfactory results. Conclusions: De novo symptoms were common after transvaginal RPS with polypropylene mesh, but most of them were moderate and resolved within six months postoperatively and seldomly had a long-term negative impact on their quality of life. The impact of dyspareunia on patients’ sexual function requires further research.

Key words: New symptoms; Polypropylene mesh; Reconstructive pelvic surgery; Pelvic floor dysfunction; Life quality.

Introduction

Vaginal reconstructive pelvic surgery (RPS) is a major surgical procedure for treating severe pelvic organ prolapse (POP), which aims to not only recover the function of pelvic organs but also obtain long-lasting reconstructive effects with the premise of minimising surgical trauma. Traditional RPS can strengthen the reconstructive effect by adopting autologous tissue to strengthen the pelvic floor, but unfortunately the recurrence rate after POP is high, with about one-third of patients requiring reoperation [1]. It has become an urgent clinical need to replace autologous tissue with newly developed synthetic materials. Until recently, French surgeons have taken the lead in adopting synthetic mesh for reinforcement after vaginal RPS surgery [2]. Such surgery usually requires a pre-selected piece of synthetic mesh to retain the pelvic wall to support prolapsed organs using puncture needle in casing. Although the application of synthetic mesh in RPS usually occurs after hernia repair surgery, the performance demands on the material are harsh. There must be sufficient support strength, good compliance, and flexibility to avoid discomfort in patients with appropriate extension of the vagina; this also requires good compatibility, permanence, and low pathogenicity of material tissues. The present, more consistent views show a low pathopoiesis rate with polypropylene material woven with a low weight, large holes, and single-stranded fibres at a flexible range of 20% to 35% [3]. Polypropylene mesh has become the most common synthetic material for transvaginal RPS. However, not all the studies have reported encouraging transvaginal polypropylene mesh RPS results, so the efficacy and safety for polypropylene mesh in vaginal applications of RPS still need adequate randomised controlled trials [4]. Studies on the complications caused by mesh, including mesh exposure, infection, painful sexual intercourse, organ perforation, and vascular nerve injury have been reported, which will affect the life quality of patients, and can even be life-threatening [5]. This is also a common cause of new-onset symptoms after surgery. Since 2005, the U.S. Food and Drug Administration (FDA) has received over 1,000 related reports of serious complications caused by synthetic mesh for POP and incontinence [5], and has issued a warning [6].

Most transvaginal polypropylene mesh RPS-related re-
ports have been related to the surgical failure rate and a reduction in mesh exposure [2,7,8]. In recent years, studies on the effect of implanted network chips on the function of the vagina and adjacent pelvic floor muscle began to appear [9,10], but little research has focused on new postoperative symptoms. It is well-known that many patients with severe POP have pelvic floor dysfunction (PFD) symptoms to varying degrees, which can seriously affect the quality of life of patients. Relief of PFD symptoms and improvements in quality are important criteria to weigh whether the clinical operation was successful. Clinicians have emphasized the improvement of surgery on PFD symptoms in patients with severe preoperative POP, but often overlooked the distresses caused by common new-onset postoperative symptoms. Serious new-onset symptoms still have a negative impact on the quality of life of patients and degree of satisfaction with the treatment, and is worthy of further attention.

Therefore, this study was designed to investigate PFD symptoms and quality of life in patients who underwent RPS using vaginal polypropylene mesh because of severe POP, but also addressed new-onset postoperative symptoms and the effects of these on patient quality of life.

Materials and Methods

Study design

From May 2004 to March 2011, 114 patients with severe POP were treated with polypropylene mesh RPS. The average age of 114 patients was 64 ± 8 years, the mean body mass index (BMI) was 24.6 ± 2.7 kg/m², the average parity was 2.8 ± 1.5, and the average menopause was 14 ± 8 years. The patients had no history of hormone treatment. Of the 114 patients, 65 patients (57%) had more than one kind of medical complication, including 47 cases of hypertension, 11 cases of coronary heart disease and heart surgery, 17 cases of diabetes, four cases of cerebrovascular disease, nine cases of chronic bronchitis, two cases of chronic obstructive pulmonary disease, and one case of non-Hodgkin’s lymphoma combined with systemic lupus erythematosus. The 114 patients were staged as Stage III-IV according to the degree of POP quantification (POP-Q) method, with 84 cases of Stage III (74%) and 30 cases of Stage IV (26%). The recurrence of simple repair of the vaginal anterior and posterior wall occurred in six cases, and vault prolapse after hysterectomy in five cases. Preoperative symptoms associated with PFD are shown in Table 1. New-onset symptoms were defined as PFD symptoms in postoperative patients which troubled the daily life of patients.

Investigation methods: questionnaire

The classic questionnaire used in international women’s PFD research was adopted to evaluate PFD symptoms and their impact on life quality: the pelvic floor distress scale summary table, pelvic floor distress inventory-20 (PFDI-20), and the impact questionnaire summary of the pelvic floor impact questionnaire-7 (PFIQ-7) [11]. PFDI-20 was composed of 20 POP symptom questions, and included three subscales: POP distress inventory (POPDI-6), colorectal-anal distress inventory (CARDI-8), and urinary distress inventory (UDI-6). The PFDI-20 scoring criteria were: 0, asymptomatic; 1, symptomatic but had no effect on life quality; 2, mild impact; 3, moderate impact; and 4, severe impact.

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Before operation</th>
<th>Two months</th>
<th>Six months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFDI-20</td>
<td>62.40 ± 43.70</td>
<td>13.70 ± 7.95*</td>
<td>9.76 ± 14.81*</td>
<td>5.89 ± 12.13*</td>
</tr>
<tr>
<td>POPDI-6</td>
<td>29.58 ± 18.37</td>
<td>2.86 ± 5.62*</td>
<td>1.84 ± 4.17*</td>
<td>0.81 ± 2.24*</td>
</tr>
<tr>
<td>UDI-6</td>
<td>24.92 ± 21.76</td>
<td>8.21 ± 13.39*</td>
<td>6.85 ± 11.12*</td>
<td>3.41 ± 7.02*</td>
</tr>
<tr>
<td>CARDI-8</td>
<td>8.27 ± 12.49</td>
<td>2.54 ± 6.94*</td>
<td>1.10 ± 3.76*</td>
<td>1.66 ± 6.05*</td>
</tr>
<tr>
<td>PFIQ-7</td>
<td>79.90 ± 55.81</td>
<td>14.65 ± 5.84*</td>
<td>9.45 ± 21.23*</td>
<td>6.35 ± 18.04*</td>
</tr>
<tr>
<td>POPIQ-7</td>
<td>39.81 ± 24.95</td>
<td>3.82 ± 6.83*</td>
<td>3.47 ± 11.88*</td>
<td>1.11 ± 3.70*</td>
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<tr>
<td>UIQ-7</td>
<td>32.01 ± 28.83</td>
<td>8.23 ± 19.15*</td>
<td>5.43 ± 12.65*</td>
<td>3.46 ± 10.41*</td>
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<tr>
<td>CARIQ-7</td>
<td>8.04 ± 20.64</td>
<td>3.08 ± 13.07**</td>
<td>0.35 ± 5.32**</td>
<td>1.72 ± 7.35*</td>
</tr>
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Compared with that before operation: *p < 0.01; **p < 0.05.

The subscale scores were the sum of the subscale scores of each question/the number of questions × 25. Score range was from 0 to 100. The total scale score for the three subscale scores were added together, with a range of 0 to 300 points. A higher score indicates more severe symptoms of PFD. The PFIQ-7 also included three scales: the pelvic organ prolapse impact questionnaire-7 (POPIQ-7), colorectal-anal impact questionnaire-7 (CARIQ-7), and urinary impact questionnaire (UIQ-7). Each scale consisted of seven daily life questions to evaluate the impact of PFD symptoms on life quality. The rating criteria were: 0, no effect on life quality; 1, mild effect; 2, moderate impact; 3, severe impact. The subscale scores were the sum of the subscale score of each question/the number of questions × 100 ÷ 3; scores range from 0 to 300. A higher score indicates more severe symptoms of PFD.

Questionnaire methods

The questionnaire was performed together by non-surgical personnel and patients. The preoperative questionnaire was completed before surgery but after admission. The patient follow-up questionnaires were completed after two and six months and at one year. All patients who completed questionnaires were conscious and answered questions independently.

The classic questionnaire used in international women’s PFD research was adopted to evaluate PFD symptoms and their impact on life quality: the pelvic floor distress scale summary table, pelvic floor distress inventory-20 (PFDI-20), and the impact questionnaire summary of the pelvic floor impact questionnaire-7 (PFIQ-7) [11]. PFDI-20 was composed of 20 POP symptom questions, and included three subscales: POP distress inventory (POPDI-6), colorectal-anal distress inventory (CARDI-8), and urinary distress inventory (UDI-6). The PFDI-20 scoring criteria were: 0, asymptomatic; 1, symptomatic but had no effect on life quality; 2, mild impact; 3, moderate impact; and 4, severe impact.

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Compared with that before operation: *p < 0.01; **p < 0.05.
was used for classification data. A statistically significant.

patients, and the damage naturally healed one week after

volume was 248 ± 142 ml (50 - 800 ml). The guide pin
formed in one case. The mean operative time was
was performed in one case. The mean hemorrhage
formed in 95 cases (83.3%), external anal sphincter repair was per-
perineorrhaphy and levator myorrhaphy was performed
suspension surgery was performed in 44 cases (38.6%),
hysterectomy was performed in 102 cases (89.5%), high
mesh RPS was performed in 17 cases (14.9%), vaginal
RPS, front pelvic polypropylene mesh RPS was per-
Table 4.—PFDI-20, PFIQ-7 and subscale score in group with
and without new symptoms two months after polypropylene
mesh RPS (x ± s).

<table>
<thead>
<tr>
<th>Syndrome after</th>
<th>Two months after operation (n = 48)</th>
<th>Six months after operation (n = 48)</th>
<th>12 months after operation (n = 77)</th>
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</thead>
<tbody>
<tr>
<td>Abnormal excretion in vagina</td>
<td>16.55 ± 4.95</td>
<td>10.86 ± 17.15</td>
<td>0.12</td>
</tr>
<tr>
<td>POPDI-6</td>
<td>4.51 ± 7.08</td>
<td>1.20 ± 2.83*</td>
<td>0.003*</td>
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<tr>
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<tr>
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<td>0.40 ± 2.16*</td>
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</tr>
<tr>
<td>UIQ-7</td>
<td>8.73 ± 19.41</td>
<td>1.17 ± 19.09</td>
<td>0.80</td>
</tr>
<tr>
<td>CARIQ-7</td>
<td>3.73 ± 15.28</td>
<td>17.08 ± 10.57</td>
<td>0.72</td>
</tr>
</tbody>
</table>

*p < 0.05.

Statistical methods

SPSS 10.0 software was adopted for statistical analysis; t-tests or rank tests were used for quantitative data and the Pearson χ² test was used for classification data. A p < 0.05 was considered statistically significant.

Results

RPS with polypropylene mesh

Of the 114 patients treated with polypropylene mesh RPS, front pelvic polypropylene mesh RPS was performed in 97 cases (85.1%), while pelvic polypropylene mesh RPS was performed in 17 cases (14.9%), vaginal hysterectomy was performed in 102 cases (89.5%), high sacral ligament vaginal suspension surgery was performed in 95 cases (83.3%), tension-free urethral sling suspension surgery was performed in 44 cases (38.6%), perineorrhaphy and levator myorrhaphy was performed in 95 cases (83.3%), cystoscopy was performed in 95 cases (83.3%), external anal sphincter repair was performed in one case, and line abdominal wall hernia repair was performed in one case. The mean operative time was 180 ± 52 min (90 - 405 min) and the mean hemorrhage volume was 248 ± 142 ml (50 - 800 ml). The guide pin prickling technique caused bladder damage in two patients, and the damage naturally healed one week after indwelling catheter. A six-cm hematoma was detected on the left side of the bladder of one patient with persistent postoperative pain. The patient’s bleeding stopped and improved after local therapy, two months after surgery, and ultrasound results showed the hematoma had disappeared. Routine cystoscopic examination of one patient showed right ureteral obstruction. The urine spray of the bilateral ureter was normal, confirmed by cystoscopy after removing and replacing the right sacral ligament sutures. In three cases urinary tract infection had occurred within one week, one case had type II pulmonary infection combined with type II respiratory failure, and one case had an infection of the perineal body, followed by anti-infective therapy. The postoperative morbidity was 4.4% (5/114).

Postoperative follow-up of patients treated with polypropylene mesh RPS

Follow-up was carried out at two, six months, and at one year after surgery and was 84% (96/114), 75% (85/114) and 68% (77/114), respectively. All the POP-Q stages of the follow-up patients after one year were ≤ Stage I, and the surgical objective success rate was 100%. Nineteen patients showed vaginal mesh exposure two months after surgery (19.8%, 19/96), with an average diameter of 0.64 ± 0.55 cm (0.1 - 2 cm). Mesh exposure was detected in 13 patients six months after surgery (15.3%, 13/85), with an average diameter of 0.40 ± 0.30 cm (0.1 - 1 cm).
Mesh exposed reference was according to treatment methods recommended by Muffly and Barber, including regular observation, topical estrogen cream, and metronidazole suppository, cutting off the exposed mesh in the clinic or hospital, etc. [12]. Most exposure gradually improved based on this method after treatment and recovery, but there were still six cases of patients with exposed mesh one year later. No progress was found in any patient. One patient underwent mesh exposition excision 11 months after surgery because she could not tolerate long-term abnormal vaginal discharge; healing of the exposed parts was found two months later. The preoperative PFD symptoms of the patients were significantly relieved two months after surgery and maintained until one year after surgery (Table 1). Corresponding to this, the PFDI-20 and PFIQ-7 scores and their subscales for the patients after surgery decreased significantly compared to the preoperative scores (Table 2). 50% (48/96) of patients encountered new symptoms two months after surgery (Table 3), but to a lesser extent, the majority of new-onset symptoms were relieved and disappeared six months after conservative treatment. There was no statistically significant difference between the PFDI-20 and PFIQ-7 scores of the patients in the new-onset symptomatic group and those patients with no new symptoms two months later, but the scores of the subscale POPDI-6 and POPIQ-7 were significant (p < 0.05) (Table 4). There was no statistically significant difference between the subscale scores of PFDI-20 and PFIQ-7 in the patients of the two groups six months after surgery (p > 0.05) (Tables 5 and 6).

Abnormal vaginal discharge (35.4%, 34/96), and pelvic muscle symptoms (20.8%, 20/96) were the most common new-onset symptoms of the patients in this group. The results at two months follow-up showed that in 34 cases in the abnormal vaginal discharge group, mesh exposure was found in 14 cases (41.2%), line knots on the vaginal stump in four cases, and granulation in one case. Five patients were found with mesh exposure in 62 cases with no abnormal vaginal discharge (8.1%), a vaginal stump knot in six cases, and granulation in one case. The rates of mesh exposure of the patients in the abnormal vaginal secretion group were significantly higher than those of the patients in the no abnormal vaginal secretion group (p = 0.0003).

A total of three patients were dissatisfied one year after surgery; one patient was dissatisfied with new-onset postoperative pain in the vagina, one patient found no improvement in preoperative urinary frequency and urge incontinence and new-onset stress incontinence, and one patient found no improvement in fecal and urge incontinences. The overall satisfaction rate was 96% (74/77) one year after surgery.

Discussion

The recurrence rate of traditional surgical treatment on POP was high. The anterior vaginal wall is the most common site of recurrence, as 60% of prolapses recurred at the initial site of the vagina, and about one-third of patients required reoperation. Most current domestic and international literature shows that transvaginal polypropylene mesh RPS cannot only improve the effect of surgical repair, but also relieve the symptoms of PFD and improve life quality of patients [13-16], which is consistent with the results in this study. The efficacy of clinical research findings usually depends on whether the changes are statistically significant, but statistically significant differences do not have clinical meaning. The minimal clinically important difference (MCID) is the minimum threshold used to determine whether the change of therapeutic effect has clinical significance. Data that attain or exceed the MCID and has statistically significant changes can be considered clinically significant [17]. Barber’s research has shown that PFDI-20 and PFIQ-7 of the MCID was 45 score and 36 score, respectively [11]. In this study, the changes in PFDI-20 and PFIQ-7 before and after surgery were 48.7 points and 62.3 points, respectively, which were higher than the MCID. It also suggests that polypropylene mesh RPS can really relieve PFD symptoms and improves the life quality of patients and have clinical practice significance.

Polypropylene mesh is currently the most commonly used synthetic material in transvaginal pelvic reconstructive surgery. Although surgery can improve the effect of repair, it can also result in mesh exposure, infection, shrinkage, organ damage, and other complications, and induce new clinical symptoms. Currently, less attention has been paid to these effects in the clinic. Pham et al. have reported postoperative new-onset symptoms of RPS, such as urinary incontinence (27%), urgency (25%), urinary frequency (23%), constipation (22%), and dysuria (10%). The total new-onset symptoms rate of the patients was 42%, but the related surgery methods were non-surgical vaginal polypropylene mesh RPS [18]. Aungst et al. more systematically investigated the new-onset postoperative symptoms in patients after treatment with transvaginal polypropylene mesh RPS. The postoperative new-onset of stress urinary incontinence was 24.3%, and the rate of new-onset symptoms of pelvic muscles including sexual intercourse pain, vaginal pain, groin pain, sitting pain, and walking pain, etc. was 18.3% [19]. In this study, the occurrence rate of new-onset pelvic muscle symptoms (20.8%) was similar, but abnormal vaginal discharge (35.4%) was more common, which may be related to the higher mesh exposure rate (19.8%) in this study. Further statistical analysis also confirmed that mesh exposure was the main reason leading to abnormal vaginal discharge. Although most scholars have taken a series of measures, such as preoperative vaginal mucosa fully treated with estrogen, preventing inverted “T”-shaped incisions, placing the mesh in vaginal muscle without tension, and fully covering with the vaginal mucosa, etc., mesh exposure still occurs at a reported exposure rate of about 4.6% to 15.6% [12, 20, 21]. In this study, the slightly higher rate of mesh exposure may be related to the following factors: 1) the average age of patients was high, the average menopause was longer, and the level of estrogen in vaginal mucosa was relatively poor; 2) with more surgery, operative time was rather long; 3) the proportion of hysterectomy was
needed to be resolved. A study by Lowenstein et al. showed that the incidence of new-onset symptoms was higher and may be related to the level of satisfaction of patients regarding surgery. Some studies have shown that postoperative new-onset symptoms in RPS of patients have a direct impact on achieving the desired objectives and the satisfaction of the patients, and can even affect the quality of life of patients [18, 23]. This study showed that although there was no statistically significant difference between the total PFDI-20 and PFIQ-7 scores of the patients in the no new-onset symptom group and those of the patients in the no new-onset symptom group, the scores of the POPDI-6 and POPIQ-7 subscale of the patients in the no new-onset symptom group were significantly higher compared with those of the patients in the no new-onset symptom group (POPDI-6 = 0.003, POPIQ-7 = 0.000). This suggests that new-onset postoperative vaginal or pelvic symptoms still have a negative impact on the life quality of patients, but there were no statistically significant differences in the scores of the patients on the two groups six months after surgery. This suggests that new-onset symptoms were transient, the degree of the majority of new-onset symptoms was lower and could be eased or even disappear over time. This corresponds with the viewpoint of August et al. in that most new-onset symptoms after RPS are mild, and can be improved by conservative treatment six months after surgery [19]. In this study, the incidence of new-onset symptoms was higher and may be related to the following factors: 1) some preoperative symptoms existed before surgery and were masked by severe PFD symptoms. When surgery effectively relieved the severe PFD symptoms, the original mild symptoms reemerged and were perceived by patients; 2) after the severe PFD symptoms were resolved, slighter PFD symptoms needed to be resolved. A study by Lowenstein et al. also reported a similar phenomenon [23]. The improvement of the original preoperative symptoms of patients was poor and new symptoms will affect the achievement of the prospective target of patients, and thus affect the satisfaction of patients. Hullfish et al. mentioned the significance of the achievement of self-objectives of patients in assessing the therapeutic effect of RPS for the first time [24]. A study by Elkadry et al. also found that the satisfaction of patients with surgery was poorly correlated to traditional objective measurement results, and was related to the level of achieving self-goals [25]. According to the postoperative satisfaction survey, it is not difficult to find that new-onset symptoms of patients was one of the main causes resulting in the dissatisfaction of patients.

Dyspareunia is a common new-onset symptom after transvaginal polypropylene mesh RPS, but unfortunately the patients in this group were not sexually active, and the authors failed to obtain sufficient data for analysis. It was reported that the incidence of painful sexual acts in females with POP was quite different (8%-43%), which makes postoperative clinical evaluation of new-onset dyspareunia more difficult [26]. Lowman performed the POP survey in women after polypropylene mesh RPS, and postoperative new-onset dyspareunia occurred in 16.7% (21/57) of patients, but mild to moderate dyspareunia occurred in 75% of patients; 83% of patients with new-onset sexual intercourse pain were willing to choose this procedure again, which was similar to the previous studies. This suggests that such operations did not affect the patient’s overall sexual health [26]. However, for patients to retain vaginal function and choose transvaginal RPS with polypropylene mesh, the impact of this kind of surgery on sexual function is still worthy of addressing.

In summary, common postoperative new-onset symptoms were induced by polypropylene mesh RPS, but the general extent was low, and most symptoms could be eased or even eliminated by conservative treatment within six months. Few long-term negative effects on the life quality of patients were found. The effect of postoperative dyspareunia on sexual function needs to be further investigated.

References


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Which factors may influence the duration of misoprostol-induced abortion in the second trimester?

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Summary

Background: To investigate the factors that may affect the time interval between induction and fetal expulsion in misoprostol-induced termination of second trimester pregnancy. Materials and Methods: A retrospective analysis of second-trimester pregnancies terminated in the second trimester between October 2008 and 2010 was performed. Induction was done by administration of 400 mcg intravaginal misoprostol. The correlation between the duration of abortion and maternal, fetal, and clinical features were statistically analyzed with multivariate regression analysis. Results: One hundred and seventy-five singleton pregnancies that met the inclusion criteria were evaluated. The average gestational age at the first induction was 18.3 weeks. The mean time interval between the first induction and expulsion was 37.2 ± 21.3 (range 3 to 160) hours. Fetal expulsion occurred significantly at a later gestational age and those with a higher blood glucose level at admission. However, no correlation could be established between the duration of abortion and the number of pregnancies, deliveries, age, hemoglobin levels or platelet count. Conclusions: Misoprostol is safe and effective in induction of abortion during second-trimester pregnancies. The induction-to-abortion interval is longer in patients with hyperglycemia and advanced gestational age. Prospective, randomized studies are necessary to better understand the factors influencing the duration of abortion.

Key words: Termination of pregnancy; Mid-trimester; Abortion; Misoprostol; Duration.

Introduction

Termination of pregnancy (TOP) is one of the most common procedures in obstetrics and gynecology. There is a rise in the first- and second-trimester abortions due to the detection of chromosomal abnormalities and major structural fetal malformations by antenatal screening programs [1, 2]. Termination can be performed either by surgical evacuation or medically by prostaglandins. In general, prostaglandins have been reported to be safe and efficient for the induction of labor and termination of pregnancy [3].

A prostaglandin E1 analog, misoprostol, is widely prescribed for the prevention and treatment of peptic ulcers that may result from long-term use of non-steroidal anti-inflammatory drugs. Although not registered for the induction of abortion, misoprostol has been commonly used for this purpose. Use of labor induction agents in the second trimester are indicated mostly for congenital fetal anomalies and intrauterine fetal demise. However, use of misoprostol for second-trimester TOP is reported to be associated with uterine rupture, especially when combined with intravenous oxytocin infusion [4, 5].

In particular, the time from the initiation of abortion to expulsion is hard to predict. Dickinson had suggested that gestational age, maternal age and parity were parameters that may affect this time interval in cases where vaginal misoprostol was utilized [6]. Similarly, Wagner had stated that the only relevant contributors to estimation of the time interval in this group of patients were gestational age and previous history of spontaneous delivery [1].

The aim of the present study was to analyze the factors that could possibly influence the duration of medical termination of pregnancy in the second trimester. On the basis of the existing evidence, the authors chose 400-mcg dose of misoprostol and administered it vaginally every six hours including up to four doses [4, 5].

Materials and Methods

This retrospective study consisted of all singleton pregnancies terminated using prostaglandins between October 2008 and 2010 in the obstetrics and gynecology department of a tertiary care center. The pregnancies were terminated between 11 and 24 weeks of gestation on the basis of present or expected severe maternal distress due to fetal abnormalities. For each case, maternal and pregnancy-related features, the induction interval, clinical characteristics, and the dose of prostaglandin administered were evaluated from the hospital database. The induction interval as an outcome parameter was defined as the time interval between the first application of the prostaglandin and the end of fetal expulsion.

Pregnancies were terminated due to various major congenital anomalies or intrauterine fetal demise. Gestational week was calculated from the last menstrual period. Congenital anomalies were diagnosed by ultrasonographic examination and a written informed consent was obtained from the patients for TOP. Intrauterine fetal demise was diagnosed when no fetal cardiac function was observed during obstetric ultrasonography.

On the basis of the existing evidence, the authors chose a 400-mcg dose of misoprostol and administered it vaginally every six hours including up to four doses [6, 7]. Induction was repeated every four to six hours until relevant contractions were recorded. Patients with a history of a spontaneous delivery without previous cesarean section had received misoprostol. Prostaglandins were inserted vaginally every four to six hours until relevant contrac-
Results

Data were gathered from the medical records of 175 women with a mean age of 27.8 ± 6.1 (range 16 to 47) years. The average number of previous pregnancies and deliveries were 4.4 and 2.8, respectively. The average gestational age at the first induction was 18.3 (range 15 to 26) weeks. The mean time interval between the first induction and expulsion was 37.2 (range 3 to 160) hours. Total dose of misoprostol administered varied between 400 mcg to 1,600 mcg.

Multivariate regression analysis of maternal, fetal, and clinical variables demonstrated that total dose of misoprostol (p < 0.001), blood glucose level at admission (p = 0.028), and gestational age (p = 0.001) were correlated with the duration of abortion procedure. Whereas, age (p = 0.094), number of previous pregnancies (p = 0.513), and previous live births (p = 0.607), hemoglobin levels (p = 0.074) and platelet counts (p = 0.735) did not seem to influence this time interval (Table 1).

Discussion

TOP in the second trimester can be undertaken for various clinical indications [1]. Several methods for second-trimester abortion have been used, including hysterotomy, dilatation and curettage, hyperosmolar fluid injection into the amniotic fluid, administration of prostaglandins, oxytocin, anti-progesterone, methotrexate or a combination of these agents [2].

Misoprostol is an effective uterotonic drug commonly used for induction of abortion. Pharmacologically, the drug is a methylated analogue of prostaglandin E1 available as 200 μg and 100 μg tablets. Misoprostol stimulates the myometrium causing uterine contractions and subsequently leading to abortion [3-5]. Fever, vomiting, and diarrhea are the major adverse effects of this drug [3-5]. With regard to its few adverse effects, low cost and ease of use, misoprostol is preferred over other prostaglandins for this purpose [7]. Vaginal route is safe and effective and the peak drug concentration is reached between 60 and 120 minutes after its application. Successful termination was generally considered as expulsion of the fetus within 48 hours with a success rate ranging from 60% to 100% [2-5, 7]. Previous studies have evaluated the efficacy of misoprostol at various doses, ranging from 100 mcg to 800 mcg, using a variety of routes and intervals of administration [5, 7]. The dose of misoprostol, however, has yet to be standardized. The authors used 400 mcg to 1,600 mcg misoprostol intravaginally with no significant side-effects. Although some researchers suggest that a shorter induction-to-abortion interval might occur in patients receiving higher doses of misoprostol, others argue that poorer tolerance of those doses mitigates the benefit derived from hastening termination [3-5, 7]. Repetitive administrations of misoprostol are usually needed to induce abortion especially when used as a single agent [7-9].

In this retrospective study, it was shown that parameters with significant impact on the duration of abortion were gestational age at the time of induction, blood glucose level, and total dose of misoprostol applied. By interpretation of this data, accurate prediction of fetal expulsion within 24 h after the first induction attempt may be feasible.

The present findings are consistent with Dickinson’s study, where 1,066 terminated pregnancies were achieved with 400 mcg vaginal misoprostol suppositories. Gestational age was found to have a significant effect impact on the time interval between the first induction and fetal expulsion [6]. Contrary to the present results, they had also suggested that maternal age and previous obstetric history also had an effect on the duration of abortion. The impact of prolonged induction time associated with increasing gestational age may be attributed to the need for greater cervical dilatation due to the increasing fetal size. Grigsby et al. demonstrated that with advancing gestational weeks, there is a reduction of some prostaglandin receptors in the maternal myometrium [10]. It could therefore be argued that prolonged induction interval with gestational age could be due to a reduction in the degree of responsiveness of the uterus to prostaglandins [8]. In contrast, Lo et al., who examined 280 pregnancy terminations between 13 and 23 weeks by vaginal application of 400 mcg misoprostol, could not find a relationship between gestational age and the duration of TOP [11].

From the present results, the impact of gestational diabetes on the duration of abortion is noteworthy. The impact of blood glucose level on prolongation of the time interval may be associated with either macrosomia of fetus in diabetic mothers or decreased contractility of uterus in diabetes. The authors have not come across any publications in PubMed that mention the influence of diabetes on the duration of abortion. Therefore, this result renders the present study unique in this aspect and the authors suggest that further studies are necessary to investigate the effects of diabetes on abortion.

Table 1. — The duration between onset of induction of abortion and expulsion with respect to demographic and clinical variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average (range)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age</td>
<td>27.8 (16-47)</td>
<td>0.094</td>
</tr>
<tr>
<td>Gravida</td>
<td>4.4 (1-15)</td>
<td>0.513</td>
</tr>
<tr>
<td>Parity</td>
<td>2.8 (0-10)</td>
<td>0.607</td>
</tr>
<tr>
<td>Hemoglobin level (g/dl)</td>
<td>12.0 (6.1-18.0)</td>
<td>0.074</td>
</tr>
<tr>
<td>Platelet count (mm$^3$)</td>
<td>263 x 10$^3$ (105-528 x 10$^3$)</td>
<td>0.735</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>18.3 (15-26)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Total misoprostol dose (mcg)</td>
<td>733 (400-1600)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Blood glucose level (g/dl)</td>
<td>93.2 (51-187)</td>
<td>0.028*</td>
</tr>
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</table>

* Statistically significant.
In contrast to these findings, the effect of the previous obstetric history on the duration of abortion procedure was observed by Jannet [12]. The time interval between the first induction and fetal expulsion was significantly shorter in multiparous than in primiparous women [13, 14]. This was explained by the more rapid ripening of the cervix after a previous spontaneous delivery [1, 13, 14]. Maternal side effects of oral prostaglandins, such as diarrhea, nausea, vomiting and were not significant in the present series where misoprostol was intravaginally administered.

The results also showed that gestational age was associated with the time interval from induction to complete abortion. This finding is in parallel with data from literature [1, 6]. However, the authors could not find a correlation between this time interval and parity. This is contradictory to some publications implying that nulliparous women required a significantly longer period of time to completely terminate fetal expulsion than multiparous women presumably due to alterations in cervical compliance [10,11].

The authors found that the total dose of misoprostol was increased in parallel to the prolongation of abortion process. However, this finding is not surprising since repeated doses of prostaglandins are administered in case the intended response is not achieved.

The main limitation of this study is its retrospective character. Further prospective research on this issue may yield more precise data on the parameters affecting the duration of abortion.

Conclusion
Misoprostol is safe and effective for induction of abortion in second-trimester pregnancies. The time interval between induction and abortion is prolonged with advanced gestational age and higher blood glucose levels. Prospective randomized studies are necessary to better understand the factors influencing the duration of abortion.

References

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Pre-pregnancy counseling in Lagos: a report on the first 1,000 cases

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Summary

Objective: To assess the activity of a pre-pregnancy counseling clinic in terms of investigations, counseling, treatment, and subsequent pregnancy outcome. Materials and Methods: Prenatal diagnosis and therapy were given in a tertiary university hospital in Lagos offering referral services for fetal medicine. Design: Review of the first 1,000 couples who referred to the centre from various centres attended from 1992 to 2007. Main outcome measures were assessed through types of referrals, value of diagnostic tests, and subsequent pregnancy outcomes. Results: The main types of referral were previous miscarriage (48.2%), previous fetal abnormality (10.8%), chronic maternal illness (25.1%), and others (15.9%). Routine investigation showed high serological toxoplasmosis, rubella, cytomegalovirus, herpes simplex I, herpes simplex II (TORCH) positive antibodies carrier rate. Subsequent pregnancy outcome did improve in the chronic maternal diseases and previous miscarriage group. Conclusions: This study illustrates the importance of making an accurate assessment of previous problem and current health as a means of determining both maternal and fetal risks in a subsequent pregnancy.

Key words: Pre-pregnancy; Counseling; Clinic; Activity; Report.

Introduction

The mission of a pre-pregnancy counseling clinic or service is to provide education and facilitate healthcare of individual who believe they are at risk for (or known to have) a genetic disease. These individuals might also believe (or known to have) an increased risk to pass on a genetic disease to their offspring. To provide this service properly the counseling team must address: education related to specific disorders including natural history and treatment options, availability of genetic testing when appropriate, genetic risk assessment, reproductive options, and psychosocial implications of information provided [1-3]. This was one of the several considerations behind the establishment in Lagos in 1992 of which was probably the first pre-pregnancy clinic in Nigeria and West African Region. The authors report their experience from 1992 to 2007 at this clinic in the first 1,000 cases reviewed, which convinced them of its value in assessing factors that can contribute to the better management of pregnancy during the different disease states.

Materials and Methods

The clinic is staffed by one consultant obstetrician gynecologist, one staff nurse/midwife, and one laboratory scientist. The obstetrician personally examined all patients. Structured questionnaires were given to the patients to answer and all were interviewed. The questionnaires were well-compiled and were returned immediately. Aside from this, patients were asked to discuss after lecture given and films were shown on medical disease, infertility, pregnancy, breast and endometriosis problems, and voluntary serological screening for all toxoplasma, rubella, cytomegalovirus, herpes simplex I, herpes simplex II (TORCH) were offered. Venous blood samples were taken, centrifuged, separated, and stored at -20°C until batch assayed using TORCH-IgG kits. Over the years, the proportion of the authors' own patients attending the clinic has gradually increased. After 1993 it fluctuated between 81% to 93% on an annual basis.

Results

An attempt was made in Table 1 to analyze the main types of referrals. Commonly, there was more than one problem but an assessment was made regarding which was the most important that led the woman to seek consultation. Four hundred eighty-two (48.2%) patients sought consultation due to previous miscarriage and 108 (10.8%) patients due to chronic illness. Table 2 shows the frequency of previous miscarriage. History of two-times abortion was the highest. Table 3 shows the results of the pregnancy outcome in those with chronic maternal disease and previous miscarriage in whom the authors have data on. The take-home baby rate data was 62.6% (n = 92 out of n = 147) patients who had a follow-up of two to three years after antenatal management. Table 4 shows the types of maternal illnesses. Genotype problem was the most important, 56.6% (142 of 251) cases. Table 5 shows the positivity rate to different pathogens IgG: toxoplasmosis IgG (68.3%), rubella IgG (83.6%), cytomegalovirus IgG (80.0%), hepatitis BsAg (17.8%), herpes simplex type 2 (75.7%), mumps IgG (51.5%), varicella zoster IgG (56.5%), chlamydia trachomatis IgG (82.1%), and syphilis IgG (13.3%).

Discussion

The results of the present study show a large number of women collected over the years with a new form of management, but certain points seem to be very important. The authors run aside from the clinic, a separate lecture and question and answer session, and a Wednesday weekly class. Patients are allowed to go for voluntary blood group, rhesus type, serotype, viral, and fasting glucose screening,
after initial medical obstetrics and gynaecological history are taken. Hormonal studies are also undertaken. If necessary, patients are referred to medical specialist which is another outstanding feature that originates from the analysis, and this is the large number of patients who are concerned about previous premature labor or late spontaneous abortion since low birth weight is the most common associated factor of perinatal mortality; this anxiety deserves attention [4, 5] and supports the findings of other authors [6, 7]. In this group, second time abortion topped the list which did not change management. The second largest group is that with chronic maternal illnesses who are concerned about their chronic disease state. Patients with problems of hemoglobinopathy formed the largest group in an environ-

<table>
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<tr>
<th>Types of problems</th>
<th>No.</th>
<th>Percentage</th>
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<tr>
<td>Previous cesarian</td>
<td>88</td>
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<tr>
<td>Previous vacuum</td>
<td>3</td>
<td>1.9</td>
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<td>Previous forceps</td>
<td>7</td>
<td>4.4</td>
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<tr>
<td>Maternal trauma at delivery</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Anaesthesia difficulties</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>General anaesthesia</td>
<td>14</td>
<td>8.8</td>
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<td>Epidural anaesthesia</td>
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<tr>
<td>Episiotomy</td>
<td>27</td>
<td>16.98</td>
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<td>Bleeding problem</td>
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<td>Sex selection</td>
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<td>Lamaze Reid technique</td>
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<td>1.26</td>
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<tr>
<td>Program planned delivery</td>
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<td>1.9</td>
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<tr>
<td>Multiple delivery</td>
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<td>Chronic maternal disease</td>
<td>62</td>
<td>45</td>
<td>38</td>
<td>7</td>
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<tr>
<td>Previous miscarriage</td>
<td>85</td>
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<td>54</td>
<td>4</td>
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<table>
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<tr>
<th>Maternal illness</th>
<th>No.</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Genotype SS/SC/AS/AC</td>
<td>142</td>
<td>56</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>51</td>
<td>20.3</td>
</tr>
<tr>
<td>Renal disease</td>
<td>17</td>
<td>6.8</td>
</tr>
<tr>
<td>GDM/IDDM</td>
<td>41</td>
<td>16.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types of pathogen</th>
<th>No. of patients</th>
<th>IgG Positive</th>
<th>Rate %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>381</td>
<td>68.3</td>
</tr>
<tr>
<td>Rubella</td>
<td>752</td>
<td>629</td>
<td>83.6</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>809</td>
<td>648</td>
<td>80.0</td>
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<tr>
<td>Hepatitis BsAg</td>
<td>897</td>
<td>160</td>
<td>17.8</td>
</tr>
<tr>
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<td>828</td>
<td>627</td>
<td>75.7</td>
</tr>
<tr>
<td>Mumps</td>
<td>446</td>
<td>230</td>
<td>51.5</td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>421</td>
<td>238</td>
<td>56.5</td>
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<tr>
<td>Chlamydia trachomatis</td>
<td>749</td>
<td>615</td>
<td>82.1</td>
</tr>
<tr>
<td>Syphilis</td>
<td>827</td>
<td>110</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 1. — Other previous labour problems (n = 159).

Table 2. — Types of problems.

Table 3. — Pregnancy outcome in chronic maternal disease and previous miscarriage group (n = 147) at two to three years follow-up.

Table 4. — Chronic maternal illness (n = 251).

Table 5. — Seropositivity IgG rate (Elisa Method).

References


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A suppository for treating cervical erosion and its preparation method

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Summary

Objective: To study a suppository for treating cervical erosion, its preparation method, and to observe its therapeutic effect on cervical erosion in animal models. Methods: Twenty rats were divided into five groups: blank control group, model group, and three different therapeutic concentration groups. Phenol slurry was injected through the vagina to create cervical erosion animal models. After seven days of drug treatment, the difference of cervical histopathology was observed and compared between different groups. Results: Compared with the control group, the model group showed obvious cervical erosion, inflammatory cell infiltration, vascular dilatation, and congestion. Among the administration group, the high administration group had the least inflammation and cell infiltration in the mucous membrane. Conclusions: There is a significant effect of therapeutic drugs in rats with cervical erosion, which is related to the concentration of drugs.

Key words: Double-layer suppository; Cervical erosion; Rats; Pathological changes.

Introduction

Cervical erosion is one of the most common diseases in women, which has a certain relationship with the occurrence of cervical cancer [1]. Cervical erosion is a condition in which the endocervical columnar epithelium protrudes through the external os of the cervix and into the vaginal portion of the cervix, undergoes squamous metaplasia, and transforms into stratified squamous epithelium. Squamous epithelium is covered by columnar epithelium, resulting in a lower resistance to disease because the former is thinner [2].

The most common drug treatment for cervical erosion is a suppository. With the study of suppositories, the double-layer suppository has been shown to be helpful in the treatment of cervical erosion. The suppository consists of two layers, with each layer containing different drugs that successively take effect [3].

Basic fibroblast growth factor (bFGF) is the first clinical growth factor and it can significantly accelerate the formation of granulation tissue and re-epithelialization [4, 5]. Studies have shown that a variety of growth factors gather in local wounds [6], however they are unstable, especially in refractory ulcer wounds [6]. Exogenous bFGF can improve the amount of other growth factors, such as epidermal growth factor (EGF) and transforming growth factor (TGF) when it plays the role in the healing of wounds [7].

Combined with the characteristics of the suppository and drugs, curcuma, borneol, and bFGF can successively take effect with the purpose of extending the duration of action, improving therapeutic effects, and reducing side-effects.

The aim of this study was to prepare a suppository to treat cervical erosion. The three-layer suppository was applied to facilitate successful drug uptake and was used in cervical erosion animal models. The effect of the suppository was measured according to the histopathology of the model groups.

Materials and Methods

Experimental preparation

Twenty clean grade Sprague Dawley (SD) female rats purchased from the Experimental Animal department in Wenzhou Medical College were used with a weight of 150 g to 200 g. The protocols used were consistent with Ethical Principles for Animal Research adopted by the Wenzhou Medical College of Animal Experimentation.

Experimental content: preparation of suppository

This suppository is a three-layer suppository based on hollow double-layer suppository mold: the inner layer is medical absorbent cotton, the middle layer is a gelatin glycerol sustained-release layer with bFGF as the primary therapeutic drug, and the outer layer contains borneol and acts as the sterilisation layer (Figures 1 and 2).

To make the outer layer: 20 g of semi-mixed fatty acid glyceroester was fragmented and melted by 70°C water bath heating, before maintaining the temperature at 50°C and cooling. Curcuma and borneol were ground into a fine powder, and one g of each was mixed with a suppository base before adding it to the suppository mold. The latter was then inserted before solidification and natural cooling.

To make the middle layer: 20 g of gelatin, glycerol, and distilled water were taken in a ratio of 6:3:1 (gelatin: glycerol: distilled water). These were melted by 75°C water bath heating, before mixing with one g of curcuma and adding them to the suppository mold. The temperature was maintained at 40°C and cooling.
bFGF of a corresponding amount was added prior to natural cooling. To make the inner layer: the medical absorbent cotton was formed into a cylinder and placed into the mold.

Experimental methods of animals: animal experiments

Thirty ml of liquid phenol, 40 g of arabic gum, and 50 ml of distilled water were taken to prepare a phenol slurry (w = 37.5%) [5]. Phenol slurry (0.2 ml) was injected into the rat vagina via a stomach tube (injected at different times, 0.1 ml (twice), once/3d, and four times. After the creation of models, the rats were divided into four groups with four rats randomly assigned to each group, including a model group, and an administration group. The blank group was given a similar volume of distilled water. Three days after modeling, the administration groups was given the corresponding amount of drugs (divided into three groups of low grade according to the difference in the density, with a content of bFGF of 100μμg, 10μμg, and 1μμg, respectively). The animals were treated daily for seven days, and were then sacrificed on day eight. The uterine tissue was dissected observed, and information was recorded. The tissue was fixed in formalin and the samples were sliced into paraffin sections, before being stained with Hematoxylin and Eosin (H&E). Light microscopic observation was then performed.

Specimen collection and processing occurred at 24 hours after the last administration when all rats were placed under intraperitoneal anesthesia using chloral hydrate (three ml/kg). The rats were then fixed and the uterine tissue was extracted after dissection. The tissue was fixed in 10% formalin for three days, then placed into an ethyl alcohol series, embedded in paraffin, sectioned at six μμm and stained with H&E, before being observed by microscopy and photographed.

From the day of the modeling, the vaginal and cervical secretions of the rats were observed, indicating whether there was less activity or piloerection [8].

Histopathological changes, namely in the squamous cells were classified as either partially thickened (more than two to three layers); obviously thickened; wildly thickened or unsmooth mucosa [9].

Columnar epithelium translocation was classified according to the following criteria: few regional squamous epithelial were erosive, adjacent columnar epithelial were proliferative; columnar epithelial were obviously proliferative and replaced the squamous epithelial; and squamous epithelial shed.

Inflammatory cell infiltration was classified according to the following criteria: a small amount of inflammatory cell infiltration in the mucous membrane; a moderate amount of inflammatory cell infiltration in the submucosal interstitium; or a vast array of inflammatory cell infiltration in the intestinal mucous membrane.

Results

The preparatory procedure finally resulted in a suppository with three layers (Figure 3). Transfiguration temperature for the outer layer was completely melted within 20 min, and the middle layer was completely melted within 50 min.

During the entire experiment, the weight variations of the animals were recorded and are shown in Table 1. After the cervical erosion animal model establishment, the conditions of the exterior entrance of the vagina were observed (Figure 4). Most of the rats had symptoms of vaginal swelling after model establishment and some of them showed symptoms of purulence. At the same time, they had other symptoms, such as less activity, piloerection, loss of appetite, and weight loss. After a period of therapy, the vaginal secretions of administration group gradually reduced, and four days after administration, the exterior entrance of the vagina of the administration group was basically dry (Figure 5). There was no difference seen in the appearance of the blank group.

After the uterine tissue was extracted, it was morphologically observed. Vascular congestion and edema were observed in the model group. There was some degree of improvement in the administration group (Figure 6).

By using light microscopy to observe H&E uterine tissue sections, interstitial edema, inflammatory infiltration, and squamous hyperplasia were seen in the model group. Compared with the model group, the blank group had no obvious symptoms. Compared with the high administration group, the degree of squamous epithelial thickening of the low administration group was greater. Among the administration group, the low administration group had the most inflammatory cell infiltration in the mucous membrane (Figure 7).

Discussions

Cervical erosion is a common gynecological disease which is difficult to treat. Current treatment methods are of poor efficacy, with long-lasting treatment cycles, so patients do not easily continue the medication. These factors all render cervical erosion difficult to cure, but a hollow triple-layer suppository can overcome these difficulties. The drug of the outer layer releases first, to achieve the purposes of sterilization, then the inner drug is released; therefore, this can extend the duration of its action.

This experiment used a phenol glue solution to establish the model of rat cervical erosion, in a manner that was simple and economic. After modeling, most of the rats showed symptoms of vaginal swelling, and some of them had symptoms of purulence. At the same time, they showed symptoms such as less activity, piloerection, loss of appetite, and weight loss. After a period of therapy, the vaginal secretions of the administration group gradually reduced, and after administration, the exterior entrance of the vagina of the administration group was basically dry, with no difference compared to the blank group in appearance. The clinical symptoms, physical signs and pathological changes were basically in accordance with the clinical states, therefore providing a scientific way to study drugs.

In the model group, conditions presented included interstitial edema, inflammatory infiltration, and squamous hyperplasia. Compared with the model group, the blank group
showed no obvious symptoms. Compared with the high administration group, the degree of squamous epithelial thickening of the low administration group was greater. In comparison with the administration group, the low administration group had the most inflammatory cell infiltration in the mucous membrane. Therefore, the suppository was useful in the treatment of cervical erosion and was related to the concentration of the drugs.
Figure 6. — Uterine tissues: groups 1, 2, and 3 are the low, medium, and high administration groups. Group 4 is the model control group, and the normal group is the blank group.
In conclusion, the three-layer suppository had a significant therapeutic effect on rats with cervical erosion, which was related to the concentration of the drugs: the higher the concentration, the better the therapeutic effects.

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Maternal and umbilical cord oxygen content and acid-base balance in relation to general, epidural or subarachnoid anesthesia for term elective cesarean section

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Summary

Purpose: To compare maternal and neonatal oxygenation and acid-base status after elective cesarean section (CS) under different anesthetic techniques. Materials and Methods: Three hundred and eighty parturients undergoing elective cesarean section were randomly assigned to receive general (GA, n = 140), epidural (EA, n = 117) or subarachnoid anesthesia (SA, n = 123). Blood gases, oxygen content, and acid-base status parameters were measured in maternal artery and umbilical cord vessels. Neonatal Apgar scores were also reassigned to receive general (GA, n = 140), epidural (EA, n = 117) or subarachnoid anesthesia (SA, n = 123). Blood gases, oxygen content, and acid-base status parameters were measured in maternal artery and umbilical cord vessels. Neonatal Apgar scores were also recorded. Results: Umbilical artery pH, HCO3−, and actual base excess (ABE) were significantly higher in the GA compared to SA group (p < 0.001, p < 0.05, and p < 0.05, respectively). Umbilical vein ABE was lower in the SA compared to GA and EA groups (p < 0.05). Oxygen content in maternal artery was higher in the GA and EA groups compared to the SA group (p < 0.05). Neonatal oxygen content in both cord vessels was higher in the GA group compared to EA and SA groups (p < 0.05). Umbilical venous-arterial difference of PO2, oxygen content, and Apgar scores did not differ significantly among groups. Conclusion: Neonatal oxygenation and acid-base status values were better preserved when GA was administered for elective CS compared to regional modalities. Apgar scores and neonatal outcomes were not affected by the anesthetic technique.

Key words: Anesthesia; Cesarean section; Fetal oxygenation; Fetal acid-base status.

Introduction

Regional anesthesia is considered the technique of choice for cesarean deliveries in normal and complicated pregnancies and has replaced general anesthesia (GA) in the majority of cases [1]. Nevertheless, regional anesthesia cannot always be provided, even in elective cesarean deliveries, as in cases of maternal refusal, inability to cooperate, coagulation disorders, infection at site of injection, and true allergy to local anesthetics.

General and regional anesthetic techniques have potential advantages and disadvantages. Advantages of GA include rapid induction of anesthesia, decreased incidence of hypotension, and superior ventilation control. Epidural (EA) and subarachnoid anesthesia (SA) minimize the risk of maternal aspiration and maternal death due to a difficult airway, while avoiding neonatal depression. On the other hand, regional techniques may cause maternal hypotension, due to an extended sympathetic blockade [2].

The impact of the anesthetic technique on neonatal outcome is essential. Umbilical cord acid-base status is a reliable indicator of fetal oxygenation and well being at birth; moreover it has been associated with long-term outcome as well [3]. For most clinicians it is the gold standard for the assessment of uteroplacental function and it may exclude the diagnosis of birth asphyxia in approximately 80% of depressed newborns at term [4]. The findings regarding the impact of anesthetic technique on umbilical cord blood gas values are contradictory [5–8]. Moreover, only few studies associate maternal oxygencation and blood gas values to the cord acid-base status and neonatal outcome parameters [9, 10]. The present study was designed to investigate the influence of GA, EA, and SA on neonatal blood gas values and acid-base status. The authors’ hypothesis was that the anesthetic technique for elective cesarean section (CS) has no effect on fetal oxygenation and acid-base status.

Materials and Methods

After obtaining approval from the Institutional Review Board, 380 parturients scheduled for elective CS gave written informed consent to participate in the study. Women received randomly GA, EA, or SA by the use of sealed envelopes describing group allocation. All cesarean deliveries were performed in the morning. In all cases, anesthesia was provided by an experienced consultant anesthesiologist.

One hundred and forty parturients (37%) received GA, 117 (31%) received EA, and 123 (32%) received SA. Exclusion criteria were gestational age < 38 weeks, cardiotocographic abnormalities, obstetric or medical complications, oxytocin for labor stimulation, neonatal congenital malformations, predicted or known difficult airway, and parturient’s request for a specific type of anesthesia.

All women received ten mg of metoclopramide and 50 mg of ranitidine intravenously 15 minutes before anesthesia. Standard monitoring was applied (electrocardiogram, non-invasive blood pressure measurement, pulse oximetry), and a capnograph was additionally used in the GA group. Parturients were breathing 50% oxygen via a Venturi facemask at 15 l/min, according to manufacturer.

In the GA group, after rapid sequence induction, anesthesia was maintained with one percent sevoflurane and 50% N2O/oxygen mixture. Neuromuscular blockade was achieved...
with vecuronium 0.1 mg kg⁻¹, while opioids were avoided before delivery. Mechanical ventilation was adjusted to maintain an end-tidal CO₂ (ET-CO₂) 30-35 mmHg.

In regional anesthesia groups, hydroxyethyl starch (6%) 500 ml was administered preoperatively. Epidural anesthesia was performed with a 18G Tuohy needle at L₂-L₃ or L₃-L₄ intrathecal space and an epidural catheter was inserted. A test dose of 2.5 ml lidocaine two percent was followed by ropivacaine 0.75% in incremental doses of six ml, along with fentanyl one μg kg⁻¹ targeting to a sensory block up to T₄ dermatome.

Subarachnoid anesthesia was performed with a 27G pencil – point needle at L₂-L₃ or L₃-L₄ intrathecal space and 1.6 - 2.0 ml of 0.5% isobaric levobupivacaine was administered, based on mother’s body weight and height. Additionally, one μg kg⁻¹ of fentanyl in a volume of 10 ml normal saline was administered epidurally. Ephedrine 2.5 mg was given immediately after intrathecal levobupivacaine to prevent hypotension.

In all women, arterial blood pressure was measured every minute until delivery and ephedrine was given in 2.5 mg increments if hypotension occurred. The total amount of ephedrine administered to each parturient was recorded. Hypotension was defined as systolic blood pressure below 100 mmHg or a decrease of more than 30 mmHg from the baseline value (measurement before any intervention). Skin incision-to-delivery and uterus incision-to-delivery time intervals were also recorded.

All parturients were placed in the supine position with a right hip wedge during operation. At delivery, maternal arterial blood sample from radial artery, as well as umbilical arterial and venous blood samples were withdrawn from a doubly-clamped cord segment. Blood gases, pH, HCO₃⁻ and actual base excess (ABE) were determined in an ABL-30 gas analyzer. Oxygen content (CTO₂) was also calculated for all blood samples according to the formula: CTO₂ = (0.03 × PO₂) + (1.31 × Hb × SO₂ %). Apgar scores were recorded five minutes after delivery by a neonatologist. Oxygen administration to the neonate, tracheal intubation, transfer to intensive care unit, and neonatal deaths were recorded as parameters of the neonatal outcome.

Statistical analysis was performed with SPSS (Statistical Program for Social Science). Analysis of variance or the non-parametric Kruskal Wallis test were used where appropriate. Multiple comparisons tests were performed between means using a Bonferroni test when appropriate. The 95% confidence interval was at a significance level of 0.05; p values were two-tailed and a p < 0.05 was considered significant.

Results

Data from 380 parturients were analyzed. The initial number of women assessed for eligibility to participate in the study was 438. Thirty-three parturients requested a specific type of anesthesia, while 11 of the EA group and 14 of the SA group were excluded due to inadequate anesthetic block.

Maternal characteristics, delivery times, and ephedrine doses are shown in Table 1. Maternal pH was significantly lower in the GA group (p < 0.001), while maternal ABE did not differ among three groups. Maternal PaO₂ and PaCO₂ were significantly higher in the GA vs EA and SA groups. Maternal CTO₂ was significantly higher in GA and EA compared to SA group.

<table>
<thead>
<tr>
<th>Parity</th>
<th>GA (n = 140)</th>
<th>EA (n = 117)</th>
<th>SA (n = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76</td>
<td>70</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>43</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Maternal weight (kg ± SD)</td>
<td>65 ± 14</td>
<td>71 ± 12</td>
<td>71.5 ± 13</td>
</tr>
<tr>
<td>Cause of CS</td>
<td>Previous CS</td>
<td>81</td>
<td>70</td>
</tr>
<tr>
<td>Other (IVF, fetal position)</td>
<td>59</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>Gestational age (weeks ± SD)</td>
<td>39 ± 1</td>
<td>38.9 ± 0.7</td>
<td>38.6 ± 0.4</td>
</tr>
<tr>
<td>Skin incision to delivery time (min)</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>(mean, range)</td>
<td>(5-8)</td>
<td>(5-9)</td>
<td>(6-9)</td>
</tr>
<tr>
<td>Uterus incision to delivery time (sec, mean, range)</td>
<td>75</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>Total dose of ephedrine (mg ± SD)</td>
<td>0.75 ± 1.0</td>
<td>5.80 ± 6.26</td>
<td>9.10 ± 6.26</td>
</tr>
</tbody>
</table>

Table 1. — Maternal and cesarean section (CS) characteristics in the general anesthesia (GA), epidural anesthesia (EA), and subarachnoid anesthesia (SA) group.

| MA-PO₂ | 224.56 ± 86.77 | 151.28 ± 38 | 157.36 ± 53.51 |
| MA-PCO₂ | 35.03 ± 3.88 | 29.25 ± 5.05 | 29.64 ± 4.16 |
| MA-pH | 7.38 ± 0.03 | 7.40 ± 0.02 | 7.43 ± 0.03 |
| MA-ABE | -3.74 ± 1.42 | -3.52 ± 2.52 | -3.56 ± 1.83 |
| MA-CTO₂ | 15.52 ± 0.18 | 15.75 ± 0.30 | 14.92 ± 0.35 |
| UA-PO₂ | 15.6 ± 5.48 | 9.29 ± 4.41 | 9.2 ± 4.06 |
| UA-PCO₂ | 53.3 ± 5.02 | 53.2 ± 8.44 | 55.29 ± 8.33 |
| UA-pH | 7.29 ± 0.02 | 7.27 ± 0.03 | 7.26 ± 0.06 |
| UA-CTO₂ | 25.50 ± 1.7 | 24.75 ± 2.8 | 24.25 ± 1.8 |
| UA-ABE | -1.71 ± 1.3 | -2.5 ± 2.3 | -3.45 ± 2.6 |
| UA-PO₂ | 3.67 ± 0.57 | 1.61 ± 0.39 | 1.43 ± 0.29 |
| UA-PCO₂ | 27.7 ± 7.66 | 21.43 ± 6.55 | 23.05 ± 6.72 |
| UA-pH | 46.2 ± 4.83 | 43.8 ± 7.54 | 43.9 ± 5.59 |
| UA-CTO₂ | 3.73 ± 0.02 | 7.35 ± 0.04 | 7.33 ± 0.04 |
| UA-PO₂ | 23.84 ± 1.5 | 23.52 ± 2.3 | 23.14 ± 1.9 |
| UA-ABE | -2.15 ± 1.0 | -2.06 ± 1.7 | -3.35 ± 2.2 |
| UA-CTO₂ | 9.68 ± 1.0 | 9.67 ± 1.37 | 7.51 ± 0.90 |
| UA-ABE | 12.45 ± 0.23 | 11.57 ± 4.30 | 14.91 ± 5.98 |
| UA-CTO₂ | 7.08 ± 3.27 | -9.20 ± 3.90 | -11.89 ± 4.77 |

Table 2. — Blood gas/acid base measurements in the general anesthesia (GA), epidural anesthesia (EA), and subarachnoid anesthesia (SA) group. Values are expressed as mean ± SD.

Umbilical artery pH, HCO₃⁻, and ABE values differed significantly between the GA and SA groups (p < 0.001 for pH, p < 0.05 for HCO₃⁻, and ABE). Umbilical artery PO₂ and CTO₂ were higher in the GA compared to both regional modalities (p < 0.001). Umbilical vein pH did not differ among groups, but umbilical vein PO₂ and CTO₂ were significantly higher in the GA group (p < 0.05). Umbilical vein HCO₃⁻ and ABE were significantly lower in the SA group.

The umbilical venous-arterial difference of PO₂ and CTO₂ were similar in all groups, while the umbilical ve-
Table 3. — Neonatal outcome parameters in the general anesthesia (GA), epidural anesthesia (EA), and subarachnoid anesthesia (SA) group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GA (n = 140)</th>
<th>EA (n = 117)</th>
<th>SA (n = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apgar score at 5th min &lt; 7</td>
<td>12 (8.5%)</td>
<td>8 (6.8%)</td>
<td>10 (8.1%)</td>
</tr>
<tr>
<td>Oxygen administration to the neonate</td>
<td>17 (12.1%)</td>
<td>13 (11.1%)</td>
<td>15 (12.2%)</td>
</tr>
<tr>
<td>Mask ventilation of the neonate</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endotracheal intubation</td>
<td>10 (7.1%)</td>
<td>7 (6%)</td>
<td>9 (7.3%)</td>
</tr>
<tr>
<td>Transfer to a special unit for further treatment</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neonatal deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Statistical significance (p < 0.05) for comparisons between GA and EA groups.
† Statistical significance (p < 0.05) for comparisons between GA and SA groups.
‡ Statistical significance (p < 0.05) for comparisons between EA and SA groups.

Fetal acidemia (umbilical artery pH < 7.20) was found only in the groups of regional modalities; four cases (3.4%) were found in the EA and nine cases (7.5%) in SA group. However, none of these newborns presented Apgar score < seven at five minutes. Apgar scores and outcome variables did not differ among the groups (Table 3).

Discussion

The results in this study showed that GA for elective CS was associated with better maternal and fetal oxygenation and also a favorable umbilical cord acid-base status. Although short-term neonatal outcomes were not clinically affected, regional anesthesia, particularly SA, was associated with impaired acid-base status and oxygenation. Also, previous reports [5, 6, 10, 11] have implicated regional modalities in neonatal hypoxemia and acidemia, but with no correlation to maternal oxygenation and acid-base status.

The significance of fetal oxygenation at delivery has attracted the interest of many investigators [12-14]. Ramanathan et al. have shown that maternal hyperoxygenation (up to 100%) improved fetal oxygen stores during CS under EA [14]. Oxygen administration in laboring women during fetal distress has long been used to improve fetal oxygen saturation [12]. However, high maternal-inspired oxygen fraction (FiO2: 0.5) in CS under regional anesthesia may increase free radical activity and lipid peroxidation in both mother and fetus [13]. On the contrary, administration of 100% oxygen to parturients undergoing elective CS under GA improves fetal oxygenation without increasing free radical activity and lipid peroxidation compared to lower FiO2 (0.3 and 0.5) [15]. The clinical significance of all these findings remains, to date, unclear.

The authors calculated CTO2 in maternal and umbilical cord blood samples for a most accurate evaluation of oxygenation. They maintained maternal FiO2 stable (0.5) in the three groups and found a better maternal CTO2 in the GA and EA groups compared to the SA group. Impaired pulmonary function tests with a restrictive ventilatory defect have been described in parturients undergoing CS under SA [16]. In the present study, maternal arterial pH was lower in the GA group than in the regional anesthesia groups, perhaps due to the higher maternal PaCO2, as ABE was similar among groups. Dyer et al. have also reported higher maternal PaCO2 in pre-eclamptic parturients receiving GA compared to those receiving SA [5]. The higher PaCO2 values during GA are probably due to mechanical ventilation in this group and the lack of adaptation of their pregnancy-induced hyperventilation. The powerful adrenergic stimuli of intubation could be an additional or alternative reason. Apart from physiological, psychological, and emotional factors may also account for parturients’ hyperventilation during CS, resulting in further PaCO2 decrease in regional anesthesia groups.

In the GA group, CTO2 in both umbilical vessels was significantly higher vs regional anesthesia groups. These results are similar to those reported in previous studies [10, 11]. A new finding of this present study is that the EA may aggravate fetal oxygen transfer, according to the lower CTO2 in the umbilical vein, although maternal CTO2 was similar in the GA and EA groups. This finding is supported by previous results suggesting that fetal intrapartum oxygen saturation was affected after initial or top-up epidural analgesia [17]. In this case, a high maternal FiO2 may be quite significant in improving fetal oxygenation. The FiO2 in the present study was 0.5 in all groups, but Ramanathan et al. found that PO2 in the umbilical vein was improved when parturients under EA breathed high-oxygen concentrations up to 100% [14].

The umbilical venous-arterial PO2 and CTO2 difference was found similar among groups, indicating that metabolism, fetal oxygen consumption, and fetal cardiac output are not influenced by the anesthetic technique, or ephedrine administration, at least at the doses given in the present study (mean dose < 10 mg). Ephedrine produces α- and β-sympathomimetic effects and has been associated with fetal acidosis [18], especially when administered at high-bolus doses ≥ 10 mg [19]. Ephedrine has been associated with fetal acidosis by increasing fetal oxygen demand and CO2 production due to β-adrenergic stimulation [20]. The present results do not indicate this mechanism, since oxygen consumption as assessed by CTO2 and umbilical artery PCO2 did not differ significantly among the groups. It should be noted though, that in the present study, the mean total dose of ephedrine administered in the SA group was quite small [19, 21].

Another finding of the present study was a higher difference of umbilical venous-arterial PCO2 in the GA compared to the EA and SA groups. This difference was possibly due to an increased umbilical venous PCO2 because of the higher maternal PaCO2 found.

The HCO3- and ABE values in both umbilical vein and artery of the GA and EA groups did not differ, as in previous studies [7, 22]. However, there was a significant difference in HCO3- and ABE concentrations in the umbilical vein and artery between the GA and SA groups, indicating an "acidotic" tendency of metabolic nature in the latter. A higher incidence of fetal acidemia occurs in

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cesarean deliveries under regional – especially subarachnoid – vs general anesthesia [6-8, 10, 19]. Maternal arterial hypotension is the most common cause of fetal acidemia, as uteroplacental circulation has not adequate autoregulatory mechanisms and uteroplacental blood flow is decreased [6, 10].

The duration and severity of maternal hypotension due to subarachnoid blockade is of particular importance. Fetal bradycardia may occur when hypotension persists for more than four minutes [23], while hypotension lasting even less than two minutes may cause fetal academia [24]. It has also been found that the maximum reduction of maternal systolic blood pressure significantly affects umbilical arterial pH [18], although Robson et al. did not show a direct correlation between the severity of hypotension and fetal acidemia after birth [23]. Maayan-Metzger et al. found retrospectively that term neonates tolerate quite well short periods of hypotension (three to five min) during CS under regional anesthesia, without perinatal complications [2]. It should be noted that maternal blood pressure cannot be used as a predictor of fetal outcome; hence, usually it is treated promptly and the vast majority of hypotensive episodes during CS are of short duration. However, reductions of maternal cardiac output representing a great risk for the fetus do not necessarily manifest as a maternal blood pressure decrease, because of the changes in peripheral vascular resistance. The maximum percentage change in maternal cardiac output and umbilical artery pulsatility index correlated with umbilical artery pH [25].

Subarachnoid anesthesia has been associated with a decrease in cardiac output even in the absence of hypotension [25], while the epidural administration of local anesthetics in divided doses reduces this risk [22]. Thus, it is not surprising that a decrease of maternal cardiac output with an increase of umbilical artery pulsatility index, indicating a reduced uteroplacental blood flow, is found after SA but not after EA [25, 26]. Roberts et al. found severe fetal acidemia (pH ≤ 7.19) in 12% of patients after EA, 18% after combined spinal-epidural, and 24% after SA [10]. A lower incidence of fetal acidemia was found in the present study (3.3% after EA and 7.3% after SA), probably because of close monitoring and prompt treatment of hypotension.

In the present study the authors found no difference among the groups regarding uterus incision-to-delivery time intervals. Prolonged uterine incision-to-delivery interval has been found to significantly affect umbilical arterial pH and standard base excess, while induction-to-delivery and skin incision-to-delivery intervals were not significant predictors of neonatal acid-base status [18]. The authors also found no significant differences in immediate neonatal outcomes among the three anesthetic groups, as evaluated by five-min Apgar scores or the need for oxygen administration to the neonate or mask assisted ventilation. This finding is consistent with previous reports [27, 28]. However, there is significant controversy regarding this area. Several studies report a higher incidence of low one min Apgar scores in neonates exposed to GA, as compared with regional techniques [5, 7, 10]. Nevertheless, the adverse effects of general anesthesia on Apgar score, if present, are usually short-lived, and easily managed [19], while hypotension has also been associated with low Apgar scores at one min [29]. In either case, it should be noted that Apgar scores correlate poorly with neurologic outcome and as a subjective measure has limited diagnostic value in fetal asphyxia. Although fetal pH alone cannot serve as a prognostic index for neurologic outcome, it reflects the status of the neonate at delivery and as an objective parameter is more preferred for this purpose [6]. Routine determination of umbilical cord blood gases in every birth has been proposed by Thorp et al. more than 20 years ago, for medical and legal reasons [30].

A limitation of the present study is the randomization of parturients to receive general or neuraxial anesthesia, since regional techniques are on the whole safer for the mothers. Regarding this issue, the present study was approved by the Institutional Review Board and the complications of both anesthetic techniques were discussed with the parturients before their written informed consent was obtained. Also, parturients requesting a specific type of anesthesia were excluded from the study. Moreover, all cases were elective CS of the morning list, had no morbidity or predicted/know difficult airway, and anesthetics were administered by an experienced anesthesiologist. The authors consider that in this setting, the parturients who received general anesthesia were not exposed to a higher risk.

In conclusion, the current results suggest that the type of anesthesia does not significantly influence the early neonatal outcome in elective cesarean deliveries. However, maternal and fetal oxygenation and umbilical cord acid-base status appear to be superior when GA is administered compared to regional modalities, especially SA. Finally, differentiations in fetal oxygen consumption or indications for changes in fetal oxygen consumption or indications for changes in fetal cardiac output were not observed with regards to anesthetic techniques.

References

Maternal and umbilical cord oxygen content and acid-base balance in relation to general, epidural or subarachnoid anesthesia etc.


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The expression and role of oxidative stress markers in the serum and follicular fluid of patients with endometriosis

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²Reproductive Medicine Centre, the Third Affiliated Hospital of Guangzhou Medical University, Guangzhou (China)

Summary

Objective: To investigate the expression and role of oxidative stress markers in the serum and follicular fluid of patients with endometriosis. Materials and Methods: A prospective case-control study was conducted in 42 patients who underwent in vitro fertilization-embryo transfer (IVF-ET). They were divided into Group I: patients with endometriosis (n = 20) and Group II: patients with tubal factor infertility (n = 22). All patients underwent a long gonadotropin-releasing hormone (GnRH) agonist protocol for pituitary downregulation followed by controlled ovarian hyperstimulation. Level of reactive oxygen species (ROS), superoxide dismutase (SOD), and vitamin E (VE) were measured by enzyme-linked immunosorbent assay (ELISA). The results of IVF-ET between the two groups were compared. Results: The ROS levels in both serum and follicular fluid of the study group were significantly higher than in the control group. The serum levels of SOD and VE in the study group were significantly lower than those in the control group, but there was no difference in follicular fluid levels of SOD and VE between the two groups. Furthermore, the mature oocyte and fertilization rates in the study group were significantly lower than those of the control group. However, the levels of ROS, SOD, and VE in serum and follicular fluid were not significantly correlated with outcome following IVF-ET. Conclusion: Patients with endometriosis have increased oxidative stress, as well as lower mature oocyte rates and fertilization rates. Nevertheless, there is no evidence that the oxidative stress status is directly related to the outcome of IVF treatment.

Key words: Oxidative stress; Endometriosis; In vitro fertilization; Fertilization rate; Pregnancy rate.

Introduction

Endometriosis is a benign disease with malignant tumors and one of common gynaecological diseases causing pelvic cavity pain, menoxenia, infertility, and approximately 30%-50% of these cases evolve into infertility [1]. The underlying mechanism and curative effects of endometriosis-induced infertility remains unclear and unsatisfactory. In vitro fertilization-embryo transfer (IVF-ET) has been regarded as the main treatment of endometriosis-associated infertility, whereas the pregnancy rate was still significantly lower than that of patients with tubal factor infertility [2]. The mechanism of endometriosis-associated infertility and the reason why the women had low pregnant rate following IVF-ET have been the focus for all the clinicians, and oxidative stress is included.

Oxidative stress (OS) is defined as the unbalance between internal oxidative and anti-oxidative reactions, more inclined to oxidation, leading to inflammatory infiltration of neutrophil, increased secretion of protease, and a large amount of intermediate products, which is characterized as the increase in reactive oxygen species (ROS) and the decline or deficiency of anti-oxidants [1]. OS played a certain role during pregnancy maintenance, normal reproduction process, and premature initiation [2-4]. Other previous findings indicated that OS was associated with pathological and physiological mechanism of birth defects and abortion induced by preeclampsia, hydatid mole, and free radicals [5-8]. Although the relationship between endometriosis and OS remains debatable, relevant data indicated that OS was found in the abdominal cavity in endometriosis women. However, whether OS is correlated with the pathogenesis of endometriosis-associated infertility or not remains to be further elucidated. The influence of OS upon reproduction potentiality of human beings has increasingly attracted attention [9-11]. Thereafter, whether OS exists in follicular fluid or granulocytes of women suffering from endometriosis, and whether it is harmful to oocytes quality leading to negative consequences to clinical outcomes have been the main research topic in recent years. Currently, there has been no report investigating the balance status between oxidation and anti-oxidation in serum and follicular fluid of patients with endometriosis-induced infertility, and its effect on the outcome of clinical trials. This clinical trial aimed to investigate the expression and role of OS markers in serum and follicular fluid of women with endometriosis.

Materials and Methods

Subjects

The endometriosis patients and those with tubal factor infertility receiving IVF-ET in Reproduction Center in the Third Hospital, affiliated to Guangzhou Medical College from August to October, 2009 were enrolled in this study.
Sampling methods

All patients underwent a long gonadotropin-releasing hormone (GnRH) agonist protocol for pituitary downregulation followed by controlled ovarian hyperstimulation. Serum sampling included periphery blood which was collected on the day of retrieving oocytes, and then centrifuged at 1,000 × g for 20 min. The separated serum was assigned into sterile EP tubes and stored at -70°C. Follicular fluid sampling included laboratorian’s retrieval of the first tube of follicular fluid uncontaminated by blood following oocytes retrieval. The obtained follicular fluid was centrifuged at 1000 × g for ten min. The supernate was collected and assigned into sterile EP tubes and stored at -70°C. The SOD, ROS, and VE concentrations in both serum and follicular fluid of patients between two groups were detected by using enzyme-linked immunosorbent assay (ELISA). The main clinical indexes included: retrieved oocytes, mature oocytes rate, fertilization rate, good-embryo rate, implantation rate, and clinical pregnancy rate.

Statistical analysis

All the data obtained were statistically analyzed using SPSS 13.0 software package. Categorical data were handled by Chi-Square test. Measurement data were analyzed by independent sample t-test. Correlation analysis between experimental data and clinical evaluation indexes were expressed by Spearman correlation coefficient. A test level of α = 0.05 was considered as statistical significance.

Results

Patient characteristics

No significant difference was noted between the study and control groups in terms of mean infertility time, and baseline follicle-stimulating hormone (FSH) level (p > 0.05), suggesting there was no statistical significance between two groups and all the patients were comparable (Table 1).

ROS, SOD, and VE levels

ROS serum level in the study group was significantly increased compared with that in the control group (p < 0.05), and SOD and VE serum concentrations were significantly decreased than those in the control group (p < 0.05), which are shown in (Table 2). ROS level in follicular fluid in the study group was significantly increased compared with that in the control group (p < 0.05), whereas SOD and VE concentrations slightly decreased than those in the control group with no significant difference (p > 0.05) (Table 3). In addition, ROS, SOD, and VE levels in follicular fluid were significantly decreased than their counterparts in serum in both two groups (p < 0.05) (Tables 4 and 5).

IVF-ET

No significant difference in retrieved oocytes was noted between two groups (p > 0.05). Mature oocyte and fertilization rates in the study group were significantly lower compared with those in the control group (p < 0.05). Good oocyte rate, implantation rate, and clinical pregnancy rates in the study group declined compared to those in the control group, whereas no significant difference was noted between the two groups (p > 0.05) (Table 6).

ROS, SOD, VE levels, and evaluation factor of IVF-ET

Correlation analysis of ROS, SOD, and VE levels and evaluation factor of IVF-ET outcome revealed that ROS level in serum and follicular fluid negatively correlated with mature oocyte and fertilization rates. SOD level positively correlated with mature oocyte, fertilization, implantation, and clinical pregnancy rates. VE level positively correlated with mature oocyte, fertilization, good-embryo, implantation, and clinical pregnancy rates. However, no significant difference was noted (p > 0.05) (Table 7).

Discussion

When internal oxidative level was extraordinarily high, a large amount of oxidants was depleted, and the original oxidation-anti-oxidation balance was destroyed, leading to OS status. As the OS elevated in systemic circulation, the OS level in local environment might be enhanced accordingly, such as, peritoneal fluid, follicular fluid, etc. Until now, the exact relationship between OS and endometriosis has been debatable. Murphy et al. [12] indicated that endometriosis originated from OS, or there was relationship between them. For pelvic endometriosis cases, macrophage activation in the peritoneal cavity induced OS, yielding an abundance of peroxides. They also found that lipoprotein level in peritoneal fluid of endometriosis women was increased compared with that of normal counterparts. In addition, VE level in peritoneal fluid was significantly lower than that in serum, indicating that the protective action of anti-oxidants in peritoneal fluid was inferior to that in serum. Szczepan-
The environments surrounding oocytes played vital roles in regulating oocyte quality, fertilization, and embryonic development. Under normal circumstances, the redundant ROS in follicular fluid was eliminated by the anti-oxidation system existing in mitochondria of granulocytes, maintaining the biological balance between oxidation-antioxidation reactions, and preventing oocytes from damages induced by ROS. When the host organism was in pathological state, granulocytes presented OS, and host organism released a substantial amount of ROS into follicular fluid. The redundant ROS in follicular fluid was eliminated by the anti-oxidation system existing in mitochondria of granulocytes, maintaining the biological balance between oxidation-antioxidation reactions, and preventing oocytes from damages induced by ROS. When the host organism was in pathological state, granulocytes presented OS, and host organism released a substantial amount of ROS into follicular fluid. Previous clinical investigations have proved that the fertilization rate of the patients with endometriosis-associated infertility following IVF or ICSI was lower compared with their counterparts with tubal factor infertility.

Table 1. — Patient background in the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>32.0 ± 2.8</td>
<td>32.4 ± 3.1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mean infertility time (years)</td>
<td>3.3 ± 2.7</td>
<td>4.7 ± 2.7</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mean baseline FSH level (U/l)</td>
<td>5.6 ± 1.4</td>
<td>5.1 ± 1.5</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of ROS, SOD, and VE levels in serum between the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS (ng/ml)</td>
<td>5.49 ± 1.39</td>
<td>3.93 ± 1.22</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>11.38 ± 4.44</td>
<td>18.99 ± 6.80</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>VE (μmol/l)</td>
<td>17.66 ± 4.89</td>
<td>23.34 ± 8.14</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of ROS, SOD, and VE levels in follicular fluid between the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS (ng/ml)</td>
<td>1.35 ± 0.38</td>
<td>0.53 ± 0.88</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>7.65 ± 1.25</td>
<td>9.26 ± 2.70</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>VE (μmol/l)</td>
<td>6.16 ± 1.95</td>
<td>6.88 ± 2.45</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 4. — Comparison of ROS, SOD, and VE levels between serum and follicular fluid in the study group.

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Follicular fluid</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS (ng/ml)</td>
<td>5.49 ± 1.39</td>
<td>1.35 ± 0.38</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>11.38 ± 4.44</td>
<td>7.65 ± 1.25</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>VE (μmol/l)</td>
<td>17.66 ± 4.89</td>
<td>6.16 ± 1.95</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 5. — Comparison of ROS, SOD, and VE levels between serum and follicular fluid in the control group.

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Follicular fluid</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS (ng/ml)</td>
<td>3.93 ± 1.22</td>
<td>0.53 ± 0.88</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>18.99 ± 6.80</td>
<td>9.26 ± 2.70</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>VE (μmol/l)</td>
<td>23.34 ± 8.14</td>
<td>6.88 ± 2.45</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 6. — Evaluation indexes of the outcome of IVF-ET in the study and control groups.

<table>
<thead>
<tr>
<th>Evaluation indexes</th>
<th>Study group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean retrieved oocytes</td>
<td>12.6 ± 6.9</td>
<td>12.2 ± 6.4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mature oocytes rate</td>
<td>86% (211/244)</td>
<td>92% (256/278)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>70% (171/244)</td>
<td>77% (216/278)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Good-embryo rate</td>
<td>28% (47/169)</td>
<td>31% (66/214)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>20% (8/40)</td>
<td>21% (11/52)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>30% (6/20)</td>
<td>36% (8/22)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 7. — Correlation analysis between the outcome of IVF-ET and ROS, SOD, and VE levels in serum and follicular fluid.

<table>
<thead>
<tr>
<th>Evaluation indexes</th>
<th>ROS</th>
<th>Serum</th>
<th>VE</th>
<th>SOD</th>
<th>Follicular fluid</th>
<th>VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrieved oocytes</td>
<td>-0.01</td>
<td>0.10</td>
<td>0.06</td>
<td>0.004</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Mature oocytes rate</td>
<td>-0.27</td>
<td>0.29</td>
<td>0.22</td>
<td>-0.25</td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>-0.13</td>
<td>0.14</td>
<td>0.12</td>
<td>-0.14</td>
<td>0.18</td>
<td>0.25</td>
</tr>
<tr>
<td>Good-embryo rate</td>
<td>-0.04</td>
<td>0.05</td>
<td>0.17</td>
<td>-0.04</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>0.01</td>
<td>0.17</td>
<td>0.22</td>
<td>0.02</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>0.04</td>
<td>0.14</td>
<td>0.21</td>
<td>-0.03</td>
<td>0.27</td>
<td>0.23</td>
</tr>
</tbody>
</table>
or other patients [17-19]. Alternative findings indicated that the decline in implantation rate of endometriosis patients might be associated with impaired oocytes quality [20, 21]. In addition, other findings revealed the relationship between low oxygenation and the reduction in development potential of oocytes, mainly characterized by the increased defect rate in oocytes cytoplasm, impaired cleavage, and abnormal segregation of oocytes’ chromosome [22]. Meantime, ROS increased the production of embryonic segments by accelerating cell apoptosis [23]. Saito et al. [24] found that the 8-hydroxy-deoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (4-HNE) levels in granular cells within endometriosis women were significantly enhanced compared with those within other factor-related infertility cases (tubal factor, male spouse factor, and unknown factors). Moreover, 8-OHdG was regarded as a common evaluation index for oxidation DNA damages and OS, and 4-HNE was a product by lipid peroxides, suggesting that significant OS existed in follicular fluid of endometriosis patients. Simultaneously, they also found that 8-OHdG concentration contained in granular cells negatively correlated with good-embryo rate, and 8-OHdG level in granular cells within endometriosis women were significantly increased compared with those within other factors-related infertility cases (tubal factor, male spouse factor, and unknown factors), collectively indicating granular cells had OS which reduced fertilization rate and embryo quality. In addition, 8-OHdG directly affected the oocytes quality in endometriosis women, which was possibly one of the reasons explaining why endometriosis evolved into infertility. Campos et al. [25] found that the serum VE level in endometriosis patients was decreased compared to that in normal controls prior to or after ovulation stimulations. Moreover, the serum MDA levels in endometriosis subjects significantly increased following induced ovulation. However, there was no statistical significance between the endometriosis and control groups in terms of the VE and MDA levels in serum and follicular fluid on the day of oocytes retrieval. To investigate the existence and influence of OS upon oocytes quality in follicular fluid of endometriosis patients, the ROS, SOD, and VE levels in follicular fluid between the study and control groups were detected and compared in this clinical trial. The obtained outcomes showed that the endometriosis patients had a significant rise in follicular fluid ROS level but a slight decline in SOD and VE levels compared with their counterparts in the control group, strongly suggesting the existence of OS in follicular fluid of endometriosis women. Besides, mature oocyte and fertilization rates in the study group were significantly decreased than those in the control group, and ROS level in both serum and follicular fluid negatively correlated with mature oocyte rate and fertilization rate, which indicated that OS indeed affected the oocytes quality, leading to a reduction in oocyte developmental potential and a negative influence on mature oocyte and fertilization rates.

Previously, whether OS existed in endometriosis and endometriosis-associated fertile patients has been widely debated, mainly due to a lacking agreement in the selection and detection methods of OS markers, and the discrepancies in inclusion criteria of the enrolled subjects. Thereafter, establishing a unified standard is the key. The following studies should expand sample size, select, and detect the OS markers especially sensitive to endometriosis patients.

References

The expression and role of oxidative stress markers in the serum and follicular fluid of patients with endometriosis


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Surgical treatment outcomes of serious chronic tubo-ovarian abscess: a single-center series of 20 cases

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1Departments of Obstetrics and Gynecology, 2Department of Biochemistry, Shimane University School of Medicine, Izumo (Japan)

Summary
In recent years, Shimane University Hospital has begun to see patients with pelvic inflammatory disease (PID) which has become severe and chronic after insufficient conservative treatment in primary or secondary medical care facilities. Serious chronic tubo-ovarian abscess (TOA) is complicated by intraperitoneal inflammatory adhesions to surrounding organs, so that it is difficult to determine the original anatomical position of organs at surgery. Forcible synechotomy can result in damage to the adhering organs and insufficient drainage after surgery can cause recurrence of inflammation. In order to increase the chances for a successful surgical treatment, careful preparation, such as preoperative administration of antibiotics and ureteral stent insertion are necessary. In addition, the chances for recurrence of inflammation can be lessened by thorough intraperitoneal irrigation and insertion of a drainage tube.

Key words: Pelvic inflammatory disease; Tubo-ovarian abscess.

Introduction
Thanks to the recent development of effective antibiotics, most cases of pelvic inflammatory disease (PID) are being cured by conservative management. The careless use of antibiotics, however, sometimes causes recurrence of infection resulting in serious chronic tubo-ovarian abscess (TOA), in which the prognosis is extremely poor. TOA is the consequence of extensive suppurative infection and is often resistant to treatment. Once the severity is advanced, conservative care is no longer effective and surgical treatment is required [1, 2].

Empirical knowledge tells us that emergency surgery for PID without adequate preparation cannot achieve a good result due to serious adhesions and friability of the pelvic organs; the patient often ends up with a recurrence of infection. It is becoming clear that pelvic inflammation should be controlled by using strong antibiotics and prepare for surgery by placing ureteral stents, then proceed with surgical treatment for a higher curative rate.

Materials and Methods
Treatment outcomes of serious chronic TOA patients requiring operations in Shimane University Hospital from November 2006 through November 2011 were analyzed: the patients' medical background, pathogenic bacteria, medical records, and operative reports were reviewed. The validity of the treatment policy for TOA was then examined.

Of the 20 serious chronic TOA cases which required surgical treatment, 17 cases (85.0%) originally received conservative treatment at private clinics. When their disease was not controlled, they were admitted to Shimane University Hospital.

Patients' background
Table 1 summarizes the patients' background. The average age was 47.4 years (33-80). Pregnancy history: nulliparous two cases (10%), parous 18 cases (90%). Married: 19 cases (95%), menopause: four cases (20%), infertility: five cases (25%), usage of intrauterine device (IUD): two cases (10%), past history of pelvic operation: 11 cases (55%), past history of PID: four cases (20%).

Findings at diagnosis
Table 2 summarizes the findings at diagnosis. Almost all patients (14/20, 70%) presented with a palpable mass at diagnosis. Pelvic peritonitis was present in 85% of all cases (19/20). Patients with TOA showed high serum white blood cell count (WBC) and C-reactive protein (CRP) levels on laboratory examination. The average diameter of the masses was 74.7 mm (18-180). Computed tomography (CT) and magnetic resonance imaging (MRI) showed that 55% (11/20) of patients had an adnexal abscess accompanied by ovarian tumors. In those cases, 60% (12/20) occurred in the right ovary; 40% (8/20) of cases were complicated by endometriosis.

Pathogenic bacteria
The detection rate was 30% (6/20) in cultures from the ovarian mass and 35.0% (7/20) in cultures from the cervical duct. The gram-negative bacilli of aerobic bacteria were the most frequently detected, while the pathogenic bacteria in most cases were anaerobic. New quinolone-resistant bacteria were seen in some cases, but the rate of chlamydia IgG detection was unexpectedly low (15.0%, 3/20).

Use of antibiotics and other drugs
Most of the TOA patients in this study had already received antibiotic treatment, so it was not possible to standardize the antibiotic treatment from the start. In general, typical salpingitis is controlled by β-lactam derivatives while more powerful antibiotics, such as fourth generation cephalosporins and carbapenems, are used for TOA. In this study, all patients were treated with these powerful antibiotics, with new quinolones or amino-
glycosides used concurrently for 44.1% of patients. In addition, 50% (10/20) of the patients were treated with immunoglobulin preparations.

**Results**

Table 3 summarizes the treatment results. The average length of hospital stay was 22.3 (9-54) days. Patients were required to stay an average of 4.9 (0-19) days in the hospital before surgery and 17.7 (7-49) days afterwards.

The average operation time was 215 minutes, longer than that of conventional total abdominal hysterectomy and salpingo-oophorectomy. The average blood loss was 910 ml, a relatively large quantity for an operation for benign disease. Postoperative laboratory examination showed average hemoglobin levels of 9.0 g/dl (5.4-11.6). Fifty percent (10/20) of patients required intraoperative blood transfusion. Abscess drainage alone, salpingo-oophorectomy, and total hysterectomy + salpingo-oophorectomy were performed for 5% (1/20), 30% (6/20), and 65% (13/20) of patients, respectively.

All nine patients who required bilateral salpingo-oophorectomy and were older than 45 years, showing extended inflammation to their uterus, received total hysterectomy + bilateral salpingo-oophorectomy in order to prevent the recurrence of inflammation. Fifteen percent (3/20) of patients required intestinal tract resection due to severe adhesions.

There has been one recurrent case in the past five years. That patient received only abscess drainage due to poor surgical conditions: the operation was performed in an emergency situation and because of the extensive inflammation, it was impossible to determine the anatomical relationship of the organs for proper surgical treatment. Several days after her surgery, CRP increased again and CT showed reformation of the abscess. The authors placed ureteral stents and then performed a radical operation. Since this experience, ureteral stenting as preoperative management has been the standard, when possible. Use of ureteral stenting in this series was 65% (13/20). Joint surgery with gastrointestinal surgeons was required in 65% (13/20) of patients who needed a radical operation. Postoperative drainage was provided in 90% (18/20) of patients. It took 5.6 days on average, ranging from two to nine days, until drainage became no longer necessary. Patients with intestinal adhesions required longer periods of drainage.

Since September 2008, the authors have been performing thorough pelvic and peritoneal irrigation with a large quantity of physiological salt solution for prevention of abscess recurrence. They have followed this protocol with over 5,000 ml of solution for 14 (70%) patients in this series and with 5,000-10,000 ml solution for 11 (55%) patients. Since commencing this irrigation protocol, the authors have not experienced a single recurrence.

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>47.4 (33-80)</td>
</tr>
<tr>
<td>Married</td>
<td>19/20 (95%)</td>
</tr>
<tr>
<td>History of gravity, parity</td>
<td>18/20 (90%)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>4/20 (20%)</td>
</tr>
<tr>
<td>Previous pelvic surgery</td>
<td>11/20 (55%)</td>
</tr>
<tr>
<td>History of PID</td>
<td>4/20 (20%)</td>
</tr>
<tr>
<td>Use of IUD</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Previous infertility</td>
<td>5/20 (25%)</td>
</tr>
</tbody>
</table>

Table 2. — Clinical and laboratory data.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palpable mass</td>
<td>14/20 (70.0%)</td>
</tr>
<tr>
<td>Pelvic peritonitis</td>
<td>19/20 (85.0%)</td>
</tr>
<tr>
<td>Mass diameter on sonography</td>
<td>74.7 (18 - 180)</td>
</tr>
<tr>
<td>Mean body temperature (°C)</td>
<td>38.7°C (37.0 - 39.8°C)</td>
</tr>
<tr>
<td>Mean WBC/ml</td>
<td>15,622 (10,930 - 27,600)</td>
</tr>
</tbody>
</table>

Table 3. — Treatment results.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of period in hospital (days)</td>
<td>22.3 (9-54)</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>215 (59-368)</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>910 (10-2150)</td>
</tr>
<tr>
<td>Use of ureteral stent</td>
<td>13/20 (65.0%)</td>
</tr>
<tr>
<td>Operation with digestive surgeons</td>
<td>13/20 (65%)</td>
</tr>
<tr>
<td>Positive culture from the cervix</td>
<td>7/20 (35%)</td>
</tr>
<tr>
<td>Antibiotic treatment in hospital (I.V.)</td>
<td>12/20 (60.0%)</td>
</tr>
<tr>
<td>Single agent, %</td>
<td>8/20 (40.0%)</td>
</tr>
<tr>
<td>Multiple agents, %</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In general, women with a history of childbirth show a higher incidence of pelvic abscess, compared with nulliparous women [3]. IUD usage is considered to be one of the causes of TOA. In this study, most patients had a history of childbirth. The number of women who used an IUD, however, was unexpectedly low. Half of the patients had a history of pelvic surgery, such as ovarian resection and appendectomy, and one-third had a past history of PID. It became clear that parous women and those with a history of pelvic surgery or PID tended to suffer from TOA at a higher frequency than women without this history. In addition, since 40% of the patients had endometriosis; this could constitute a predisposition for infection [4]. One of the reasons TOA occurs more frequently on the right side may be the anatomical positioning of other organs which could cause infection, such as in the appendix. When TOA is caused by IUD insertion, the pathogenic bacteria can be actinomyces [5]. In most cases of TOA, actinomyces are identified in the operative specimens [6].

The origins of TOA may vary. Some infections may originate from the cervix or endometrium and ascend through the fallopian tubes, some spread hematogenously from the uterus or through the lymphatic system, and some occur as a consequence of abdominal infection, such as appendicitis or diverticulitis. There are several causes that predispose to TOA: 1) Examination or treatment of the uterine cavity such as IUD placement, hysterosalpingography, hysteroscopy, dilatation and curettage, and embryo transfer; 2) Transvaginal treatments such as tamponade, artificial fertiliza-
Surgical treatment outcomes of serious chronic tubo-ovarian abscess: a single-center series of 20 cases

Intraoperative findings of severe chronic TOA. The ovaries, uterus, and intestinal tract have become firmly adhesed.

While uncomplicated salpingitis can be cured by second-generation β-lactam derivatives, some infections may develop resistance to these antibiotics and advance to serious chronic TOA due to resistant bacteria. These cases should be treated with strong antibiotics, such as carbapenems, in order to control the inflammation resulting from infection. Ideally, appropriate antibiotics should be administered as soon as pathogenic bacteria are identified by culture. Only then, if appropriate antibiotics do not control the infection should surgical treatment be undertaken [7, 8]. Identification of the pathogenic bacteria is essential for determining antibiotic treatment; however, in most clinical situations, empiric therapy is begun without waiting for the result of bacteriologic cultures.

Shimane University Hospital is a tertiary care facility; most cases of TOA evaluated in this hospital had acquired drug resistance to initial conservative treatment given by previous clinicians, progressing to serious chronic TOA. The operations for these patients were quite difficult due to severe adhesion formation. Incorrect use of antibiotics (inappropriate antibiotic choice, incorrect duration of treatment, etc.) during conservative care might cause PID or TOA to progress to serious chronic TOA. Making a prompt decision to treat serious chronic TOA surgically is vital for curative resection [9].

In the present facility, surgical treatment was preceded by several days of antibiotic treatment to avoid long hospitalization. No cases showed evidence of recurrence of inflammation, and the average hospitalization period was 17.7 days (7-49). Surgery for serious chronic TOA is often challenging for the gynecologist because severe inflammatory adhesions are present, and the operation often requires support from a general surgeon, gastrointestinal surgeon, or urologist (Figure 1). The authors sometimes encounter cases in which operative techniques common to surgeries for gynecologic malignancies are needed due to severe adhesions to surrounding organs and the possibility of organ injury [8].

In one study, 8.4% of 71 TOA cases in which total hysterectomy plus adnexectomy was performed were accompanied by intestinal injury [8]. This report advises that ureteral stenting is useful for performing the operation safely. In addition, it should be mentioned that the friability of inflammatory tissue causes unexpected bleeding and intestinal edema. Securing central venous access is essential. Finally, postoperative infection is another complication that should be carefully monitored. There are two kinds of infection resulting from surgery: surgical site infection (SSI) and peritoneal infection.

When the operation is clean (vs clean-contaminated, contaminated, or dirty), the incidence of SSI is below two percent. SSI increases to 40% in cases involving infectious diseases [10]. Careful observation is imperative in the postoperative period. Fever, change of skin color, and emergence of pain can be signs of postoperative infection. The main cause of SSI is thought to be bacterial contamination during the operation by the irrigation fluid from the abdominal cavity or subcutaneous tissue. Indigenous bacteria on the patient’s skin or leakage of bacteria from the gastrointestinal lumen can enable contamination during the operation [11-13]. Other reports have claimed that patients’ decreased immunity after prolonged surgery and blood transfusion were capable of causing SSI as well [13-15].

In order to avoid SSI, bacterial contamination should be strictly prevented during surgery. In the present study, the authors found that copious irrigation of the abdominal cavity could reduce the incidence of SSI, yet some studies report it could lead to SSI. The authors did not find this a causal relationship between irrigation and SSI. The incidence of SSI is reported to be nearly 0% for upper gastrointestinal surgery. In contrast, 27% of lower intestinal surgical cases are complicated by SSI [14, 16, 17]. What can be learned from these reports is that careful observation of the surgical site is very important for TOA operations, especially when lower intestinal resection is performed. In addition, an abscess can be formed at the vaginal cuff or within the pelvic cavity. Since phlegmonous inflammation involving the vaginal cuff is usually accompanied by unbearable pain, careful attention should be paid to patients’ complaints.

In the case of TOA, inflammation is expected to spread nearly to the pelvic wall, so antibiotics with a broad spectrum, effective for gram-positive, gram-negative, and anaerobic bacteria, should be used from the beginning [7, 9]. The reason for postoperative abscess recurrence in the abdominal and pelvic cavities is thought to be insufficient drainage, caused by unhygienic operative technique or hematoma formation [10]. When continuous fever or abdominal pain emerge after surgery for serious chronic TOA, the possibility of abscess recurrence should be immediately considered and imaging studies such as CT scanning carried out.
Conclusions

Although PID can seem to be cured by oral antibiotics, it sometimes recurs and becomes chronic. Initial treatment for PID should be addressed with extreme prudence; once PID advances to TOA, radical surgery is often required. Strong broad-spectrum antibiotic treatment while awaiting surgery can facilitate adhesiotomy during the operation; this is preferable to an emergency operation without adequate preparation. A pelvic abscess tends to involve the urinary tract when it expands. Ureteral stenting can be useful to protect the urinary tract. Finally, thorough irrigation of the abdominal cavity at the end of the operation and inserting a drainage tube after concluding the surgery are important to prevent abscess recurrence and wound infection.

References

The determination of high-risk pregnancy: the use of antenatal scoring system

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²Ege University School of Medicine, Department of Obstetrics and Gynecology, Izmir (Turkey)

Summary

Aims: A standardized antenatal scoring system is not available in order to detect high-risk pregnancies at primary health care units in Turkey. The authors aimed to evaluate the applicability of the “Knox scoring system” in Turkey. Materials and Methods: One hundred and twenty-nine pregnant women were included in study. They were assessed upon admission and then the same women were reassessed at the onset of labor by Knox scoring system. Results: The Knox scoring system identified 65 pregnant women (50.4%) as high-risk upon admission while 22 pregnant women (17.1%) as high-risk at the onset of labor. Twelve pregnant women (9.3%) (one case of perinatal death and 11 cases of perinatal morbidity) had poor perinatal outcomes during the study period. The Knox scoring form administered upon admission yielded 58% sensitivity, 50% specificity, and 10% positive predictive value, while 91% sensitivity, 90% specificity, and 50% positive predictive value at the onset of labor. The diagnostic value of the Knox scoring system for determining high-risk pregnancies was not found statistically significant regarding admission (ROC value: 0.655; p > 0.05), while statistically significant regarding the onset of labor (ROC value: 0.946; p < 0.05). Conclusions: The use of the Knox scoring system for determining high-risk pregnancies seems to be effective at the onset of labor.

Key words: Antenatal scoring system; High-risk pregnancy; Knox scoring system.

Introduction

Physiological changes that occur during pregnancy may narrow the line between health and disease. Due to maternal and fetal diseases and anomalies, some complications may develop during pregnancy and at labor. Pregnancy-related deaths occurring among women in the antepartum and peripartum periods are an important health problem in developing countries [1]. First pregnancy, high parity, interpregnancy intervals shorter than two years, advanced maternal age, short maternal stature, low body mass index (BMI), inappropriate weight gain during pregnancy, poor obstetric history, anemia, smoking during pregnancy, history of stillbirth, and malnutrition are some of the worldwide accepted risk factors [1, 2]. Turkey Demographic and Health Survey (TDHS) demonstrated that 69.4% of the pregnancies in the country were classified under the risk category [3]. Accordingly, high-risk pregnancies remain a significant problem in Turkey [4]. Early identification of risk factors associated with pregnancy and providing relevant and timely treatment may reduce maternal and fetal mortality rates, and improve pregnancy outcomes. Hence, high-risk pregnancies should be identified and deliveries should be carried out in the referring hospital [5, 6].

The factors of antenatal risk can be estimated in several ways. The informal (clinical) antenatal risk assessment was classified by Hobel et al. [7] as level 1 obstetric risk assessment. The accuracy of level 1 assessment depends on the experience of healthcare professionals. Level 2 risk assessment uses the presence or absence of single risk factors to decide whether a person is at risk. In this level 2 assessment, a large number of women will be considered high-risk and all risk factors are considered to have an equal effect on outcome. Level 3 assessment assigns each factor a statistical weighting to reflect the fact that different risk factors have differing levels of effect. The use of statistical weighting is potentially more effective than clinical weighting because it excludes experimental bias.

The risk scoring system is one of the available methods for predicting situations at risk associated with pregnancy in the prenatal period. Hence, a wide range of risk scoring systems diversely evaluating these risks are available in the literature [8-12]. Among these, the scoring system developed by Knox et al. in 1993 in New Zealand, is one of the methods exhibiting the highest predictive value [11].

In Turkey, pregnancies at risk are roughly determined according to the patient’s history at the prenatal period without using a standardized risk scoring system in primary health care units. This current study aimed to evaluate the applicability of the “Knox Scoring System”, which has been developed to identify pregnant women at risk according to the circumstances in Turkey.

Materials and Methods

In this study the Knox scoring system was used to determine pregnancy-related risks by using 27 significant antenatal variables. The Knox scoring system was applied upon admission and at the onset of labor at the level III hospital in Izmir, Turkey. The pregnant women in this study were 159 and recently diagnosed before 20 weeks of gestation and filled out a scoring form. Within the next few days, 11 cases resulted in abortion, while 19 pregnant women were lost at follow-up. When the remaining 129 pregnant women applied to the same hospital to give birth,
they filled out the scoring form again. The study was approved by the local ethics committee. Oral and written informed consent was taken from each participant.

During the first questioning, variables that could be detected at the beginning of the pregnancy were asked (minimum 14, maximum 25 inquiries). The purpose of this survey was to determine the prenatal care plan (frequency of routine follow-up) of the pregnant woman according to her risk status, furthermore, determining the relevant patient care unit. During the second questioning, the Knox scoring system was entirely administered at the onset of labor. It was intended to re-evaluate the degree of risk and refer high-risk pregnancies requiring a higher level of care to specialized units.

The Knox scoring form, is a statistically-weighted risk scoring system using data on 27 pregnancy-specific significant risk factors. It consists of six sections covering socio-demographic data, individual characteristics, previous obstetric, gynecological and medical history, any health problems experienced during pregnancy (gestational diabetes, etc.), and gestational week at birth. Considering the social structure of the country, ethnicity was excluded from socio-demographic variables, and the validity of the Knox scoring form was evaluated via 26 items. A high-risk pregnancy was identified using the exact sum of logistic coefficients used by the Knox score (> 0.4 upon admission and > 2.75 at the onset of labor). A poor outcome in this analysis was defined as perinatal mortality (any death after 20 weeks of pregnancy or during the first week of life) or perinatal morbidity (defined as a stay of longer than five days in a neonatal unit).

Receiver-Operating Characteristic (ROC) analysis was performed with the construction of ROC curves to identify the cut-off values.

### Results

The Knox scoring system identified 65 pregnant women (50.4%) as high-risk and 64 (49.6) as low-risk during the admission visit. At the onset of labor, the Knox scoring system identified 22 pregnant women (17.1%) as high-risk and 64 (49.6) as low-risk during the study period. Seven of these cases upon admission and 11 of these cases at the onset of labor were predicted by the Knox scoring system with positive predictive values of 10.8% (7/65) and 50% (11/22), respectively.

According to these results, the Knox scoring form administered upon admission yielded 58% sensitivity, 50% specificity, and 10% positive predictive values, while 91% sensitivity, 90% specificity, and 50% positive predictive values at the onset of labor (Tables 1 and 2). Of the pregnant women enrolled in the study, 59.7% achieved normal vaginal deliveries, whereas 40.3% had cesarean section. No significant maternal morbidity was noted by the participants.

The diagnostic value of the Knox scoring system for determining high-risk pregnancies was not found to be statistically significant regarding admission (ROC value: 0.655; p > 0.05), while statistically significant regarding the onset of labor (ROC value: 0.946; p < 0.05).

<table>
<thead>
<tr>
<th>Poor perinatal outcome</th>
<th>Good perinatal outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk</td>
<td>7</td>
<td>58</td>
</tr>
<tr>
<td>Low-risk</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>117</td>
</tr>
</tbody>
</table>

Sensitivity: 7 / 12 (58%), Specificity: 59 / 117 (50%), Positive predictive value: 7 / 65 (10%).

<table>
<thead>
<tr>
<th>Poor perinatal outcome</th>
<th>Good perinatal outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Low-risk</td>
<td>1</td>
<td>106</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>117</td>
</tr>
</tbody>
</table>

Sensitivity: 11 / 12 (91%), Specificity: 106 / 117 (90%), Positive predictive value: 11 / 22 (50%).

### Discussion

The identification of pregnancies at risk is one of the most important components concerning prenatal care and moreover, considered very helpful both for the patient and for the general health system [8]. Through such an assessment, high-cost medical examinations and treatments would be reserved for high-risk pregnancies, while low-risk pregnancies can be managed with minimal interferences in normal delivery rooms [11, 13].

According to the Knox scoring data obtained upon admission, half of the pregnant women were at high-risk (65 high-risk / 64 low-risk); therefore, the validity of the Knox scoring system was not found statistically significant. This could be explained with some distinctive data regarding the Knox scoring variables in that of the pregnant women enrolled in this study group, 67% displayed primary school or a lower education status, 14% had less than 50 kg body weight, 17.8% smoked during pregnancy, 15.5% noted shorter than one year interpregnancy interval, and 9.3% had Rh- blood group. In addition, poor obstetric outcomes with respect to previous pregnancies were frequently reported. Moreover, this study was conducted in a level III hospital, where high-risk pregnancies were commonly referred for early diagnosis and treatment, as well as for follow-up.

The sensitivity, specificity, and positive predictive values the authors obtained from the Knox scoring form administered at the onset of labor, were similar to the data reported by Knox et al. [11] in New Zealand (sensitivity 90%, specificity 87%, positive predictive value 42%).

The positive predictive value obtained by Knox et al. [11] in the scoring form administered at the onset of the labor was reported as the highest to date. In this present study, the positive predictive value obtained upon admission was similar to the value of Knox et al.

Mohamed et al. [12] compared the data of Knox scoring system administered both upon admission and at late pregnancy (36 weeks gestation) with data of the scoring system they used in England. Upon admission, Knox scored 11.7% of women as high-risk, while their system scored 48.9%. At 36 weeks gestation, Knox scored 1.4% of the same pregnant women as high-risk, while their scoring system iden-
tified 37.9%. The positive predictive value they found with the Knox scoring system (18%) was very close to the value reported by Knox et al. [11] (16%), but higher than the value of their scoring system (12%). However, sensitivity was determined higher with their scoring system (90%) compared to the Knox scoring system (30%), and both were rather different than the value reported by Knox et al. [11] (62%). In the present study, sensitivity was 58% and positive predictive value was 10% upon admission, which were rather low compared to the values reported by Knox et al. [11]. The significant difference in the findings of two groups may be correlated with the majority of the high-risk pregnancy in this study group. Additionally, as stated by Mohamed et al. [12], it is probable that the predictive accuracy of any score can be altered by the frequency of poor outcome in the population tested. The results of previously studies are summarized in Table 3.

Low-risk pregnant were almost half of this study group upon admission (49.6%), while 82.9% at the onset of labor. Mohammed et al. [12] also reported low-risk in half of their pregnancy cases, both with Knox scoring system and also with their own scoring system. Recognition of the low-risk pregnancies is also important. Comparison of midwife/general practitioner-managed care vs obstetrician/gynaecologist shared care showed similar clinical efficacy [1, 14].

Pregnancies at risk should be differentiated from normal pregnancy cases through antenatal care. These should be monitored frequently in appropriate conditions. However, there is no perfect system for predicting pregnancy complications, which can develop at any stage. Therefore, beginning from the antenatal period, risk assessment should be performed on a regular basis with certain periods throughout pregnancy, and repeated at the onset of labor.

The authors did not achieve statistically significant validity with the data of Knox scoring system administered upon admission. This could be explained as the study was conducted in a level III hospital, where high-risk pregnancies were commonly referred for early diagnosis and treatment, as well as to follow-up. This situation was a limitation in the present study. The positive predictive value reported by Knox et al. was higher compared to data of all the other studies conducted. The authors found a similar positive predictive value when applied at the onset of labor. The results obtained at the onset of labor demonstrated that more accurate evaluation of the pregnant women could be managed with this scoring system, thus unnecessary referral to level III hospitals could be reduced.

In Turkey, a standardized scoring system is not available in order to detect high-risk pregnancies. Randomized controlled studies with larger sampling size are required to be conducted especially in primary health care units. In the light of these data, new scoring systems relevant to the conditions in this country, can be further developed.

### Table 3: The result of antenatal scoring systems.

<table>
<thead>
<tr>
<th>Gestation at scoring</th>
<th>% in risk group</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Number of items in score</th>
<th>Prevalence of poor outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knox et al. Booking</td>
<td>31</td>
<td>63</td>
<td>72</td>
<td>16</td>
<td>15</td>
<td>7.6</td>
</tr>
<tr>
<td>Onset of labor</td>
<td>16</td>
<td>87</td>
<td>90</td>
<td>42</td>
<td>27</td>
<td>7.6</td>
</tr>
<tr>
<td>Mohamed et al. Knox</td>
<td>12.7</td>
<td>30</td>
<td>90</td>
<td>17.6</td>
<td>27</td>
<td>6.8</td>
</tr>
<tr>
<td>system Booking</td>
<td>36. gestational weeks</td>
<td>1.4</td>
<td>54</td>
<td>12.1</td>
<td>50</td>
<td>6.8</td>
</tr>
<tr>
<td>Current study</td>
<td>36. gestational weeks</td>
<td>38</td>
<td>50</td>
<td>10</td>
<td>14</td>
<td>9.3</td>
</tr>
<tr>
<td>Booking</td>
<td>11</td>
<td>58</td>
<td>50</td>
<td>10</td>
<td>14</td>
<td>9.3</td>
</tr>
<tr>
<td>Onset of labor</td>
<td>50</td>
<td>91</td>
<td>90</td>
<td>50</td>
<td>26</td>
<td>9.3</td>
</tr>
</tbody>
</table>

### References


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Investigation on maternal physiological and psychological factors of cheilopalatognathus

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Summary

Objective: Case-control study on mothers of cheilopalatognathus children was conducted, to investigate the maternal physiological and psychological factors for occurrence of cheilopalatognathus. Materials and Methods: One hundred ten mothers of cheilopalatognathus children who were scheduled for one-stage surgery were selected as a research group, and 110 mothers of normal children served as a normal control group at the same time. Trait Anxiety Inventory (T-AI), Life Events Scale (LES), Trait Coping Style Questionnaire (TCSQ), Type C Behavior Scale (CBS), adult Eysenck Personality Questionnaire (EPQ), and homemade general questionnaire survey were employed for the investigation. Results: Compared with the control group, the scores for negative event tension value, anxiety, and depressive factors were higher in the study group ($p < 0.05$); while the scores for positive event tension value, intellect, optimism, and social support factors were lower ($p < 0.05$). Regression analysis found that physiological factors included were five: education, changes in body weight during pregnancy, the intake amount of milk and beans, and intake of healthcare products, and supplementary folic acid taken or not, while the psychological factors included were four: positive event stimulation, negative event stimulation, the amount of social support, as well as introvert and extrovert personalities. Conclusion: The study results suggest that pregnant women’s physiological and psychological factors can cause changes in cheilopalatognathus incidence, which is expected to be guidance for healthcare during pregnancy, to prevent the occurrence of cheilopalatognathus.

Key words: Cheilopalatognathus; Physiological and psychological factors; Maternal factors; Investigation.

Introduction

Cheilopalatognathus is a congenital deformity of mouth and face. Cheiloschisis is caused by the un-fusion of maxillary process with the probiam about six weeks after conception. Simple clef palate palatate caused by the un-fusion of two plates of the skull that form the hard palate (roof of the mouth) during eight to nine weeks after pregnancy [1-3], and it is the most common oral and maxillofacial congenital malformations, occurring on average in about one in 700 live births in China [4, 5].

Its occurrence is due to, during embryonic development of the maxillofacial region, the effect of multiple pathogenic factors such as genetics, nutrition, endocrine, infection, physical and chemical damage, drugs, alcohol, and tobacco, leading to blockage of the normal development and fusion of the embryo, thus resulting in deformity [6-8]. Cheilopalatognathus not only seriously affects the facial appearance, but also directly affects the development as the mouth and nasal cavity are connected. Severe cheiloschisis in children will affect feeding, and the children cannot be normally fed by breastfeeding, resulting in malnutrition in children. The children will present a speech impediment during growth and development.

A large number of studies on the etiology of cheilopalatognathus have been reported, involving studies on a number of environmental factors, genetic modes, and multiple gene loci, as well as the interaction of environmental and genetic factors [9, 10]. These studies have raised a number of important clues to the etiology and have provided a solid foundation for further research. However, most of the conclusions of the study reported were inconsistent, or need to be confirmed by further study [8, 11]. In particular, what physiological and psychological factors of the mother during pregnancy will produce a significant effect on the fetus, to which there is no consistent understanding and conclusions [12-14].

Some studies have reported that, the older age of the mother is a high risk factor for occurrence of cheilopalatognathus [15]. However, more studies have not found that the elder age of the mother is a high risk factor for occurrence of it [1, 16-18]. On the contrary, some studies have found that the younger age of the mother on the contrary, would be a high risk factor for occurrence of cheilopalatognathus [19, 20]. At the same time, the intake of vasoactive drugs such as pseudoephedrine, aspirin, ibuprofen, amphetamine methamphetamine, cocaine or psychedelic drugs and smoking can lead to high incidence of cheilopalatognathus [9, 21-25]. Anti-convulsant drugs such as phenobarbital, tridione, valproic acid, and dilantin will also lead to high incidence of cheilopalatognathus [6, 26-33], but there is also questioning that this is caused by the drugs or potential epilepsy [31]. Isotretinoin may also be responsible for its
Results

Comparison of Trait Anxiety Inventory (T-AI)

With the T-AI, the score for the study group was higher than that of the control group, and the difference was statistically significant ($p < 0.05$; Table 1).

Comparison of Life Events Scale (LES)

Negative event stimulation in the study group was significantly higher than that in the control group; the positive event stimulation in the study group was significantly lower than that in the control group, and the differences in both the positive and negative life event stimuli between the two groups were statistically significant ($p < 0.05$); while in the total amount of life event stimuli, there was no significant difference between the study and control groups, and the difference was not statistically significant (Table 2).

Comparison of Trait Coping Style Questionnaire (TCSQ)

Score for the negative coping (NC) of the study group was lower than that in the control group, and the difference between the two groups was statistically significant ($p < 0.05$); there was no significant difference in positive coping (PC) score between the study group and control group, and the difference was not statistically significant (Table 3).

Comparison of C-type Behavior Scale score

Difference in the scores for anxiety, depression, intellect, optimism, and social support factor between the study and control groups was significant, and the difference was statistically significant ($p < 0.05$); whereas, the scores for two factors of anxiety and depression in the study group were higher than that in the control group, and lower than that in the control group for factors of intellect, optimism, and social support (Table 4).

Comparison of adult Eysenck Personality Inventory (EPI)

The differences in scores for factors of Extrovert (E), neuroticism (N), and lie (L) between the study and control groups were significant, and the differences were statistically significant ($p < 0.05$); the E factor, score of the study group was lower than that of the control group. For the N factor and the L factor, the score of the study group was higher than that of the control group; while for the P factor, difference in score of the study group and control group was not significant, and the difference was not statistically significant (Table 5).

Logistic stepwise regression analysis for maternal physiological and psychological high risk factors

The logistic stepwise regression analysis was used for screening of risk factors, and the psychological risk factors eventually entering the model included four: positive event stimulation, negative event stimulation, social support, as well as introversion and extroversion.

The regression equation was as follows: $Y = -0.318 + 0.069 \times \text{positive event stimulation} - 0.040 \times \text{negative event stimulation} + 0.196 \times \text{social support} + 0.110 \times \text{introversion and extroversion}$ (Table 6).
Table 1. — Comparison of scores for trait anxiety inventory of the study group and control group (x ± s).

<table>
<thead>
<tr>
<th>Investigation item</th>
<th>Experimental group</th>
<th>Control group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-AI</td>
<td>45.55 ± 7.595</td>
<td>39.49 ± 7.105</td>
<td>5.585</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of scores for life event scale of the study group and control group (x ± s).

<table>
<thead>
<tr>
<th>Quantity of stimulus</th>
<th>Experimental group</th>
<th>Control group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>of gross life events</td>
<td>26.25 ± 29.489</td>
<td>26.01 ± 28.738</td>
<td>0.054</td>
<td>0.957</td>
</tr>
<tr>
<td>of negative events</td>
<td>19.74 ± 25.480</td>
<td>10.91 ± 18.447</td>
<td>2.769</td>
<td>0.006</td>
</tr>
<tr>
<td>of positive events</td>
<td>6.51 ± 7.881</td>
<td>15.10 ± 16.392</td>
<td>-4.337</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of scores for introversion-extraversion questionnaire of the study group and control group (x ± s).

<table>
<thead>
<tr>
<th>Investigation item</th>
<th>Experimental group</th>
<th>Control group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>29.33 ± 9.049</td>
<td>32.51 ± 7.321</td>
<td>-2.591</td>
<td>0.010</td>
</tr>
<tr>
<td>PC</td>
<td>24.71 ± 8.220</td>
<td>24.61 ± 5.023</td>
<td>0.100</td>
<td>0.920</td>
</tr>
</tbody>
</table>

Table 4. — Comparison of scores for type C behavior scale of the study group and control group (x ± s).

<table>
<thead>
<tr>
<th>Investigation item</th>
<th>Experimental group</th>
<th>Control group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>44.25 ± 7.008</td>
<td>41.53 ± 6.853</td>
<td>2.675</td>
<td>0.008</td>
</tr>
<tr>
<td>Depression</td>
<td>45.18 ± 6.127</td>
<td>41.43 ± 5.618</td>
<td>4.320</td>
<td>0.000</td>
</tr>
<tr>
<td>Anger</td>
<td>20.47 ± 3.358</td>
<td>19.28 ± 4.830</td>
<td>1.383</td>
<td>0.115</td>
</tr>
<tr>
<td>Anger introversion</td>
<td>13.29 ± 3.090</td>
<td>13.29 ± 2.816</td>
<td>0.008</td>
<td>0.994</td>
</tr>
<tr>
<td>Anger extroversion</td>
<td>14.06 ± 3.764</td>
<td>14.71 ± 3.187</td>
<td>1.250</td>
<td>0.213</td>
</tr>
<tr>
<td>Intelect</td>
<td>35.74 ± 6.694</td>
<td>38.08 ± 4.420</td>
<td>-2.952</td>
<td>0.004</td>
</tr>
<tr>
<td>Control</td>
<td>15.07 ± 3.864</td>
<td>15.49 ± 3.023</td>
<td>0.798</td>
<td>0.426</td>
</tr>
<tr>
<td>Optimistic</td>
<td>20.04 ± 3.617</td>
<td>21.01 ± 3.116</td>
<td>-1.991</td>
<td>0.048</td>
</tr>
<tr>
<td>Social support</td>
<td>14.34 ± 3.374</td>
<td>16.10 ± 2.534</td>
<td>-4.115</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 5. — Comparison of scores for eysenck personality questionnaire scale of the study group and control group (x ± s).

<table>
<thead>
<tr>
<th>Investigation item</th>
<th>Experimental group</th>
<th>Control group</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>4.75 ± 2.981</td>
<td>4.75 ± 3.054</td>
<td>-0.010</td>
<td>0.992</td>
</tr>
<tr>
<td>E</td>
<td>9.90 ± 3.771</td>
<td>12.11 ± 4.424</td>
<td>-3.711</td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td>11.39 ± 5.502</td>
<td>9.83 ± 5.033</td>
<td>1.995</td>
<td>0.047</td>
</tr>
<tr>
<td>L</td>
<td>14.01 ± 3.500</td>
<td>12.39 ± 3.429</td>
<td>3.180</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 6. — Estimates of the independent variables and relevant parameters in the equations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity of positive events</td>
<td>0.069</td>
<td>0.017</td>
<td>16.258</td>
<td>0.000</td>
<td>1.071</td>
</tr>
<tr>
<td>Quantity of negative events</td>
<td>-0.040</td>
<td>0.011</td>
<td>13.952</td>
<td>0.000</td>
<td>0.961</td>
</tr>
<tr>
<td>Social support</td>
<td>0.196</td>
<td>0.058</td>
<td>11.243</td>
<td>0.001</td>
<td>1.216</td>
</tr>
<tr>
<td>Introversion-extroversion</td>
<td>0.110</td>
<td>0.045</td>
<td>6.056</td>
<td>0.014</td>
<td>1.116</td>
</tr>
</tbody>
</table>

Table 7. — Estimates of the independent variables and relevant parameters in the equations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational background</td>
<td>-1.211</td>
<td>0.259</td>
<td>21.885</td>
<td>0.000</td>
<td>0.298</td>
</tr>
<tr>
<td>Weight change during pregnancy</td>
<td>0.792</td>
<td>0.366</td>
<td>6.688</td>
<td>0.030</td>
<td>2.208</td>
</tr>
<tr>
<td>Milk and beans intake</td>
<td>-0.715</td>
<td>0.308</td>
<td>5.383</td>
<td>0.020</td>
<td>0.489</td>
</tr>
<tr>
<td>Healthcare products intake</td>
<td>0.717</td>
<td>0.243</td>
<td>8.725</td>
<td>0.003</td>
<td>2.048</td>
</tr>
<tr>
<td>Whether folic acid was added</td>
<td>2.166</td>
<td>0.555</td>
<td>15.201</td>
<td>0.000</td>
<td>8.720</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.534</td>
<td>1.533</td>
<td>1.001</td>
<td>0.317</td>
<td>0.216</td>
</tr>
</tbody>
</table>

Regression analysis was conducted on the social and biological risk factors, and the risk factors eventually entering the model included five, namely: education, changes in body weight during pregnancy, intake amount of milk and beans, and intake of healthcare products, as well as folic acid supplementation or not.

The regression equation was as follows: Y = -1.534 × X1 + 0.792 × X2 + 0.717 × X3 + 2.166 × X4 + 0.216 × X5 + 1.211 × X6 + 0.792 × body weight change during pregnancy - 0.715 × intake amount of milk and beans + 0.717 × intake of health care products + 2.166 × folic acid supplementation or not (Table 7).

Discussion

The present results have proved that: 1) for pregnant women with a high degree of anxiety, depression, negative event stimulation, negative coping manner, neurotic personality aspects and strong lie nature, cheilopalatognathus may occur; 2) for pregnant women with active coping manner, positive event stimulation, intellect, optimism, and a high degree of social support, the incidence of cheilopalatognathus may be reduced; 3) the education qualification, folic acid supplementation or not, intake of healthcare products, intake amount of milk and beans, and body weight change during pregnancy were all important factors for cheilopalatognathus, and they showed cheilopalatognathus, and early childhood lip cleft palate with the possibility of the degree of influence in descending order. Among them, the level of education qualification directly affects maternal self-regulation, highly-educated mothers were generally good at self-psychological adjustment, and were not often distressed by negative emotion, and thus the incidence of cheilopalatognathus can be reduced. Generally speaking, the family environment of highly-educated mothers was relatively better, being able to provide better material conditions for their pregnancy to ensure adequate supply of nutrition, which was of positive effect for the good development of the fetus.

Body weight changes during pregnancy caused restlessness in pregnant women. The oversensitivity of the pregnant women to the change would also lead to intensified anxiety; while persistent anxiety and tension would induce the secretion of adrenocorticotropic hormone, which would increase the incidence of fetal cheilopalatognathus.

In general, supplementation of a certain amount of folic acid and healthcare products and intake of a certain amount of milk and beans during pregnancy can guarantee the balanced nutrition of pregnant women to effectively reduce the incidence of cheilopalatognathus. However, this study showed that intake of healthcare products and supplementation of folic acid had on the contrary, a negative impact on the occurrence of cheilopalatognathus in early childhood, and the possible explanation is that this might be associated with excessive anxiety in mothers of cheilopalatognathus.
children during pregnancy, and the excessive anxiety and worry led them to an overdose of healthcare products and folic acid supplements in order to obtain good results. This excessive use of healthcare products and folic acid supplementation caused by anxiety covered up the act that the intake of them had a positive effect in reducing the occurrence of cheilopalatognathus in early childhood, resulting in the image of the negative impact showed in the data. Therefore, supplementation of a certain amount of folic acid and healthcare products during pregnancy, as well as intake of a certain amount of milk and beans can effectively reduce the incidence of cheilopalatognathus.

The results show that, the T-AI score in the group of cheilopalatognathus children was higher than that in the group of normal children, indicating that the anxiety level of mothers of cheilopalatognathus children was relatively high, which provides a basis for analysis of their characteristics or traits from the perspective of personality. Trait anxiety individuals often show a relatively continuous anxiety in life; for pregnant women, this ongoing anxiety would cause psychological stress in pregnant individuals, and would adversely affect the secretion of hormones and neurotransmitters, eventually leading to the generation of cheilopalatognathus in infants.

Analysis of life event stimulation showed that, negative event stimulation in the study group in life was higher than that in the control group, while the positive event stimulation in life in the study group was lower than that in the control group. This reflects the fact that, as compared to mothers of normal children, the maternal population of children with cheilopalatognathus encountered more negative event stimulation in their lives. Generally, negative life events experienced by the individuals would have a negative impact on the individuals, and would bring much psychological pressure on the individuals, accompanied by a series of adverse psychological factors such as anxiety, depression, obsession, hostility, and fear in their lives. This results in a very unfavorable environment for the development of the fetus. Therefore, the pregnant woman must be actively guided to make reasonable adjustments to their own state of mind and pay attention to prenatal care.

From the results of TCSQ, the negative coping score of maternal group of normal children was higher compared to that of maternal group of cheilopalatognathus, and there was no difference in active coping between the two groups. This may be related to the lying nature of this part of population in the maternal group of cheilopalatognathus children that was higher in terms of personality lie. Due to the relatively high lying nature of maternal group of cheilopalatognathus children, they deliberately avoided this part of the negative information when answering the questionnaire, eventually leading to these pseudo-images of lower score on positive coping of the group and no significant differences between the two groups.

At the same time, the scores for anxiety and depressive factor of mothers of cheilopalatognathus children in CBS were consistently higher than that of the control group of mothers of normal children, and intellect, optimism, and social support factors in the CBS were significantly lower than that in the control. In addition, there was no significant difference in scores for anger, introversion and extraversion, anger, and control factors between the two groups. Thus, there was a more obvious tendency for type C behavior in the maternal group of the cheilopalatognathus children, which has played a negative impact on the occurrence of cheilopalatognathus symptoms in young children. The analysis showed that they demonstrate more widespread and deeper anxiety and depression in their daily lives, which was related to the lack of intellect in them to face conflict and frustration of the event. Meanwhile, relative lack of optimism promoted the manifestation of the type C behavior. In addition, they felt less social support. This on one hand may reflect the fact that they received less social support; on the other hand, it is more likely that their subjective sensitivity to social support given by the surrounding population was poor. The former reflects the adverse impact of external factors on the manifestation of type C behavior; the latter is more likely to reflect the higher expectations of maternal group of children with cheilopalatognathus for social support as compared with maternal groups of normal children; thus it is difficult for them to obtain subjective satisfaction from the general social support, which explains the impact of this phenomenon on the fact that mothers of cheilopalatognathus children showed more common type C behavior. With regards to the result that there was no significant difference in anger, introversion and extraversion between the two groups, analysis showed that it was related to the consistent degree of anger control in the two groups.

Results of EPQ questionnaire score showed that score for introversion and extraversion dimension of mothers of cheilopalatognathus children was significantly higher, indicating that the personality traits of mothers of cheilopalatognathus children tend to be introversion. This type of individuals is accustomed to introspection, relatively would pay more attention to inner experience, making it easy to be in a continuously anxious and depressive state, thus negatively impacting fetal development. Score for neuroticism of mothers of cheilopalatognathus children was also significantly higher, which indicates the cheilopalatognathus mother was not only in a negative state of mind at this stage of pregnancy, they would often feel anxiety, fear, melancholy and worry about their daily lives, and there would be a strong emotional response, so that a more irrational behavior would occur, which was closely linked with their corresponding personality traits. However, the score for mental quality dimension of both populations showed no significant difference, which reflects that although it has been affected by negative factors, the mother’s behavior and mental performance of the cheilopalatognathus children were still within the normal range, and there would be less unusual behaviour. Score for lie dimension of mothers of cheilopalatognathus children was significantly higher than that in the control group. This indicates that after the birth of cheilopalatognathus children, the mother would have larger mental pressure, and the sensitivity to the negative information would generate psychological discomfort. As a positive and effective solution could not be found, the correspon-
ding avoidance mechanism would be activated in face of the negative information.

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References


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The effects of hormone therapy on ischemia modified albumin and soluble CD40 ligand levels in obese surgical menopausal women

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Summary

Purpose: To determine the effects of hormone therapy (HT) on ischemia modified albumin (IMA) and soluble (s)CD40 ligand in obese surgical menopausal women. Materials and Methods: A total of 52 obese surgical menopausal women with a body mass index (BMI) > 30 kg/m² were admitted to the study. Twenty-seven women received estradiol hemihydrate two mg and 25 did not receive any menopausal therapy. At baseline and after three and six months of treatment, IMA and sCD40 ligand levels were measured. Results: There were no significant differences among the groups for any variables at baseline. No difference in change in the serum sCD40L levels was found in obese surgical menopausal women after three and six months of HT. Serum IMA levels were statistically lowered in obese women with HT after six months of treatment. Conclusion: HT may have a beneficial reduction in IMA levels in obese surgical menopausal women.

Key words: Hormone therapy; sCD40 ligand; Ischemia modified albumin; Body mass index; Surgical menopause.

Introduction

Cardiovascular disease (CVD) is the leading cause of death and morbidity in women aged 45 years and older [1]. The incidence of CVD and stroke increase after menopause because of the menopause transition is associated with an increased body weight [2], physical inactivity, high blood pressure, diabetes, and high cholesterol. In women with surgical menopause and who do not take estrogen, their risk for heart disease may also be higher. However, according to the Women’s Health Initiative (WHI), randomized controlled trial, HT or estrogen therapy are not indicated for prevention of coronary artery disease (CAD). In addition, the Heart and Estrogen/progestin Replacement Study (HERS) [3] showed that there was no beneficial reduction of CAD incidence in postmenopausal women with CAD who received HT. On the other hand, the Nurses’ Health Study demonstrated an approximately 11% risk reduction for primary CVD in postmenopausal women using HT compared with women who had never used HT, irrespective of duration of use [4].

The ligand for CD40 (CD40L) is a membrane glycoprotein on activated T cells that induces B cell proliferation and immunoglobulin secretion. Activated platelets express CD40L on their plasma membrane and release the soluble fragment sCD40L. The interaction between platelet surface CD40L and endothelial cell CD40 leads to the activation of endothelium contributing to atherothrombosis [5]. Increased plasma levels of soluble CD40 ligand have been related with increased risk of unstable angina, myocardial infarction (MI), diabetes, CAD and atherothrombotic events [6].

Ischemia modified albumin (IMA) is a sensitive and early biochemical marker of ischemia that is produced when circulating serum albumin contacts ischemic heart tissues. Postmenopausal obesity is associated with elevated serum IMA possibly due to obesity associated oxidative stress. IMA measurement could provide an assessment of atherosclerotic burden in postmenopausal women [7].

In this study, the authors’ aim was to determine the serum concentrations of IMA and soluble (s)CD40 ligand in surgical menopausal women on HT with a body mass index (BMI) > 30 kg/m².

Materials and Methods

After following the process of screening, 59 subjects were randomized to the treatment and to a control group receiving no therapy (calcium 500 mg) according to a computer-generated randomization table. Seven patients (three women in the HT receiving group and four women in the placebo group) did not complete the study. The reasons for non-completion included generalized discomfort following medication at six months (n = 1), failure of compliance (n = 6). Therefore a total of 52 women aged 40-58 years, who were obese (BMI > 30 kg/m²) and who had undergone total abdominal hysterectomy with bilateral salpingo-oophorectomy (surgical menopause) for one month previously due to benign gynecologic conditions, were included into this prospective, controlled clinical study. The patients had previously taken HT or treatment for cholesterol within the past year, women with diabetes, cancer, liver, renal or hematological disease, smokers, or other medical disorders were excluded. In summary, 27 women received estradiol hemihydrate two mg, q.d. and 25 women were not on HT.

Subjects underwent testing at baseline and after three and six months of therapy. Height (cm) and weight (kg) were measured to calculate BMI as weight (kg)/height (m²). The study protocol was approved by the Medical Ethics Committee of this University and a written informed consent was obtained from each patient who
participated in this study. This work was supported by the Research Fund of Karadeniz Technical University, project number: 2009.114.001.2

Biochemical analysis was performed in the Department of Clinical Biochemistry. After obtaining blood samples in plain tubes containing separation gels, the samples were allowed to clot for 30 minutes and centrifuged before separating the serum. The samples were then immediately frozen and stored at -80 °C for assays IMA.

Levels of human Serum sCD40L were determined by enzyme-linked immunosorbent assay kit, according to the manufacturer’s protocols. The absorbance of samples was measured at 450 nm using a tunable microplate reader. The results were expressed as ng/ml.

Reduced cobalt to albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method of Bar-Or et al. [8]. Two hundred μl of rat serum were placed into glass tubes and 50 μl of 0.1% cobalt chloride (CoCl₂.6H₂O) in H₂O was added. After gentle shaking, the solution was left for ten minutes to ensure sufficient cobalt albumin binding. Fifty microliters (μl) of dithiothreitol (DTT) (1.5 mg/ml H₂O) was added as a colorizing agent, and the reaction was quenched two min later by adding 1.0 ml of 0.9% NaCl. A colorimetric control was prepared for preoperative and postoperative serum samples. In the colorimetric control samples, 50 μl of distilled water was substituted for 50 μl of 1.5 mg/ml DTT. Specimen absorbance was analyzed at 470 nm by a spectrophotometer. The color of the DTT-containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSUs).

Statistical methods

Statistical analyses were performed by using Statistical Package for Social Sciences version 13.0.1. Results are expressed as means ± SD. The data were assessed for normal distribution by using Kolmogorov-Smirnov test. Mann-Whitney U-test was used to compare the variables obtained from patient and controls groups. Analysis of changes from baseline to six months was carried out with the t-test for paired samples. An overall analysis used a classification cut-off p ≤ 0.05.

Results

Subject characteristics, biochemical measurements at inclusion time, and after three and six months of HT of obese women with surgical menopause are shown in Table 1. There were no significant differences between the pre-treatment groups for any variables shown. Compared with the untreated control group, although a small decreased values of sCD40L were found in the HT group after three and six months, no significantly difference was noted (p > 0.05, p > 0.05, respectively).

After three months of treatment, with respect to the measurement of IMA, there was no significant changes between HT and control groups (p > 0.05) (Table 2). After six months of treatment, IMA level was significantly increased in the untreated control group (p < 0.05) (Table 2). After three and six months of treatment, platelet counts were significantly decreased in the HT group (p < 0.01, p < 0.01, respectively) (Table 2).

Discussion

Results of the present study showed that plasma CD40L levels were not statistically decreased in obese surgical menopausal women after three and six months of hormone therapy. On the contrary, serum IMA levels in obese patients with HT were statistically lower than those of obese women without HT after six months of treatment.

Central obesity is associated with unfavorable changes in CVD risk factors (increasing coagulation, decreasing fibrinolytic factors, and insulin resistance), and with increased platelet activation leading to the release of proinflammatory mediators. Menopause is associated with an increased risk of obesity and a shift to an abdominal fat distribution with associated increase of cardiovascular risk, oxidative stress with the alterations in the metabolic and endocrine status.

It was estimated that > 95% of the circulating sCD40L is derived from platelets [9]. Some studies have shown a positive correlation between sCD40L and platelet counts [10], but others have demonstrated no relationship [11]. A role of platelets in the evolutionary phase of the atherosclerotic plaque can be related to the adhesion of exposed subendothelium after endothelial injury and to the releasing vasoactive substances that induce smooth muscle cell migration and proliferation [12] and can promote foam cell formation even in the absence of hyperlipidemia [13]. Obesity is characterized by the presence of a prothrombotic state with a combination of increased thrombin generation [14], platelet hyperactivity, and decreased fibrinolysis [15] suggesting that platelet activation plays a central role to accelerate atherothrombosis by an interaction with central obesity. However there is no direct clinical evidence that platelets contribute to coronary atherosclerosis [16]. sCD40L levels > 3.71 ng/ml were associated with a 2.8-fold increase and sCD40L levels > 5.54 ng/ml were associated with a 10.62-fold increase in cardiovascular risk in a nested case-control study in healthy, middle-aged women [17]. Since this study population was limited by the small number of cases, lack of statistical power may have limited the probability to detect any relationship. Secondly, it remains unclear whether HT-associated increases or de-

| Age (years) | 48 ± 4.74 | 49 ± 4.24 |
| Time since menopause (years) | 5.3 ± 1.51 | 5.9 ± 6.04 |
| BMI (kg/m²) | 33 ± 4.07 | 32 ± 3.17 |
| Glucose (mg/dl) | 87 ± 1.01 | 86 ± 1.86 |
| Total cholesterol (mg/dl) | 203 ± 40.05 | 198 ± 57.29 |
| Triglycerides (mg/dl) | 149 ± 76.80 | 143 ± 55.02 |
| HDL (mg/dl) | 49 ± 1.56 | 48 ± 9.43 |
| LDL (mg/dl) | 137 ± 34.99 | 151 ± 35.73 |
| IMA | 0.44 ± 0.08 | 0.49 ± 0.22 |
| Soluble CD40 ligand (ng/ml) | 5.15 ± 3.49 | 5.66 ± 2.85 |
| Platelet (x10³/l) | 255,200.00 ± 533,44.00 | 277,925.93 ± 42,051.18 |

Values are mean ± SD and %.

HT: hormone therapy. IMA: ischemia modified albumin.

* There were no significant differences among treatment groups for any variables shown. BMI: body mass index. HDL: high-density lipoprotein. LDL: low-density lipoprotein.
According to the present study results, it appears that HT oral estrogen therapy increased CRP levels, while transoral regimens do not appear to raise CRP [23]. Moreover, resolution [22], rather than an inflammatory response as noninvolves first-pass hepatic up-regulation of CRP expression [22], the association between HT use and elevated CRP likely the months of HT in the present study may be explained with increased CRP levels, the decreased IMA level after six months of HT after six months of treatment. Despite the fact oral estrogen therapy might be associated with increased CRP levels, the decreased IMA level after six months of HT after six months of treatment, it remains unclear whether HT-associated decreases in IMA can account for the protective cardiovascular effects of HT. In addition, the effect of HT on sCD40L and IMA levels might be changed if the exclusion criteria of not having any women with some risk factors associated with early cardiovascular events such as hereditary influence, diabetes, hypertension, and smoking could be included into the present study. Moreover, the results of HT on cardiovascular system are not uniform and this variability may be explained with single therapy with estrogen, route of medicaments, age of subjects, analysis of the overall sample (men and women), and the time of sampling. The complexity of the pathway of atherosclerosis and atherothrombosis process, and the fact that platelet activation and proinflammatory mediators, including sCD40L and IMA, sometimes correlate with each other, rendering it difficult to study the effects and determinants of each separate factor.

In conclusion, it is not clear whether HT confers coronary risk reduction through an inflammation-sensitive mechanism. It seems reasonable to consider that initiation and continuation of HT should be based on established non-coronary benefits and risks, and patient preference. However, based on the results of this study including serum IMA levels, were statistically lower in obese women with HT after six months of treatment, and the authors also suggest that HT may be useful in obese surgical menopausal women. Larger prospective studies are needed to further investigate the effect of HT on serum markers of neutrophil and platelet activation.

Table 2. — Body mass index and hematological measurements at inclusion time and after three and six months of treatment in obese surgical menopausal women.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Women not on HT (n = 25)</th>
<th>Women on HT (n = 27)</th>
<th>p_a</th>
<th>p_b</th>
<th>p_c</th>
<th>p_d</th>
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<tbody>
<tr>
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<tr>
<td>0</td>
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<td>6</td>
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<td>IMA</td>
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<td>0.43 ± 0.08</td>
<td>0.49 ± 0.22</td>
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<td>&lt; 0.05*</td>
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<tr>
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<td>0.46 ± 0.16</td>
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<td>0.41 ± 0.16</td>
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<td>Soluble CD40 ligand (ng/ml)</td>
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<td>5.15 ± 3.49</td>
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<tr>
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<td>6.41 ± 2.64</td>
<td>4.81 ± 3.80</td>
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<td>Platelet (x10³/l)</td>
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<tr>
<td>0</td>
<td>255,200.00 ± 53,344.00</td>
<td>277,925.93 ± 42,051.18</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.01*</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
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<td>274,240.00 ± 99,594.71</td>
<td>248,370.37 ± 46,469.57</td>
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<tr>
<td>6</td>
<td>278,240.00 ± 103,724.50</td>
<td>240,962.96 ± 53,088.56</td>
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</tr>
</tbody>
</table>

Values are presented as mean ± SD. *: Significant. HT: hormone therapy. BMI: body mass index. IMA: ischemia modified albumin. p_a: month 3 in comparison with 0 in surgical menopausal women with estradiol hemihydrate two mg. p_b: month 6 in comparison with 0 in surgical menopausal women with estradiol hemihydrate two mg. p_c: month 3 in comparison with 0 in surgical menopausal women without HT. p_d: month 6 in comparison with 0 in surgical menopausal women with estradiol hemihydrate two mg. Student’s paired t-test used.

Increases in sCD40L levels can account for the adverse cardiovascular effects of HT in future. sCD40L is expressed in both adipocytes and stromal adipose fraction of obese patients. Adipocyte CD40 is biologically active, inducing adipokine secretion mediated by T-cell adipocyte interaction and T-cell CD40L [18]. As this study population was limited to obese surgical women, the authors speculated that the statistically mean difference of sCD40L could be documented if the study sample had also included non-obese women. Further work is necessary to determine the exact mechanism of sCD40L on platelet activation and arterial thrombosis between the processes of thrombosis and inflammation in obesity.

The determination of serum IMA levels may provide earlier information of the presence of CAD before high sensitivity C-reactive protein (hsCRP) elevation [19]. In the present study, focusing on surgical menopausal women, only serum IMA levels in obese patients with HT were statistically lower than those of obese women without HT after six months of treatment. Although there is no consensus on the impact of HT on CRP levels, it is well-recognized that women using HT are characterized by increased plasma CRP levels [20, 21]. The results in the present study suggest that there is an association of surgical menopausal obesity with high IMA levels, possibly due to obesity-associated oxidative stress and serum IMA levels that were statistically lower in obese patients with HT after six months of treatment. Despite the fact that oral estrogen therapy might be associated with increased CRP levels, the decreased IMA level after six months of HT in the present study may be explained with the association between HT use and elevated CRP likely involves first-pass hepatic up-regulation of CRP expression [22], rather than an inflammatory response as nonoral regimens do not appear to raise CRP [23]. Moreover, oral estrogen therapy increased CRP levels, while transdermal therapy did not modify CRP production [20, 24]. According to the present study results, it appears that HT benefit from the decreasing levels of IMA and may have a protective effect on CAD.

Based on the results of this study, including sCD40L was not associated with reduced levels of an inflammatory marker predictive of CVD in obese surgical menopausal women with HT, and consequently, serum IMA levels were statistically lower in obese women with HT after six months of treatment, it remains unclear whether HT-associated decreases in IMA can account for the protective cardiovascular effects of HT. In addition, the effect of HT on sCD40L and IMA levels might be changed if the exclusion criteria of not having any women with some risk factors associated with early cardiovascular events such as hereditary influence, diabetes, hypertension, and smoking could be included into the present study. Moreover, the results of HT on cardiovascular system are not uniform and this variability may be explained with single therapy with estrogen, route of medicaments, age of subjects, analysis of the overall sample (men and women), and the time of sampling. The complexity of the pathway of atherosclerosis and atherothrombosis process, and the fact that platelet activation and proinflammatory mediators, including sCD40L and IMA, sometimes correlate with each other, rendering it difficult to study the effects and determinants of each separate factor.

In conclusion, it is not clear whether HT confers coronary risk reduction through an inflammation-sensitive mechanism. It seems reasonable to consider that initiation and continuation of HT should be based on established non-coronary benefits and risks, and patient preference. However, based on the results of this study including serum IMA levels, were statistically lower in obese surgical menopausal women with HT after six months of treatment, and the authors also suggest that HT may be useful in obese surgical menopausal women. Larger prospective studies are needed to further investigate the effect of HT on serum markers of neutrophil and platelet activation.
References


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Reference charts and equations of fetal biometry for normal singleton pregnant women in Shaanxi, China

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Summary

Objective: To construct reference charts and equations of fetal biometry for singleton pregnant women in Shaanxi, China. Materials and Methods: This was a cross-sectional study involving 6,832 singleton pregnant women. One set of fetal ultrasonographic measurement data between the 16th to 41st gestational weeks (GW) was randomly selected from each pregnant woman, and biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) were recorded. Mean and standard deviation (SD) of BPD, AC, and FL were fitted by polynomial. Centile = Mean + Z × SD was used to calculate centiles. Differences in the 50th centile of BPD, AC, and FL between Hong Kong, Korean, Italian and Shaanxi fetuses were compared. Results: Mean of BPD, AC, and FL were well-fitted by quadratic polynomial, SD of BPD, AC and FL were fitted by linear regression. Equations for estimating mean and SD for BPD, AC, and FL from GW were obtained. Centiles for BPD, AC, and FL were calculated. From the 21st GW, the differences in BPD, AC, and FL between Hong Kong, Korean, Italian, and Shaanxi fetuses became larger. Conclusion: Fetal biometry reference charts and equations for estimating fetal size and GW could be used in obstetrics practice and research in Shaanxi, China.

Key words: Biparietal diameter; Abdominal circumference; Femur length; Reference charts; Shaanxi.

Introduction

Every year, at least 60% of the four million neonatal deaths that occur worldwide are associated with low birth weight (LBW), caused by intrauterine growth restriction (IUGR), preterm delivery, and genetic/chromosomal abnormalities [1]. An objective assessment of fetal growth has enormous utility in prenatal care. Ultrasound examination and measurement of fetal biometry has been proven to be a useful and accurate examination in evaluating fetal growth and developing status, estimating fetal age, and delivery date [2-4]. However, selecting appropriate reference charts is very important in defining “abnormal fetal growth”. Fetal growth and development is a continuous process. The most apt style of reference standard for fetal growth and development assessment is gestational weeks (GW) related centile charts. Although fetal biometric charts and equations for various populations using a recommended method [5] were published in the medical literatures [6-11], these centile charts may be inappropriate for other populations, because fetal biometry varies significantly by population’s characteristics, such as race, geography, etc [12, 13].

In China, national reference values for biparietal diameter (BPD), head circumference (HC), abdominal circumference (AD), and femur length (FL) used in clinical practice are tabulated as x ± SD according to GW, and all these references were established in the later 1980s [14]. Reference centiles for fetal biometry fitted by appropriate method are not available in daily obstetric care practice in mainland China. Shaanxi is a larger province in north-west of China, with a population of about 38.0 million. In recent five years, the birth defect rate is about 120/100,000 and ranks third among the 31 provinces or autonomous regions in mainland China, and the incidence of LBW is about ten percent, which is two times higher than that of the whole country [15]. Cross-sectional reference centile charts and equations for Shaanxi population using appropriate methods have not previously been published. The aim of this study was to construct reference charts and a prediction model using the recommended method [7] based on multiple two-dimensional (2D) ultrasound measurements of fetal BPD, AC, and FL from the 16th to 41st GW. The authors also wanted to compare the difference between these charts and published reference charts of other populations.

Materials and Methods

Subjects

This was a cross-sectional study conducted in obstetric departments of five maternal and child care hospitals located in different areas in Shaanxi province, China. Subjects were 6,832 Chinese women routinely scheduled for ultrasound examinations in obstetric departments between January 1st to December 31st, 2010.

Inclusion criteria were the following: (1) both parents ethnically are Chinese; (2) the date of the first day of the last normal menstrual period, and regular menstrual cycles (26-30 days) prior to pregnancy are remembered exactly; (3) difference in gestational age according to last menstrual period and according
to fetal crown-rump length (CRL) measurement in the first trimester of \( \leq \) four days [16].

Exclusion criteria were the following: (1) maternal diseases, such as hypertension, pre-eclampsia, diabetes mellitus, renal disease; (2) multiple pregnancies; (3) fetuses with congenital malformation, chromosomal abnormality, or IUGR; (4) uncertain date of last menstrual period, and irregular menstrual cycles.

Measurements

In each hospital, all examinations were performed by an appointed ultrasound physician with at least five years obstetric ultrasound experience. All the five ultrasound physicians were pre-trained. Ultrasound equipment was not the same type in this study, but were routinely calibrated by the Department of Biomedical Engineering of each hospital using an American Institute of Ultrasound in Medicine standard test object at intervals not exceeding three months.

Strict criteria for the characteristics of the image and caliper placement were defined at the beginning of this study according to standard methods [3, 4] and were described as follows: to maximize the accuracy of caliper placement, all measurement data were taken from images obtained at the largest possible magnification. BPDs were obtained from a transverse axial plane of the fetal head showing a central midline echo broken in the anterior third by the cavum septum pellucidum and demonstrating the anterior and posterior horns of the lateral ventricle. BPD was measured from the distal calvarial wall. AC was measured on a transverse circular plane of the fetal abdomen at the level where the spine, descending aorta, anterior third of the umbilical vein, and stomach bubble could be seen in the same plane. FL was measured from the greater trochanter to the lateral condyle, with both ends clearly visible and at a horizontal angle < 45°. Each ultrasound file was specially marked to ensure that data from the same pregnant woman were not entered into the study more than once. Each parameter was measured three times, and the mean was calculated and recorded on database specifically designed for this study. Last menstrual date, delivery date, ultrasound examination date, and other social-economic characteristics were also recorded in this database.

The present study was approved by the Fourth Military Medical University Ethics Committee for Human Research, and all the subjects signed an information consent form.

Statistical methods

The database was checked carefully during and after input to ensure data quality. To reduce correlation between measurements of repeated ultrasonography within a pregnancy, only one set of ultrasonography measurements was randomly selected from all the ultrasonography data during each pregnancy by using a computer program. The selected ultrasonography data were used for further analysis. The selected ultrasonography date and last menstrual date were used to calculate GW, rounding up when gestational age more than three days and down at three or less days. From the frequency table of each set of measurements, the authors found the number of validated data for BPD, AC, and FL in many GW before 16th were less than 50, so they only constructed reference charts for BPD, AC, and FL between the 16th to the 41st GW, all with a validated measurement number above 150.

Statistical analysis was performed using the Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). In order to obtain centiles for fetal biometric measurements, a multi-step procedure based on a regression model [17] was used. The procedure began from the equation for centiles:

\[
\text{Centile} = \text{Mean} + Z_{\alpha/2} \times SD
\]

where \( \text{Mean} \) and \( \text{SD} \) are respectively the mean and standard deviation of fetal measurements for each GW, \( Z_{\alpha/2} \) is \( \pm 1.88 \) for the 3rd and 97th centiles, \( \pm 1.645 \) for the 5th and 95th centiles, \( \pm 1.281 \) for the 10th and 90th centiles, and \( \pm 0.6745 \) for the 25th and 75th centiles. Firstly, means were modeled by fitting a polynomial curve to the original data. Models were chosen based on the coefficient of multiple determinations (\( R^2 \)) and Sum of Squares Due to Error (\( SSE \)). \( R^2 \) closer to 1 and \( SSE \) closer to 0 indicated a better fitting. Then, the residuals, which indicated the difference between fitted mean and raw mean of original data, were used for deriving the variability as a function of GW. After removing the algebraic signs from the residuals, they were regressed on GW by using a linear model. The fitted values multiplied by a corrective constant equal to \( \pi^{1/2} \) gave the GW-specific \( SD \) estimates. These values, together with the fitted mean obtained in the first step, were thus substituted in the centile equation to obtain any centiles for BPD, AC, and FL. Equations for estimating GW from BPD, AC, and FL were also obtained by fitting polynomial.

Centiles of this study and those published by Italian, Korean, and Hong Kong studies fitted by the recommended method were compared. The mean fetal measurements for Hong Kong were calculated from the published equations [18], for Korea [19], and Italy [20], referenced from the published centile tables. Because the smallest GW for Italians is the 17th week, so the 50th centile between the 17th to the 41st GW were compared to reflect the difference in fetal size between Italian, Korean, Hong Kong, and Shaanxi populations.

Results

General characteristics of the subjects

Fetal biometric measurements from 6,832 singleton preg-
areas occupied 47.3%, and the mean age of the subjects was 51.2%, the first delivery occupied 80.4%, from urban
pregnancies were analyzed. Among all the fetuses, males occupied 51.2%, the first delivery occupied 80.4%, from urban

Table 3. — Centiles for AC (cm) of Shaanxi singleton pregnancies.

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<tr>
<th>GW</th>
<th>3rd</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
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Table 2. — Centiles for BPD (cm) of Shaanxi singleton pregnancies.

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Table 5. — Sum of squares due to error (cm²) of each centile for BPD, AC, and FL.

<table>
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<tr>
<th>Centile</th>
<th>BPD</th>
<th>AC</th>
<th>FL</th>
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<tr>
<td>3rd</td>
<td>0.11</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>5th</td>
<td>0.06</td>
<td>0.10</td>
<td>0.07</td>
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<tr>
<td>10th</td>
<td>0.07</td>
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<td>0.06</td>
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<td>50th</td>
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<td>0.09</td>
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<tr>
<td>90th</td>
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<td>0.05</td>
<td>0.04</td>
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<tr>
<td>95th</td>
<td>0.07</td>
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<tr>
<td>97th</td>
<td>0.09</td>
<td>0.11</td>
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</table>

Equations for estimating mean and SD of BPD, AC, and FL from GW

Mean BPD, AC, and FL were all well-fitted by quadratic polynomial. The residual for BPD, AC, and FL were fitted by straight line. Regression equations (mean BPD, AC, or FL as dependent variable respectively, GW as independent variable) represented the relationship between fetal biometry and GW. The equations and corresponding R² are shown as follows: the equations for estimating the SD of BPD, AC, or FL from GW are also presented:

<table>
<thead>
<tr>
<th>BPD</th>
<th>AC</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4.393 + 0.567 × GW - 0.006 × GW²</td>
<td>-10.389 + 1.457 × GW - 0.01 × GW²</td>
<td>-1.523 × (0.353 - 0.002 × GW)</td>
</tr>
</tbody>
</table>

27.4 ± 4.8 years. Not all the three measures were obtained in some fetuses. The numbers of valid measurements for BPD, AC, and FL at each GW are shown in Table 1.
FL = -4.086 + 0.434 × GW - 0.004 × GW² (R² = 0.982)
SD for FL = 1.253 × (0.309 + 0.002 × GW).

Using these equations, any centiles for BPD, AC, and FL could be calculated for each GW. Mean and the 3rd, 5th, 10th, 25th, 75th, 90th, 95th and 97th centiles for BPD, AC, and FL are shown in Tables 2 to 4. SSE between fitted centiles and raw centiles for BPD, AC, and FL are shown in Table 5, and all the SSE were close to zero, indicating all the models were well-fitted.

Equations for estimating GW from BPD, AC and FL

For estimation of GW, all the data were satisfactorily fitted by a quadratic polynomial model. The equations for estimating GW from BPD, AC or FL are as follows:
GW = 10.171 + 1.16 × BPD + 0.2 × BPD² (R² = 0.992)
GW = 9.652 + 0.492 × AC + 0.014 × AC² (R² = 0.993)
GW = 7.317 + 4.278 × FL - 0.009 × FL² (R² = 0.986)

Comparison of the 50th centile for BPD, AC, and FL between Shaanxi and other populations

Figure 1 shows that the 50th centile for BPD of Hong Kong and Italian fetuses were very close to that of Shaanxi before the 21st GW, whereas those of Hong Kong and Italy increased quickly. Upon the 31st GW, Hong Kong exceeded the 95th centile curve of Shaanxi, and increased continu-
Discussion

In this study, centiles for BPD, AC, and FL of Shaanxi singleton pregnant women between the 16th to 41st GW were calculated using a recommended method [5, 17]. Equations for estimating BPD, AC, and FL from GW, and for estimating GW from BPD, AC, and FL were obtained by fitting polynomial. These equations were all well-fitted. Using these equations, centile charts (including the 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th centiles) for BPD, AC, and FL were constructed. There were significant differences in the three fetal biometric measurements between Hong Kong, Korea, Italy, and Shaanxi.

Several studies have demonstrated racial variations in fetal growth [11-13, 21]. It was reported that fetuses of Turkish and Moroccan women had a shorter FL, smaller AC than those of Belgian women, and in Africa, AC and BPD of Nigerian fetuses were smaller than those of British fetuses [12]. The present results showed that there might be some differences in fetal size among pregnant women of Italy [20], Korea [19], Hong Kong [18], and Shaanxi. The 50th centiles for BPD of Shaanxi were significantly lower than that of the other three areas and the 50th centiles of AC and FL of Shaanxi were close to that of Hong Kong before 21st GW, and from 21st GW, the difference became larger. These differences could be explained by the different ethnic origin of the four studies in some extent. However, the larger difference between Hong Kong and Shaanxi, Korea, and Shaanxi might be explained as the larger difference in economic developing levels of the three study areas, because the three populations are all east-Asians, especially, Shaanxi and Hong Kong, are all Chinese.

Although there are many fetal biometric reference centile charts for different populations available, but most of them have some shortcomings [5-10], for example, data obtained from one hospital perhaps do not represent the whole characteristics of pregnant women in the country or region, sample size in many GW are too small to assure the precise of centiles, repeated measurements on the same fetus, formation of ‘super normal’ datasets by inappropriate exclusion of complicated pregnancies, and statistical methods used to fit centiles without considering the variability of measurements with gestational age [22]. All these shortcomings contribute to the fact that these reference centiles could not reflect realistically and objectively pregnant women to some extent. Appropriate methods have been published [5] and fetal biometry charts and equations for various populations using the correct method are now available in medical literatures [6-11, 18-20].

In order to make the present results more easily compared with Hong Kong, Koreans, and Italians, the design and statistical methods were the same as those of the three countries or regions [18-20]. The authors selected a cross-sectional design using only one set of ultrasonic fetal biometrics for each fetus, and centiles were fitted by using a multi-step procedure based on regression model. These measures can ensure the variability of measurements with GW [5].

Conclusion

The authors have constructed equations for estimating fetal BPD, AC, and FL based on a large sample of cross-sectional measurements of Shaanxi singleton pregnant women using a recommended method. According to these equations, any centiles for BPD, AC, and FL could be calculated based on GW and GW could be estimated based on BPD, AC, and FL, as well as the uncertainty in days. The reference charts were different from those of Hong Kong, Korea, and Italy. The authors believe that the reference charts and equations were well-fitted, and are ready for clinical use and research among Shaanxi pregnant women.

Acknowledgment

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References


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Association between periodontal disease and adverse pregnancy outcomes in a cohort of pregnant women in Jordan

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Summary

Background: The relationship between periodontal disease (PD) and adverse pregnancy outcomes remain unclear. The authors’ objective was to assess the risk of adverse pregnancy outcomes in Jordanian women with periodontal disease compared to those without. Materials and Methods: Between April 2009 and June 2010, 277 pregnant women with no systemic diseases at gestational age < 20 weeks were enrolled in the study. Dental and oral health examination was performed at enrollment after demographic, medical, and obstetrical information were recorded. Pregnancy outcomes were obtained by phone contact and review of medical records. Results: The incidence of periodontal disease in the pregnant women enrolled was 31%. Women with PD were at higher risk for developing preeclampsia (PE), preterm birth (PB), and low birth weight (LBW). The rate of PE in women with PD was 18.6% compared to 7.3% in the control group (p = 0.005) (OR = 2.7, 95% CI: 1.2, 6.0). The OR for PB was (4.4, 95% CI: 1.7, 11.7) and for LBW was (3.5, 95% CI: 1.6, 7.5). Conclusions: PD is associated with increased risk of PE, PB, and LBW in healthy Jordanian women.

Key words: Pregnancy outcome; Periodontal disease; Preeclampsia; Preterm birth; Low birth weight.

Introduction

Adverse pregnancy outcomes are variable and can impact maternal physical and mental state because of pregnancy loss, long hospital stay, and the need of medical interference. Also the fetus and then the neonate may be adversely affected with great increase in demand on medical and financial resources. In spite of all the advances in perinatal care, adverse pregnancy outcomes like preeclampsia (PB), low birth weight (LBW), and preeclampsia (PE) remain as the main contributors to neonatal and maternal morbidity and mortality. Etiology of spontaneous PB is multifactorial and though many causes have been identified [1], 30%-40% remain with no identifiable cause. Inflammation has been suggested as a major factor in the final common pathway of PB and urogenital infection is considered to be a major pathogenic pathway for PB [2]. Interestingly periodontal disease (PD) was not associated with any of the selected markers of upper genital tract inflammation [3] and treatment of lower genital tract infection during pregnancy has not consistently lowered the rate of PB [4]. It has been suggested that prostaglandin estra-diol (E2) levels and nitric oxide levels in the gingival cervical fluid may have some relationship to delivery before term [5, 6]. The association between PD and PB and LBW remains controversial but a growing body of evidence supports this hypothesis [7, 8]. PE is a pregnancy-specific problem with multiple risk factors. Although the etiology of PE is not clear, circulating levels of TNF-α and IL-6 are increased in women with PE compared to pregnant women with no PE [9]. IL-6 and IL-8 were found to be elevated in amniotic fluid in mid-pregnancy of women later developed PE [10]. Strong association between PD and PE was found by many [11, 12] but the mechanism that correlates the two diseases is not clear. The theory that suggests a systemic inflammatory process involving cytokinase TNFα and IL-6 was not confirmed [13]. In this study the authors assessed the risk of adverse pregnancy outcomes in women with PD as compared to those without.

Materials and Methods

The study material included pregnant women randomly selected from multiple prenatal care centers from southern and northern parts of Jordan. This study was approved by the Institutional Review Board of Jordan University of Science and Technology and conducted during the period April 2009 to June 2010. Inclusion criteria were healthy women with singleton pregnancy, confirmed gestational age of 20 weeks or less (by menstrual dating and/or early ultrasonography), and had at least 20 present teeth. Exclusion criteria included women known with hypertension, diabetes mellitus or chronic medical problem, women on anticoagulant or antibiotic prophylaxis, and those with uterine or cervical abnormality.

After medical diseases history was ruled out, consent form was signed and eligible women were interviewed and information obtained regarding age, number of previous pregnancies and their outcome, years of education, employment, and smoking during pregnancy was also recorded. Body mass index (BMI) was recorded and periodontal exam was performed. Pregnancy care was carried out in the prenatal care centers. Women were followed for pregnancy outcome by direct contact and review of medical...
records for detailed information regarding, hospital admission, complications of pregnancy, gestational age and birth weight at delivery, and other neonatal outcomes. According to World Health Organization (WHO) criteria, PB is defined as delivery before completion of 37 weeks gestation, LBW is first birth weight below 2,500 grams, and PE is a pregnancy-induced hypertension with protein in the urine of 1+ or more after 20 weeks of pregnancy [14].

Periodontal examination

Periodontal examination was carefully done by a calibrated examiner using sterile examination kit for the mouth, a mirror, and standardized Hu-Friedy periodontal probe with Williams’s markings. Examination included number of missing teeth, gingival index (GI), and plaque index (PI) at four sites: mesial, distal, buccal, and lingual. Periodontal state was assessed by probing pocket depth (PPD) over six sites, percentage of bleeding on probing (BOP%) and clinical attachment loss (CAL). Periodontitis was defined as presence of four or more teeth with at least one site or more with PPD ≥ 4 and CAL ≥ 3 mm [15].

Results

Women characteristics

A total of 298 women met the inclusion criteria and agreed to participate in this study. Average age at periodontal examination was 13.8 weeks. Out of these 298 women, 277 completed the study. Their age ranged between 16 and 46 years with a mean (SD) age of 28.0 (6.1) years. More than half of these women (54.9%) had an education level less than high school and 68.2% were not employed. About 54.9% were passive smokers. Of all women, 86 (31.0%) were diagnosed with periodontitis. The socio-demographic, clinical, and obstetric characteristics of pregnant women according to periodontal status are shown in Table 1. Women with periodontitis were significantly older than women with no periodontitis and were significantly more likely to report a history of preterm delivery. Women with and without periodontitis differed significantly in order of pregnancy (p = 0.025) and did not differ in other studied medical and obstetric characteristics.

Pregnancy, delivery, and neonatal outcomes

Tables 2 and 3 show the pregnancy, delivery, and neonatal outcomes of women according to the presence of maternal periodontitis. Of the studied pregnancy and delivery outcomes, PE was more likely to occur in women with periodontitis compared to women without periodontitis (18.6% vs 7.3%; p = 0.005). Anemia, hospital admission, vaginal bleeding, postpartum fever, postpartum hemorrhage, and mode of delivery were not significantly different between women with periodontitis and those without periodontitis.

Compared to those without periodontitis, women with periodontitis were significantly more likely to deliver prematurely (19.8% vs 4.7%; p < 0.005) and more likely to give a LBW baby (22.1% vs 6.8%; p < 0.005). The other studied neonatal outcomes were not significantly different between the two groups of women.

Multivariate analysis

Separate models of binary logistic regression were conducted to determine the association between periodontitis and the three main outcome variables (PB, LBW baby, and PE). In the multivariate analysis after adjusting for significant variables in the study group including age, number of live births, pre-pregnancy BMI, previous hypertension, previous preterm birth, anemia, and women who had periodontitis had significantly higher odds to deliver prematurely, deliver babies with LBW, and develop PE during pregnancy. The odds ratios for PD, delivery of LBW baby, and PE associated with periodontitis were (OR = 4.4, 95% CI: 1.7, 11.7), (OR = 3.5, 95% CI: 1.6, 7.5), and (OR = 2.7, 95% CI: 1.2, 6.0), respectively (Table 4).
Association between periodontal disease and adverse pregnancy outcomes in a cohort of pregnant women in Jordan

Table 3. — Neonatal outcomes according to maternal periodontitis.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No (n = 191)</th>
<th>Yes (n = 86)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>9 (4.7)</td>
<td>17 (19.8)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>LBW</td>
<td>13 (6.8)</td>
<td>19 (22.1)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Admission to NICU</td>
<td>24 (12.6)</td>
<td>15 (17.4)</td>
<td>0.280</td>
</tr>
<tr>
<td>Ventilation &gt; 6 hours</td>
<td>9 (4.7)</td>
<td>10 (11.5)</td>
<td>0.035</td>
</tr>
<tr>
<td>Neonatal antibiotic therapy</td>
<td>6 (3.2)</td>
<td>4 (4.7)</td>
<td>0.539</td>
</tr>
<tr>
<td>Congenital malformation</td>
<td>4 (2.1)</td>
<td>2 (2.3)</td>
<td>0.903</td>
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</table>

Table 4. — Multivariate analysis between periodontitis and adverse pregnancy outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% confidence interval)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>4.4 (1.7, 11.7)</td>
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</tr>
<tr>
<td>LBW</td>
<td>3.5 (1.6, 7.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>PE</td>
<td>2.7 (1.2, 6.0)</td>
<td>0.011</td>
</tr>
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</table>

Adjusted for age, number of live births, pre-pregnancy BMI, previous hypertension, previous preterm birth, and anemia.

Discussion

A large portion of PBs are of unknown etiology and so is PE. Efforts are made to try and solve this dilemma in order to reduce maternal and neonatal morbidities and mortalities. PD on the other hand is common and is closely-related to mouth hygiene and dental care. In the last two decades or more, the relation of PD to PB, PE, and other pregnancy outcomes were extensively studied without conclusive evidence. Establishing the relationship between these pregnancy outcomes and PD may help in confirming a cause-effect connection later and help to implement therapeutic and prophylactic measures that may improve some of these outcomes [16, 17]. In the present study group of 277 low-risk women, who were examined at early pregnancy and completed the study, periodontitis was present in 86 (31%) of the women. The demographic and obstetric profiles of both groups, with PD and without PD were similar aside from higher parity in PD group which mostly reflects age effect (Table 1). PE was significantly higher in the group with PD than those without (18.5% and 7.3% respectively, p < 0.005) (Table 2), the OR for PE (2.7, 95% CI: 1.2, 6.0). These result were similar to findings by Siqueira et al. and Shetty et al. but not to others like Khader and Taghzouli [18, 19], who did not find evidence to support this association. Neonatal outcomes showed strong association between PD and PB. Women with PD are at higher risk of PB than those without (19.8% vs 4.7%, p < 0.005), also PD was significantly associated with LBW (22.1% vs 6.8%, p < 0.005) (Table 3) and after multivariate analysis women with PD were at higher risk for both PB (OR = 4.4, 95% CI: 1.7, 11.7) and LBW (OR = 3.5, 95% CI: 1.6, 7.5). Babies born to women with PD required more resuscitation which is related to the higher PB in this group. While similar results were found by other investigators [7, 8], others like Srinijyas et al. [20] failed to demonstrate such an association in a compara-

tively large prospective study. Chamberon in his systemic review found consistent association between PD and PB/LBW, but because of the high degree of heterogeneity between studies, he suggested that this finding should be treated with great caution [21]. The remaining controversy in results is probably related to lack of consensus on the criteria for diagnosis of PD, which may influence the frequency of complications, or due to variation in gestational age at time of periodontal assessment which could be partially affected by the hormonal changes of pregnancy on the periodontal tissue [22]. The present authors conclude that PD is associated with significantly increased risk of PE, PB, and LBW in this studied population of Jordanian women. Larger studies are recommended with consensus on criteria for diagnosis of PD and more specified pregnancy outcomes.

Acknowledgment

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References


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Anesthesia management for open fetal intrauterine surgery

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Summary

Objective: Open fetal surgery is usually performed during the second trimester in a fetus suffering from severe congenital diseases, thus enabling the pregnancy to continue until delivery. Materials and Methods: The key of this treatment is to promote uterine relaxation enough to maintain both maternal and fetal circulation stable and once surgery is completed, to offer a perfect analgesic to avoid the contractions due to pain, and finally to reduce preterm delivery. Results: Successful anesthesia is fundamental to this surgery. Conclusion: The authors have performed three cases under inhalation anesthesia combined with successful epidural anesthesia.

Key words: Open fetal surgery; Inhalation anesthesia; Epidural anesthesia; Analgesic.

Introduction

Nowadays, prenatal diagnosis allows people to better understand fetal intrauterine lesions. Successful prenatal ultrasounds have already been able to offer assistance in determining the natural pathological processes of fetal chest lesions and their pathophysiological characteristics affecting prognosis, based on which prognostic management protocols can then be proposed [1-6]. Successful prenatal ultrasounds have found that large fetal tumors can decrease in size, indicating that some fetal lesions have a self-improvement potential [7-9]. Ex utero intrapartum treatment (EXIT) can improve intrauterine lesions diagnosed late in pregnancy [10]. Open fetal surgery is also an important treatment procedure and can be applied in the treatment of predictable congenital diseases which affect fetal growth and development or even pose life-threatening consequences to the fetus, such as congenital diaphragmatic hernia, congenital high airway obstruction caused by endogenous laryngeal and tracheal deformities, exogenous airway compression, transoral tracheal intubation obstruction, severe dropsy caused by pulmonary congenital cystadenoma, bilateral tension pleural effusion, congenital chylothorax, unilateral pulmonary hypoplasia, congenital diaphragmatic hernia complicated by congenital heart disease, and so on [11-13].

Fetal surgery is a new treatment method appearing in recent years, which is based on the concept of “the fetus as a patient”. Open fetal intrauterine surgery is among these. This procedure is often used to exert surgical treatment in a fetus at mid-trimester gestation complicated by severe congenital disease, and uterus suturing is then performed for the mother’s on-going pregnancy until childbirth. However, this surgical procedure can lead to a relatively high incidence of postoperative complications. Furthermore, its required anesthesia management is also complex, in which attention must be simultaneously paid to both mother and fetus. The fetus must enter into a controlled anesthetized depth simultaneously with the mother by maintaining intraoperative blood and oxygen supplies through the umbilical cord to the fetus, adequate postoperative sedation and analgesia are required, and the uterine contractions caused by pain should also be reduced to a minimum or even avoided in order to reduce the risk of a premature birth.

In 2011, the authors successfully performed surgical deep inhalation anesthesia for three women during pregnancy, complicated by fetal pulmonary cystadenoma.

Materials and Methods

Three women in their child-bearing period were involved. Admitting diagnoses and B ultrasounds were performed in them.

Case 1

The woman had a history of two pregnancies and one delivery. The fetus was at 28+ three weeks gestation and in a right sacrotransverse position with left lower pulmonary cystadenoma. B ultrasound revealed that the fetus had a heart dextroposition but without structural anomalies in it and was in a breech position; there was no anomaly in the amniotic fluid volume; the placental maturity was 0+ degrees; and an approximate 5.6 × 5.0 × 5.0 cm hyperechoic mass in a triangle-like image was seen in the fetus’ left thoracic cavity. The mass echoes were irregular and there were several tiny cystic dark regions; it was the cause of the fetal heart dextroposition.

Case 2

The woman had a history of one pregnancy but without a delivery. The fetus was at a 28+ five weeks gestation age and in a left occipital anterior position with a congenital cystadenoma-like deformity in the left lung. B ultrasound was performed. The fetus was in a cephalic position at a gestation age equivalent to 28+ weeks. No anomaly was detected in the amniotic fluid volume. The placenta was located at the right and posterior walls with a maturity degree of 0+. An approximate 4.8 × 2.6 × 3.2 cm hyperechoic mass was detected in the fetus’ left thoracic cavity, in which a few scattered dark areas could be seen, with the maximum one of approximately 0.2 × 0.2 cm in size. No independent blood flow from the aorta was detected. The fetus’s heart was transpositioned into the right thoracic cavity due to the complete compression of the mass. Normal pulmonary tissue echoes were received from the...
upper region of the left thoracic cavity with an area of approximately 1.3 × 1.2 cm. The size of the left mass under the four chambers was around 3.5 × 3.8 cm, while that of the right lung was around 3.0 × 1.5 cm. Neither subcutaneous edema nor noticeable splanchnocoele or pericardial effusion was detected in the fetus.

Case 3

The woman had a history of one pregnancy but without a delivery. The fetus was at 29 weeks gestation and in a right occipital transverse position with a congenital cystadenoma-like deformity in the left lung. B ultrasound showed that the fetus was at a gestation age equivalent to 28 + weeks, the amniotic fluid index was 28, and the placentation maturity degree was 0 +; an approximate 7.1 × 4.6 × 5.6 cm hyperechoic mass was seen in the fetus' left thoracic cavity, in which multiple liquid anechoic areas with varying sizes could be detected; the heart and mediastina were transpositioned into the right thoracic cavity under compression, and heart oscillation could be detected. The fetus was diagnosed with pulmonary cystadeno.

Open fetal surgery (left pulmonary cystadenoma resection) under deep inhalation combined with epidural anesthesia was performed for the three pregnant women. All of them were in a physical status I-II, according to American Society of Anesthesiologists grading. They were free of endocrine system diseases and important organ diseases.

Anesthesia and surgery

Continuous epidural anesthesia and inhalation anesthesia were combined in the anesthesia management.

The women were abstained routinely from food and drink. Scopolamine (0.3 mg) was given intramuscularly 30 minutes before surgery. Two venous channels were opened for the connections to the anesthesia depth monitor. An epidural puncture was performed through the L2-3 interspace in a left-lateral position for intubation. When no blood or cerebrospinal fluid could be pumped back, two percent lidocaine at five ml as a test dose was infused. Intravenous anesthesia induction was instantly performed after a sensation block level turned up, using midazolam (0.1 mg/kg), propofol (1.5 mg/kg), fentanyl (three μg/kg), and cisatracurium besylate (0.15 mg/kg). Endotracheal catheters with an inside diameter of seven mm were successfully inserted after mask oxygen inhalation, and auscultation showed that the bilateral pulmonary respiratory sounds were clear and balanced. The catheters were fixed, and respiration was controlled with a tidal volume at ten ml/kg, a respiratory frequency at 12 times/min, and an inspired oxygen concentration at 75%. In the meantime, the isoflurane concentration was adjusted to three percent with an oxygen flux at 31 min to increase the exhaled isoflurane concentration to less than two percent. A right subclavian vein puncture was performed to establish a central venous channel for central venous pressure (CVP) monitoring. A radial arterial puncture and intubation were performed for direct arterial pressure monitoring.

Fetal position and heart rate were preoperatively determined by B ultrasound. Dopamine was pumped intravenously for intraoperative adjustment, according to maternal blood pressure and heart rate. Sufentanil (0.2 μg/kg), cisatracurium besylate (0.15 mg/kg), and 0.04% Anpo (15 drips/min) were added when the skin was cut. The fetal position and heart rate were determined again by B ultrasound before the uterus was opened. After uterine opening, one of the fetus’ arms was connected to blood-oxygen saturation and rectal temperature probes and one fetal peripheral venous channel was then opened. Anpo (a uterine contraction inhibitor) was adjusted to 25 drips/min. The fetal operative field was exposed. During operation, lactated Ranger’s solution at 40°C as amniotic fluid was continuously infused for the maintenance of the fetal external environment, body temperature, and skin humidity while B ultrasound was used for fetal heart rate and heart status monitoring. Decreased heart rates were monitored in the two fetuses with larger cystic adenomatoid masses after tumor resection, with the minimum rate of 75 beats/min. The fetuses were injected intravenously with atropine and adrenaline and then supplemented with erythrocyte suspension. Their heart rates returned to more than 145 beats/min again after infusion. After fetal operation, the exposed limb was repositioned into the uterus. Then, the inhaled isoflurane concentration was reduced to maintain an exhaled concentration at 1.5%. Meanwhile, sufentanil (0.2 μg/kg) was added intravenously for further maternal intervention. Isoflurane inhalation was stopped after the closure of the peritoneum. Postoperatively, the mother returned to spontaneous respiration and the endotracheal catheters were withdrawn under deep anesthesia. The mother was sent to the intensive care unit for 24 hours’ observation and then returned to her regular room. The mean anesthesia and maternal and fetal surgical times were three hours and 20 minutes, two hours and 30 minutes, and 29 minutes, respectively. Intraoperative supplemented crystal solution was 2,150 ml. The fetal hemorrhage volume was around four ml. The maternal hemorrhage and urinary volumes were around 50 ml and 300 ml, respectively.

Results

The women continuously received anti-infection and uterine contraction-inhibiting treatment after operation. They recovered well without obstetric complications. They presented premature ruptures of the membrane at gestational 32, 33, and 34 weeks and underwent cesarean sections, respectively. All the newborns were alive. They were ventilated mechanically after being delivered. However, the newborn of 33 weeks’ gestation age died of pulmonary maldevelopment. The other two survived and recovered well.

Discussion

Anesthesia for the mother involved in fetal surgery has a much higher risk than that for common cesarean section. The induction and maintenance of general anesthesia will affect the physiological environments of the mother’s important organs. Pregnancy changes the anatomical relations of the esophagus with the transeptae and stomach. This condition decreases the mother’s functional residual capacity and gradually increases her oxygen consumption. Pregnancy also exerts influences on the cardiovascular and central nervous systems. Supine hypotension syndrome can lead to maternal hypotension, fetal anoxia, an obvious decrease in the minimum alveolar concentration, and an increase in susceptibility to muscle relaxants [14-18].

In open intrauterine surgery, it is crucial to maintain the uterus in a relaxed state at all times. Therefore, adequate analgesia, sedation, and muscle relaxation should
be ensured and uterine contraction inhibitors should be continuously applied during perioperation. Among different uterine contraction inhibitors, Anpo has been proved to have the most specific inhibitory effect and has been widely used clinically. However, Anpo can lead to an increase in maternal heart rate, which can further influence the fetus’ heart rate. Therefore, a balance between sufficient uterine relaxation and maternal and fetal cardiovascular stability during open intrauterine surgery is necessary. However, heart rate control itself is a matter of medical debate. Some scholars believe that strict heart rate control is unnecessary because blood pressure can be adjusted through maternal heart rate changes to reduce the interventions from external drugs.

Fetal immature organ function renders anesthesia for the fetus rather difficult to perform. To perform it, reliable fetal monitoring is needed first, such as blood oxygen saturation and body temperature monitoring through the fetus’ exposed limb, B ultrasound for the fetus’ cardiac function, and so on. Maintaining fetal cardiovascular stability is the most important step in anesthesia management for fetal surgery. The fetus improves oxygenation by resorting to increases in cardiac output and blood flow redistribution, while the cardiac output depends on the fetus’ heart rate. Under stress, the hyposensitivity of the highly-aberrant tension and pressure-receptive cells in the fetus always leads to a decrease in heart rate. In addition, since the circulating blood volume in a fetus is very small, even a small amount of blood loss in surgery may cause fetal hypovolemia [19]. Based on these reasons, after a large mass was removed from the lung, mediastinal swaying which led to arrhythmia and heart failure became unavoidable in the present study. Furthermore, inhalation anesthesia can cause direct inhibition of the fetal cardiac muscle, as well as vasodilatation, which may further lead to arteriovenous shunt, ultimately destroying the balance of the cardiovascular system [20]. The inhibitory effect of inhalation anesthesia on the fetal cardiac muscle can be aggravated with the prolongation of anesthesia time. Therefore, to ensure the life safety of the fetus is another key point in fetal intrauterine surgery.

Fetal survival depends on the mother, in which maternal blood pressure maintenance plays an important role. Normally, maternal blood pressure should be maintained within a range of ten percent based on the baseline. The blood flow velocity in the fetal naval artery is influenced by fetal cardiac output and vascular resistance, so the maintenance of cardiac output plays a critical role in ensuring a sufficient oxygen supply to the fetus. Intraoperative control over the tension of the uterine smooth muscle can be fulfilled by using uterine contraction inhibitors and inhalation anesthetics. Compared with uterine contraction inhibitors, deep inhalation anesthesia can lead to uterine smooth muscle complete relaxation, which will be more convenient for surgery. Inhalation anesthesia however, has a drawback: it has an inhibitory effect on fetal cardiac vessels, which can lead to a reduction in uterine placental blood flow [20, 21]. Nevertheless, considering that almost all vasoactive drugs have a contractive effect on uterine placental vessels, deep inhalation anesthesia is still a better choice. In the future, however, a more rational application of vasoactive drugs in which the maternal blood circulation stability can be maintained on the one hand, and the influence of these drugs on the fetus can be reduced to the minimum on the other hand, remains to be explored.

In addition, postoperative analgesia for pregnant women also plays a crucial role. Thorough analgesia can reduce uterine contractions caused by pain, thus, to reduce the incidence of premature deliveries. In this study, uterine contractions caused by pain and preoccupation were basically eliminated in the three pregnant women. Fetal heart monitoring showed that the fetal heart sounds were slightly faster than normal, which is presumably correlated with the application of Anpo, and the intrauterine pressures were within the normal range.

In summary, the inhalation anesthetic isoflurane has a good effect in the anesthesia management for open fetal intrauterine surgery. However, what advantages and disadvantages does sevoflurane have as compared to isoflurane? Is it necessary to strictly control the maternal heart rate by using drugs during surgery? Can steroidal anti-inflammatory drugs be used for postoperative analgesia, and when can these drugs be used? These questions remain to be solved.

References


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Prevalence of genital warts in reproductive-aged Turkish women presenting at gynecology outpatient clinics for any reason

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Summary
The objective of this multicenter descriptive study was to calculate the frequency of genital warts among Turkish women aged 15-49 years, who visited outpatient gynecology clinics for a variety of reasons. The study was conducted in February 2011 to collect data for a minimum of 154 patients at each center, and the total sample size reached 2,967 women (95.1% completion rate). Oral informed consents were obtained. A questionnaire including data on socio-demographic characteristics and reasons for admission was administered, and a pelvic examination was performed. The overall point prevalence was 3.5% (95% CI = 3.1%-4.0%), correcting for sampling design, with the highest rates observed in the 15 to 19-year-old group. The odds of having a genital wart was 1.82 times (95% CI = 0.99-3.33) higher among non-pregnant participants than in pregnant women (p = 0.051). The overall point prevalence of genital warts among reproductive-aged women attending gynecology outpatient clinics for any reason in Turkey was 3.5%.

Key words: Genital warts; Prevalence rates; Turkish women.

Introduction
Human papillomavirus (HPV), a cause of cervical cancer, is also known to be responsible for condylomata acuminate, or genital warts [1-3]. There are approximately 40 molecularly identified HPV types, and all types can infect the anogenital tract, cause visual disturbances, and lead to psychosexual sequelae [4-6]. HPV genotypes 6 and 11 are responsible for more than 90% of genital warts [7].

Although genital warts are among the most common sexually-transmitted diseases (STDs) worldwide, quite a few articles have been published on its incidence and prevalence. The source populations, sampling techniques, socio-demographic and health-related characteristics of study participants, diagnostic methods used, data sources, and calculated indices vary across these studies, but overall, they suggest that occurrence rates of genital warts differ across populations.

Neither the prevalence of genital warts nor the associated economic cost, are known in Turkey. Some estimate of the overall prevalence rates is necessary, along with the dynamics of genital wart development across different birth cohorts, in order to investigate whether routine HPV vaccination is needed at the national level. Given the low feasibility of population-based prevalence studies of genital warts and the unavailability of a usable medical recording system in Turkey, clinically-based studies currently seem to be the best alternative to investigate the frequency of genital warts in the population to roughly estimate the percentage of reproductive-aged Turkish women with genital warts. This information, in turn, may be used to guide health policy makers in decisions of whether to incorporate HPV vaccination into the routine vaccination schedule.

The objective of the study was to calculate the frequency of genital warts among Turkish women aged 15-49 years, who visited outpatient gynecology clinics for a variety of reasons. Using the proportion of reproductive-aged women with genital warts, it was possible to estimate point prevalence rates by age group.

Materials and Methods
A multicenter descriptive study was planned to estimate the prevalence of genital warts in reproductive-aged women (15-49 years) attending selected gynecology outpatient clinics (in universities, public hospitals, and private healthcare units) across ten cities in Turkey; namely, Adana, Ankara, Antalya, Diyarbakir, Erzurum, Istanbul, Izmir, Kayseri, Samsun, and Trabzon. The cities and individual outpatient clinics were selected by convenience, based on the heterogeneity of subjects in terms of socio-demographic characteristics, healthcare-seeking behaviors, geographic regions of residence, and cooperation of the attending physicians. None of the centers selected provided any special service for genital warts, nor were they known to be referral centers for genital warts.

Sample size was enlarged for an expected design effect of two. Each participating center was requested to collect data for a minimum of 154 patients.

The study was conducted in February 2011. In each city, the study aimed to collect data in at least one university hospital, one public hospital, and one private gynecology clinic. At each center, data collection was terminated at the end of 30 days, even if the intended sample size was not reached, and the total sample size reached 2,967 women (i.e., 95.1% completion rate).
A standardized questionnaire was administered to all participants in each center. The questionnaire aimed to collect data on age, current occupation, current marital status, main reason for visiting the gynecology outpatient clinic, whether and if so where the patient had attended before with the same complaint, and type of contraceptive method currently used. The questionnaire was administered during a face-to-face interview and a pelvic examination was conducted afterwards. All eligible women presenting at one of the selected gynecology outpatient clinics were approached for the study. Participation was voluntary and oral informed consent was taken from each participant prior to completing the questionnaire. Approval for the study was obtained from the Ethics Committee of Erciyes University.

Genital wart diagnosis was made by a participating gynecologist on the basis of a standard pelvic examination. All gynecologists were aware of the main study objective, i.e. assessment of prevalence of genital warts, and were specifically requested to check for and to report presence of genital wart(s) regardless of the admitting diagnosis.

Statistical analysis

Statistical analyses included number and percent distributions and calculation of measures of central tendency and dispersion for continuous variables. Comparison of groups was performed using Student’s t-test and Chi-squared test, as appropriate. The alpha value for statistical significance was set at 0.05. The distribution of prevalence rates was calculated for five-year age bands and types of healthcare institutions. Odds ratio estimates were calculated when applicable.

Limitations of the study and measures taken to decrease their potential impact on statistical analysis

I – Data were collected from a selected set of gynecologic outpatient clinics/offices and were not necessarily representative of all healthcare settings in Turkey. In order to derive prevalence estimates that could be valid for women aged 15-49 years attending gynecology clinics throughout Turkey, some assumptions were made:

1) Participants from each selected healthcare setting (e.g. university hospital) were assumed to be representative of all patients attending similar healthcare settings throughout the population, i.e. institution-specific prevalence rates calculated from the sample could be used to estimate institution-specific prevalence rates at the national level.

2) The distribution of participants from the three clinical settings was not proportional to the distribution at the national level, for which a correction needed to be made in estimating the overall prevalence of genital warts among women attending any gynecology clinic at the national level.

Based on the records of the Ministry of Health on the total number of outpatient visits nationwide in 2010, of the total 302,984,218 visits, 235,172,924 (77.6%) were to public institutions, 20,098,754 (6.6%) were to university clinics, and 47,712,540 (15.7%) were to private clinics [8]. Of the total participants in this study, 419 (13%) visited public institutions, 1,744 (54%) visited university clinics, and the remaining 1,044 (33%) attended private clinics/offices. Therefore, the distribution of sampling from the three main types of healthcare institutions was not proportional to overall attendance patterns in the general population, and this, in turn, necessitated some adjustments in calculating overall estimates. In order to adjust for the sampling design used, sampling weights, calculated as the inverse of the “assumed” sampling proportions, were used.

Upon comparison of the five-year age bands (15-19, 20-24, 25-29, 30-34, 35-39, 40-44, and 45-49), the percentages of study participants in 15-19 and 25-34 year age groups were found to be significantly different compared to those of the general population of Turkish women in the year 2010 [9]. Gynecology clinic attendance rates were not expected to be similar across five-year age bands, and would be expected to be less common among 15 to 19-year-old women and women over the age of 40. The present findings were in line with this expectation and, thus, the authors preferred not to use any post-survey weighting adjustments.

II - Descriptive and disease-related characteristics of patients attending a specific healthcare setting may vary for several reasons. Given that the study centers provided no special care for genital warts, the authors would expect a non-differential selection bias, if any. On the other hand, if genital warts co-exist with certain gynecologic diseases, which may cause differential rates of attendance in certain settings, such a situation could have caused a differential selection bias in the study. However, neither the authors' literature search, nor expert opinions supported this.

III - The objective of the study was to make some estimations, based on clinical data, for the prevalence of genital warts in the general population of 15 to 49-year-old women residing in the community. In a survey study performed in Turkey in 2011, data from 72 hospitals of women visiting a gynecologist were collected, and the prevalence of genital warts among women aged 30-65 years was found to be 154 per 100,000 [10]. In the STD literature, authors have restricted analyses to pregnant women, as surrogates for the general population. However, studies suggest that rates calculated solely on the basis of pregnant women may either underestimate or overestimate the true risk in the population, although the final estimates for point prevalence of genital warts in the general population have been predicted based on calculations restricted to pregnant study participants who visit the clinic for routine pregnancy follow-up [11-15].

In this study population, the prevalence of genital warts among non-pregnant women was about twice as high as that among pregnant women, even after age adjustment. Therefore, prevalence in pregnant women was not deemed a good surrogate measure for prevalence in the whole female population.

Also, it was understood that this data set, even when combined with available health records, was not sufficient to estimate the prevalence of genital warts in the general population. The findings can only be considered representative of reproductive-aged Turkish women attending gynecology outpatient clinics for “any” reason.

Statistical Package for the Social Sciences (SPSS) for Windows, the Complex Samples module, was used for statistical analysis and weighted analyses, using city (n = 10) and healthcare setting type (n = 3) as “strata”, and the individual clinics where data were collected (e.g. hospital A) as “clusters”. Weights were used, as necessary.

Results

Data were collected for 2,967 women aged 15-49 years, attending 23 centers in ten cities. Table 1 presents the distribution of women included in the study by city, at the end of the 30-day study period. Not all centers were able to provide the minimum number of participants (n = 154 women). The highest completion rate was achieved in Izmir (n = 546) and Istanbul had the lowest completion rate (n =
Table 1. — Distribution of study participants by city and type of healthcare setting attended.

<table>
<thead>
<tr>
<th>City</th>
<th>Type of gynecology outpatient clinic</th>
<th>Public hospital</th>
<th>University hospital</th>
<th>Private clinic/hospital</th>
<th>Total number of participants completing the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankara</td>
<td>Public hospital</td>
<td>14 4</td>
<td>113</td>
<td>257</td>
<td></td>
</tr>
<tr>
<td>Antalya</td>
<td>-</td>
<td>154</td>
<td>70</td>
<td>224</td>
<td></td>
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<tr>
<td>Diyarbakır</td>
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<td>180</td>
<td>182</td>
<td>362</td>
<td></td>
</tr>
<tr>
<td>Erzurum</td>
<td>-</td>
<td>157</td>
<td>139</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>Istanbul</td>
<td>-</td>
<td>66</td>
<td>38</td>
<td>104</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Total N</td>
<td>-</td>
<td>385</td>
<td>1,583</td>
<td>999</td>
<td>2,967</td>
</tr>
</tbody>
</table>

Data are presented as numbers.

104). It is also important to note that not all healthcare units were represented in each city; for example, no data were collected for women attending public institutions in Adana and Istanbul, and none were collected for women attending university hospitals in Istanbul (Table 1).

The distribution of selected descriptive characteristics of study participants according to type of healthcare setting is provided in Table 2. The mean age of study participants was 32.60 years, with a standard deviation of 8.46 years. The ages ranged from 15 to 49 years, with a median age of 32 years (25% under 26 years of age, and 75% over 40 years of age). Age distributions for the three different clinical settings were similar: the mean ages were 33.31 ± 8.50, 33.30 ± 9.05, and 31.50 ± 7.25 years for those attending public institutions, university hospitals, and private care units, respectively.

The occupational classification of women was established according to the frequency and potential effect of a particular occupation on awareness of genital warts and/or related healthcare seeking behavior. The majority of the study participants were housewives, with a lower percentage among those attending private clinics/offices. The percentages of professional women working in healthcare and education sectors were quite small, yet significantly higher in private healthcare settings (p < 0.001).

At the time of the study, 80% of the participants were married, about ten percent were single, and two to four percent were divorced or separated. The marital status distribution did not change significantly according to the type of healthcare setting to which women were admitted (p = 0.850).

The most common reason for attending a gynecology clinic was menstrual dysfunction (11.1%), followed by vaginal discharge (2.9%), bleeding (2.8%), and pain (0.6%). It was notable that patients rarely mentioned genital warts as the reason for attending a gynecology clinic. Overall, 0.2% of all women (ranging from 0.2% in private hospitals to 3.2% in university hospitals) attending a gynecology clinic reported genital warts as their main reason for attendance (Table 2). Pregnancy-related reasons accounted for about one-fifth of all motivations for attending private clinics, but were less common in public health settings (7.8%). Although data were not specific enough to distinguish “admissions for routine examination” and “admissions for a previously diagnosed disease”, these two together constituted about 6.0%, 7.6%, and 13.1% of women attending public institutions, university facilities, and private institutions/clinics, respectively (Table 2).

When participants were asked whether they had visited another physician previously for the same reason stated at the time of study, the distributions varied by the type of healthcare unit, as expected. Among those who were admitted to a university outpatient clinic, two-thirds mentioned that they had previously visited a healthcare setting for the same reason. Of those, about half had a previous...
visit to a public hospital. Among those attending a public institution, about 40% had had a previous visit, and half of those attended another public institution. Among the participants attending private clinics/offices, more than half had previously visited a physician for the same reason, and more than half of these previous visits were either to the same physician or to another private clinic/office. It was observed that attendance patterns according to type of healthcare unit might vary for different reasons, including social security coverage, socio-economic status, type of disease/problem, severity of symptoms, women’s expectations of the healthcare settings, and even place of residence. Unfortunately, there was no question in the data set to investigate which reasons could have played a role in differences in preferences.

It is interesting that the proportion of pregnant women changed significantly according to type of clinical setting. Women visiting for reasons related to pregnancy made up 5.8%, 52.7%, and 41.1% of patients attending public hospitals, university clinics, and private healthcare units, respectively (p < 0.001).

It is not surprising to see that about 65.5% of all participants were not using any contraceptive method at the time of the study; 17.3% were pregnant, with higher rates in private clinics/offices. As summarized in Table 4, the prevalence of genital warts among reproductive-aged women attending gynecology clinics/offices for “any” reason ranged from 3.4% (public institutions) to 4.1% (private offices/clinics), with an overall point prevalence of 3.5% (95% CI = 3.1-4.0%).

Table 3 presents the distribution of patients in whom any genital wart was detected by the physician at the time of the study. Study participants with at least one genital wart detected at the time of pelvic exam (n = 114) had a similar age distribution (average age = 33.5 ± 8.5 years) compared with their counterparts with no genital warts (average age = 32.7 ± 8.5 years) (t-test p = 0.304). The distribution of major occupation types was not significantly different across the two groups (p = 0.395). Similarly, the distribution of marital status was similar for patients with or without genital warts: the majority of the group was reportedly married (p = 0.146). Among women with genital warts, the percentage of women who used a contraceptive method was statistically significantly higher (5.2%) than that of those who did not (3.2%) (p = 0.008).

Compared with the 514 pregnant women, the odds of having a genital wart were 1.82 times (95% CI = 0.99-3.33) higher among non-pregnant participants (Chi-squared p value = 0.051) (Table 3).

As summarized in Table 4, the prevalence of genital warts among reproductive-aged women attending gynecology clinics/offices for “any” reason ranged from 3.4% (public institutions) to 4.1% (private offices/clinics), with an overall point prevalence of 3.5% (95% CI = 3.1-4.0%).

It is noteworthy that point prevalence of genital warts varied by age among the study participants. Table 5 presents age-specific prevalence rates for genital warts. The highest rates were observed in the 15 to 19-year-old group.

<table>
<thead>
<tr>
<th>Characteristics studied</th>
<th>Women with warts (n = 114)</th>
<th>Women without genit</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupation (n = 2,967)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>76 (66.6)</td>
<td>2,004 (70.2)</td>
<td>0.395</td>
</tr>
<tr>
<td>Student</td>
<td>3 (2.6)</td>
<td>119 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Healthcare sector worker</td>
<td>2 (1.8)</td>
<td>85 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Educational sector worker</td>
<td>4 (3.5)</td>
<td>112 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>29 (25.4)</td>
<td>533 (18.7)</td>
<td></td>
</tr>
<tr>
<td>Marital Status (n = 2,948)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>92 (81.4)</td>
<td>2,484 (87.6)</td>
<td>0.146</td>
</tr>
<tr>
<td>Single</td>
<td>17 (15.0)</td>
<td>290 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>4 (3.5)</td>
<td>61 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Reason for attending the gynecology clinic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual dysfunction</td>
<td>7 (6.1)</td>
<td>322 (11.3)</td>
<td>n/a</td>
</tr>
<tr>
<td>Discharge of any type</td>
<td>-</td>
<td>87 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>-</td>
<td>82 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>-</td>
<td>19 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Control/check-up</td>
<td>-</td>
<td>275 (9.6)</td>
<td></td>
</tr>
<tr>
<td>Contraception</td>
<td>12 (10.5)</td>
<td>502 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Genital warts</td>
<td>5 (4.4)</td>
<td>3 (0.19)</td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td>1 (0.9)</td>
<td>114 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Counseling for intended pregnancy</td>
<td>2 (1.8)</td>
<td>42 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Pap smear</td>
<td>-</td>
<td>45 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Other reasons*</td>
<td>87 (76.3)</td>
<td>2,026 (71.0)</td>
<td></td>
</tr>
</tbody>
</table>

Any previous consultation for the reason described above (n = 2,949)?

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>1,307 (46.0)</th>
<th>0.036</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes: same physician</td>
<td>7 (6.5)</td>
<td>437 (15.4)</td>
<td></td>
</tr>
<tr>
<td>family medicine</td>
<td>1 (0.9)</td>
<td>74 (2.6)</td>
<td></td>
</tr>
<tr>
<td>public hospital</td>
<td>31 (28.7)</td>
<td>568 (20.0)</td>
<td></td>
</tr>
<tr>
<td>private hospital/doctor</td>
<td>18 (16.7)</td>
<td>393 (13.8)</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>1 (0.9)</td>
<td>62 (2.2)</td>
<td></td>
</tr>
</tbody>
</table>

Contraceptive method use (n = 2,922)

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>1,858 (69.8)</th>
<th>0.0081</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>113 (5.2)</td>
<td>2,809 (94.8)</td>
<td></td>
</tr>
</tbody>
</table>

Column percentages are presented. Some column totals may not be equal due to rounding. Data were not complete for all variables, thus, percentages were calculated out of total number of answers for each question. * Chi-squared test p value is presented. 1 Includes physician, nurse, midwife, dentist, medical biologist, medical technician, and laboratory technician. 2 Includes teachers and instructors. 3 The table presents the ten most common diagnoses, and those diagnoses with scarce frequencies are grouped under the “other” category.

Discussion

The lifetime rate of HPV infection among 15 to 49-year-old women in U.S.A. is up to 75% [16]. However, the majority of women infected with HPV eliminate the virus without developing clinical symptoms [4, 17], and clinically apparent genital warts reportedly affect about one percent of reproducitively active women [17]. Genital warts are reported as the most commonly diagnosed STD in the U.S.A. [18]. In the United Kingdom (2003), ten percent of women visiting all genitourinary medicine clinics were “newly-diagnosed cases” of genital warts [15], and the incidence of genital warts increased to 4.2% over a one-year period [19]. In a study of 69,147 women aged 18-45 years, residing in Denmark, Norway, Iceland, and Sweden, the self-reported prevalence of “ever” having a genital wart was 10.6%, and 1.3% reported having experienced genital warts within the 12 months preceding the survey. This same study found that the likelihood of genital warts increased along with number of lifetime sexual partners, history of...
Prevalence of genital warts in reproductive-aged Turkish women presenting at gynecology outpatient clinics for any reason

Table 4. — Distribution of selected descriptive characteristics by type of healthcare center.

<table>
<thead>
<tr>
<th>Detected genital warts</th>
<th>Public hospital (n = 385)</th>
<th>University hospital (n = 1,583)</th>
<th>Private clinic/hospital (n = 999)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw numbers of genital wart cases detected during pelvic exam in the study group</td>
<td>13</td>
<td>60</td>
<td>40</td>
<td>114</td>
</tr>
<tr>
<td>Prevalence estimates % (95% CI)</td>
<td>3.4 (3.3-3.4)</td>
<td>2.2 (2.6-5.5)</td>
<td>4.1 (2.8-8.1)</td>
<td>3.5* (3.1-4.0)</td>
</tr>
</tbody>
</table>

*Weights were used to adjust for non-proportional sampling fractions with respect to healthcare setting. 1 Point prevalence with 95% confidence intervals. 2 Represents women attending a gynecology outpatient clinic for “any” reason, with or without genital warts. 3 Adjusted for the sampling design used in the study.

Table 5. — Age-group-specific point prevalence for physician-diagnosed genital warts and odds ratios for the association between genital warts diagnosis and age.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Number of women examined</th>
<th>Age-group-specific prevalence estimates % (95% CI)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19 years</td>
<td>115</td>
<td>6.6 (6.3-6.8)</td>
<td>1.71 (1.46-2.01)</td>
</tr>
<tr>
<td>20-24 years</td>
<td>470</td>
<td>3.4 (2.5-4.6)</td>
<td>0.85 (0.72-1.00)</td>
</tr>
<tr>
<td>25-29 years</td>
<td>606</td>
<td>3.4 (3.2-3.5)</td>
<td>0.86 (0.75-0.99)</td>
</tr>
<tr>
<td>30-34 years</td>
<td>579</td>
<td>3.1 (2.5-3.9)</td>
<td>0.79 (0.74-0.84)</td>
</tr>
<tr>
<td>35-39 years</td>
<td>450</td>
<td>1.0 (0.9-1.1)</td>
<td>0.25 (0.22-0.29)</td>
</tr>
<tr>
<td>40-44 years</td>
<td>366</td>
<td>5.9 (5.7-6.2)</td>
<td>1.54 (1.31-1.81)</td>
</tr>
<tr>
<td>45-49 years</td>
<td>381</td>
<td>4.0 (3.3-4.7)</td>
<td>1.00 (reference)</td>
</tr>
</tbody>
</table>

Table, use of hormonal contraceptives, use of condoms, smoking, and higher educational attainment [20]. The cumulative incidence of genital warts in different birth cohorts, estimated on the basis of age at first diagnosis, has been found to be increased with younger age (p < 0.001), suggesting that the burden of genital warts will be increasing in upcoming years [20]. However, the cross-sectional nature of the given study restricted its ability to assess the temporality and several potential confounders (such as self-awareness, access to healthcare, diagnostic accuracy, and validity of self-reporting) which could limit the validity of the interpretations. In contrast, the study had distinct strengths, including large sample size, random selection of study participants, inclusion of participants from four different Nordic countries, and high response rates [20]. Overall, their findings suggested that the prevalence of genital warts is not low, and the associated disease burden is likely to increase in future years.

In a recent literature review of incidence/prevalence studies on genital warts, Singhal et al. [21] identified a total of 25 peer-reviewed studies and classified them into four groups according to data collection method: 1) retrospective administrative claims database or medical chart reviews (n = 10); 2) prospective physician reports (n = 4); 3) gynecological or pelvic exams (n = 5); and 4) patient self-reports (n = 6).

In countries like Turkey, where routine surveillance systems and medical record systems do not include data about disease burden, research-based calculations are important for estimating the overall burden of disease in the general population. This is also valid for investigating the overall disease burden and economic cost associated with genital warts among women in Turkey. Various methods, having particular strengths and limitations, have been used for research-based estimates of genital warts in different countries.

Research based on data collected in clinical settings represents the prevalence of warts among women visiting healthcare facilities. These findings are therefore valid only for a subgroup of women in the population, and thus will be of limited generalizability, depending on how representative the study participants are of women who have not visited a similar healthcare setting. Singhal et al. [21] reported on ten peer-reviewed publications on annual incidence and prevalence of genital warts among women, based on retrospective administrative claims database or medical chart review studies, and determined that annual incidence rates ranged from 120-231 per 100,000 and prevalence rates ranged from 128-165 per 100,000. It is important to note that this type of study provides evidence limited to data recorded in the database/medical charts, and can underestimate the true prevalence of disease in several situations, for example when two or more diseases coexist and only one diagnosis (for major symptoms) is recorded. Centripetal- and referral-filtered biases could also be a problem if not all hospitals/care centers are included in the data collection process. All of these factors may lead to information bias, most likely resulting in an underestimation of the “true” risk. Prospective studies of genital warts are less prone to information bias, if standard diagnostic tools are used and if all physicians are equally aware of the purposes of the study.

Warts often cause annoying symptoms such as burning, itching, bleeding, and pain, and psychosocial stress may result in negative self-perception, low self-esteem, embarrassment, and anxiety [22]. Despite this, not all women with warts seek medical attention for this condition. This issue is of special concern in self-report based studies. The review by Singhal et al. [21] reported the results of six peer-reviewed publications in which the rates of genital warts were based on self-reported history of physician-diagnosed genital warts. The 12-month prevalence rates were 300-1,900 per 100,000 and the corresponding lifetime prevalence was 356-12,000 per 100,000. The wide range of occurrence rates can be at least partially explained by reporting and recall bias.

Singhal et al. [21] also reviewed a total of five peer-reviewed journal articles published between 2000 and 2010, which attempted to estimate the prevalence of genital warts among women based on genital examinations, and stated that the estimated prevalence was found to be between 200 and 4,030 per 100,000. The wide range of rates suggests population-based differences in occurrence, or simply, differences in rates of availability, accessibility, and acceptability of gynecological care services provided for different populations. It is also possible that those visiting a gynecology clinic for “any” reason are more likely to have a genital wart compared with their counterparts who have not attended such a clinic. Thus, generalizability of the findings to the overall female population could be low. Also, given that patients with warts may choose to admit to several different outpatient clinics dealing with warts, includ-
ing gynecology, urology, and dermatology, all such clinics’ patients should be recruited for a comprehensive analysis of prevalence rates of genital warts. Subsequently, studies of genital warts based on outpatient clinic visits often underestimate the true prevalence of warts in the general population.

It is important to note that all studies set up on clinically-collected data will be limited in terms of generalizability and are prone to several sources of biases, such as Berkson’s fallacy, hospital access bias, ascertainment bias, and diagnostic access bias.

In contrast, population-based prevalence studies set up on “random” samples of reproductive-aged women are limited by poor response rates, given privacy concerns, and low acceptance rates for genital examination [22]. Distinct privacy concerns in the identification of genital warts in the general population have led to a scarcity in the number of such studies in the literature [23, 24], but are sufficient to deduce that rates differ widely across regions and countries, ranging from 1.4% in Spain to 25.6% in Nigeria [25, 26]. Poor awareness and recall might also lead to underestimation of the true rates of occurrence.

Despite these limitations in coverage and generalizability, most studies on frequency of genital warts have been conducted in selected populations, such as, STD clinic attendees, university students, or individuals insured through private health plans [17, 20, 27, 28].

It is important to note that, regardless of the study designs and sample populations, sole use of point prevalence rates will serve to further underestimate the annual genital wart-associated disease burden, given that some of the warts disappear spontaneously. This is of special concern in disease-associated cost estimations.

In the present study, the prevalence of genital warts among reproductive-aged women attending gynecology clinics/offices for “any” reason ranged from 3.4% to 4.1%, with an overall point prevalence of 3.5%, which corresponds well with other studies in different countries [29-33]. This finding is in line with findings of Hillemanns et al. [34], who reported a prevalence of 148 per 100,000 (new: 114/100,000 and recurrent: 35/100,000) among females aged 14-65 years who were consulting gynecologists for genital warts.

Based on genital examination, rates ranged from 200-4,030 per 100,000. Nyári et al. [32] studied 397 asymptomatic women, with a mean age of 35.5 years, attending gynecology clinics in Hungary, and found point prevalence of genital warts to be 4,030 per 100,000.

In the present study, the overall point prevalence of genital warts among reproductive women attending gynecology outpatient clinics for any reason was calculated as 3.5%. It is worth noting that point prevalence of genital warts varied by age among women attending a gynecology clinic. The highest rates were observed in the 15 to 19 year-old group. Kjaer et al. [20], in their study of prevalence in Nordic countries, reported that the average age at first diagnosis of genital warts ranged around 21-22 years; the minimum age for first diagnosis of genital warts ranged from three years in Norway to 14 years in Iceland and Sweden. In the same study, self-reported history of genital warts in the 12 months preceding the study was highest for ages < 20 years and in the 21 to 24-year-old group, with rates decreasing with increasing age. The present findings are in parallel with those of Kjaer et al. [20], other than our observation of an increase in genital warts in the 40 to 44-year-old group (5.9%).

This study is a pioneer in Turkish literature, with a specific aim to investigate genital warts in a heterogeneous group of reproductive-aged women. Given this, the study has several intrinsic limitations, analyses were conducted with extreme caution to avoid bias in the interpretation of the study findings, and issues of concern are summarized below:

1. The convenience sample, with data from ten selected cities and a total of 23 healthcare facilities enhanced the heterogeneity of women participating in the study. Yet, sampling design still limits the generalizability of the results to the whole Turkish female population of similar age. It is important to note that data were neither representative of all cities in Turkey, nor of all types of healthcare settings.

Data collection based on visits to specific healthcare settings, rather than on a random sample of the general population, further restricts the ability to generalize the study findings.

Study findings can be generalized to reproductive-aged women visiting a gynecology clinic for any reason, but not to the general female population. Given the scarcity of available evidence on national attendance rates and reasons, it is difficult to validly discuss the direction of bias in generalizing results to all women.

2. The refusal rate cannot be validly estimated and those women refusing to participate in the study might be different than those who took part in the study. However, given this, women were not informed of the main aim of “studying genital warts”, and that none had been examined differently from the others, there is no reason to believe that refusal by some participants was non-random with regard to the presence of genital warts.

3. Data cannot be used to distinguish “new” vs “existing” (either “persistent” or “recurrent”) genital warts. The frequency of lifetime (“ever”) genital warts cannot be estimated, either. As such distinction would be of value in economic evaluations, further studies should include specific questions, enabling such distinctions.

4. The descriptive nature of the study design, with no follow-up, restricts the ability of the study to evaluate causality, and to calculate incidence rates.

5. Although the study aimed to reach an equal number of women at each participating center, this goal could not be realized. The authors’ daily observations and data from previous studies reveal that women attending public institutions, universities, and private clinics may differ in terms of educational attainment, health-related awareness, socio-economic status, and even the type of health problems they suffer from. Weights were used in the study to minimize selection bias-related interpretation errors. Weights were calculated as the inverse of sampling fractions, assuming that: 1) the distribution of healthcare visits...
for “all” reasons is similar to that for “genital warts” across public health institutions, universities, and private sector clinics; 2) the characteristics of women attending study centers are similar to those attending corresponding types of healthcare settings (namely, public health institutions, universities, and private sector clinics) in the general population.

It is noteworthy to mention that women with warts might have been admitted to other outpatient clinics (such as, dermatology and/or urology clinics), and therefore the present estimates might be an underestimate of the “true” attending-based prevalence rate.

6. One of the main objectives of the study, investigation of the validity of self-report for genital warts, could not be established because the two related questions were misunderstood by participants.

Future studies should prepare more specific questions to further investigate the validity of self-report in identifying genital warts, and questions should be pre-tested in large numbers of eligible women prior to the data collection phase.

7. The non-systematic coding attending diagnosis hindered the authors’ ability to use this variable effectively in the study. In future studies, standard training of physicians and data collectors could improve the quality of the data.

Conclusion
This kind of study was conducted for the first time in Turkey. Estimation on national burden of the disease was facilitated by weighted analyses. The overall point prevalence of genital warts being 3.5 suggests that it is common in the population; and thus, effective prevention methods are required.

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References


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Sperm pooling and intrauterine tuboperitoneal insemination for mild male factor infertility

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Neogenesis IVF Centre, Marousi, Athens (Greece)

Summary

Purpose of investigation: To evaluate the efficacy of sperm pooling in the treatment of male infertility with the use of intrauterine tuboperitoneal insemination (IUTPI). Materials and Methods: A total of 169 cycles of IUTPI were performed in 69 couples with mild male factor infertility. Pooled semen samples were used in 115 cycles (Group A), whereas a single sample was used in 54 (Group B). The same mild ovarian stimulation protocols were used in all cycles. Results: The mean inseminate motile count (IMC), following sperm pooling was 6.63 x 10^6 in Group A and 3.74 x 10^6 in Group B (p = 0.0001) with a single semen sample. In total, 33 clinical pregnancies were achieved; 28 (24%) in Group A and five (9%) in Group B (p = 0.036). Conclusions: The results of this study indicate that sperm pooling may prove a useful technique in the treatment of mild male infertility when combined with IUTPI.

Key words: Sperm pooling; Mild male factor infertility; Intrauterine insemination; IUTPI; IMC; Pregnancy rates.

Introduction

Male factor infertility affects approximately 50% of infertile couples [1] either alone, or combined with female infertility. Many hormonal therapies have been proposed, in an attempt to improve the sperm count, such as clomiphene citrate [2, 3], aromatase inhibitors [4, 5], testosterone [6], and follicle stimulating hormone (FSH) [7, 8]. However, the aforementioned therapies have shown to be time-consuming, patient-specific [7, 8], and not entirely effective [9].

Couples suffering with mild male factor infertility are most of the time directly referred for in vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI) treatment due to the low success rate of intrauterine insemination (IUI). However, IUI is a less invasive and inexpensive treatment, requiring less hormonal prescriptions, and is regarded as the first line treatment according to WHO guidelines [10]. The success rate of IUI depends on sperm parameters, the woman's age, and the ovarian stimulation protocol used [11].

Regarding sperm parameters, previous studies agree that there is a direct relationship between the number of spermatozoa and IUI success rate; the higher the inseminate motile sperm count (IMC), the higher the success rate of IUI [12], with a threshold level of > 5 x 10^6/ml [11, 13, 14].

The use of more than one sperm sample for IUI, in cases of mild male infertility, was first reported in 1990 [15]. Since then, studies have shown that two sperm samples obtained on the same day or in consecutive days can improve the total motile sperm count and therefore, the outcome of IUI [15–17].

The aim of this study was to evaluate the use of sperm pooling, in couples with mild male factor infertility by combining two semen samples, followed by intrauterine tuboperitoneal Insemination (IUTPI). IUTPI is a novel IUI method that utilizes ten ml of inseminate including the sperm. The method and success rates of IUTPI have been previously described [18].

Materials and Methods

Patient selection

Retrospective analysis was conducted on 169 cycles performed between January 2010 and October 2011. Only couples with sperm concentration between 6 x 10^6/ml and 20 x 10^6/ml were included in the analysis. All female partners had a regular menstrual cycle of 25 – 33 days and had undergone a full diagnostic workup including, hysterosalpingography, prolactin, sex hormone binding globulin, thyroid hormones, and chlamydia screen. On day 2 or 3 of the cycle, transvaginal ultrasound (TVUS) check scan and baseline hormone assays including FSH, luteinising hormone (LH), and estradiol (E2) were performed.

Controlled ovarian stimulation

The female partners underwent the same controlled ovarian stimulation protocol that included, clomiphene citrate from day 2 of the cycle followed by human menopausal gonadotropin (hMG) 150IU from day 6 to day 10 of ovarian stimulation and gonadotropin-releasing hormone (GnRH) antagonists 1.25 mg from day 8 to day 10. This protocol was continued until maturing follicles reached 18 mm in diameter, when 5,000 to 10,000 IU of human chorionic gonadotropin (hCG) were administered. IUTPI was performed 36 – 40 hours after hCG administration, as previously described [18].

Sperm preparation

The male partners of the sperm pooling group (Group A) were asked to provide the first sperm sample (sample 1), by masturbation, on the day of the check scan. Following liquefaction, microscopic examination in the Makler counting chamber was performed. Cryoprotectant was then added slowly at a 1:1 ratio to the ejaculate volume and the sample was kept in room tem-
per temperature for ten minutes. It was then aliquoted in vials and kept in liquid nitrogen vapour for 25 minutes before it was plunged and stored in liquid nitrogen dewars (vapour freezing technique).

On the day of the insemination, the desired ampoules were removed from the liquid nitrogen dewars and thawed at room temperature, to be prepared, along with the fresh semen sample (sample 2) provided on the same day. Each sample was gently layered on top of two separate density gradients: a lower gradient of 90% (v/v) and an upper layer of 45% (v/v), in separate centrifuge tubes. The samples were then centrifuged at 1,500 rpm for 20 minutes, the supernatants were discarded, and the pellets that contained the motile spermatozoa from the fresh and thawed samples were combined and re-suspended in culture medium in the same centrifuge tube. A second centrifugation followed at 1,800 rpm for ten minutes and the final pellets, which contained the motile spermatozoa originating from the fresh and the frozen-thawed samples were re-suspended in ten ml of culture medium. The samples were then re-analyzed and loaded into a syringe and the insemination took place.

Male partners in Group B provided a single semen sample on the day of insemination that was processed in the same way as the fresh sample in Group A.

Statistical analysis

Pregnancy rates between Group A and Group B were calculated using the paired chi-square ($\chi^2$) test and the remaining group characteristics with the paired t test.

Results

A total of 169 cycles were included in the study, 115 in Group A and 54 in Group B. The mean age of the female partners in the two Groups, apart from the E2 levels, which were higher in Group B ($p = 0.0423$).

**Table 1. — Semen characteristics prior to sample preparation.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A (pooled samples) (n = 115)</th>
<th>Group B (single samples) (n = 54)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2.31 (± 0.63)</td>
<td>2.18 (± 0.61)</td>
<td>2.35 (± 0.72)</td>
</tr>
<tr>
<td>Concentration (spermatozoa x 10^6/ml)</td>
<td>12.80 (± 2.47)</td>
<td>13.15 (± 2.14)</td>
<td>12.53 (± 1.57)</td>
</tr>
<tr>
<td>Total sperm number (spermatozoa per ejaculate x 10^6/ml)</td>
<td>29.60</td>
<td>28.60</td>
<td>29.40</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>34.73 (± 2.05)</td>
<td>37.03 (± 1.34)</td>
<td>35.17 (± 2.44)</td>
</tr>
<tr>
<td>Degree of rapid forward progression (%)</td>
<td>28.78 (± 1.16)</td>
<td>27.55 (± 1.70)</td>
<td>26.93 (± 1.56)</td>
</tr>
<tr>
<td>Morphology</td>
<td>13.88 (± 1.14)</td>
<td>14.56 (± 1.03)</td>
<td>15.52 (± 1.19)</td>
</tr>
</tbody>
</table>

Characteristics of ovarian stimulation of the female partners in each Group, the Inseminate Motile Count (IMC) of the male partners in Group A following sperm pooling, and in Group B with a single semen sample, and showing the number of pregnancies achieved in each group.

**Table 2. — Cycle characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A (n = 115)</th>
<th>Group B (n = 54)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman’s age</td>
<td>33.68 ± 3.72</td>
<td>34.57 ± 3.23</td>
<td>0.1328</td>
</tr>
<tr>
<td>Follicles</td>
<td>3.21 ± 0.75</td>
<td>3.39 ± 0.66</td>
<td>0.1330</td>
</tr>
<tr>
<td>E2</td>
<td>683.49 ± 184.14</td>
<td>744.72 ± 175.30</td>
<td>0.0423</td>
</tr>
<tr>
<td>Endometrial Thickness</td>
<td>8.97 ± 0.79</td>
<td>9.17 ± 0.61</td>
<td>0.1022</td>
</tr>
<tr>
<td>IMC</td>
<td>6.63 ± 1.70</td>
<td>3.74 ± 1.12</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pregnancy*</td>
<td>28</td>
<td>5</td>
<td>0.036</td>
</tr>
</tbody>
</table>

*Clinical pregnancies were diagnosed ultrasonographically by the presence of a fetal heart beat.

Previous studies, where multiple semen samples had to be provided on the same or consecutive days, showed that multiple samples were very demanding for the male partners. Kücük et al. [12] reported that 35 out of 137 couples did not consent to providing two consecutive semen samples on the same day and 11 that did, were finally unable to provide a second sperm sample. The authors believe that sperm pooling with cryopreservation is more friendly and acceptable by the male partners as they are allowed to provide each sample many days apart.

Furthermore, some may argue that cryopreservation of the first semen sample in Group A, may influence semen characteristics following thawing. For this reason, all thawed samples were reviewed again to assess their microscopic characteristics before proceeding to preparation for insemination. No significant changes were recorded in the thawed samples. Recent studies further support that rapid freezing of the semen samples shows better survival characteristics compared to other methods of cryopreservation [19].

To conclude, the authors believe that sperm pooling, followed by IUTPI, is a procedure that results in higher pregnancy rates that can assist many couples who suffer from mild male factor infertility and for personal reasons do not want to proceed with IVF treatment.

Discussion

Many studies have reported the benefits of using multiple semen samples in couples suffering from mild male factor infertility, undergoing IUI. This study was designed to evaluate the combination of sperm pooling with the use of IUTPI in cases of mild male factor infertility and its effect on pregnancy rates. This retrospective analysis shows that the use of a cryopreserved semen sample along with the fresh sample provided on the day of IUTPI, significantly increases the inseminate motile count and clinical pregnancy rates per cycle.
Sperm pooling and intrauterine tuboperitoneal insemination for mild male factor infertility


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Is laparoscopic surgery safe in patients with an elevated shock index due to ruptured ectopic pregnancy?

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Summary

Purpose: To evaluate the effectiveness of laparoscopic surgery in patients with elevated shock index (SI), which is a unique determinant of acute hemorrhage. Materials and Methods: A retrospective chart review of all patients treated for ectopic pregnancy (EP) in the present gynaecology department between January 2007 and March 2011 was performed. For each measurement of heart rate (HR) and systolic blood pressure (SBP), a SI was calculated by dividing HR by SBP (normal, 0.5 - 0.7). Results: One hundred sixty patients were selected as SI above 0.7. There were 111 (69.4%) patients in the laparotomy group and 49 (30.6%) patients in the laparoscopy group. The postoperative hemoglobin (Hb) level was 8.46 ± 1.56 (g/dl) in the laparotomy group and 9.37 ± 1.52 (g/dl) in the laparoscopy group, with lower postoperative levels in the laparotomy group. The mean duration of postoperative hospital stay was 2.37 ± 0.74 days in the laparotomy group and 2 ± 0.84 days in the laparoscopy group. Conclusion: The availability of suitable operative equipment, nursing teams, and advanced laparoscopic skills, all justify operative laparoscopy for the surgical treatment of EP in women with elevated SI.

Key words: Ectopic pregnancy; Haemoperitoneum; Laparoscopic surgery; Laparotomy; Shock.

Introduction

Ectopic pregnancy (EP) is described as a condition in which the gestational sac is implanted outside the uterus. The prevalence of EP is two percent out of all pregnancies in the United States, and it is one of the leading causes of pregnancy-related deaths during the first trimester in women of childbearing age [1]. Technical advancement in the field of minimal access surgery has greatly enhanced the possibility of both diagnosing and treating this condition effectively [2]. Laparoscopic surgery is usually performed to treat EP when the patient is hemodynamically stable, beta-human chorionic gonadotropin (β-hCG) is <6,000 IU/l, history is suggestive of minimal pelvic adhesions, and pregnancy is confined within the tube [3]. However, there are studies reporting laparoscopic surgery as a treatment also in hypovolemic patients [4, 5]. There are still some conflicting results regarding performing laparoscopies and describing quantitative hemoperitoneum in patients with a ruptured EP. As we know, most cases of ectopic pregnancy present with massive bleeding in a relatively short time. However, in the case of gradual bleeding, patients remain hemodynamically stable despite a hemoperitoneum of 1,000 to 1,500 ml [6]. According to this data, the authors used the shock index (SI) which is a composite of heart rate (HR) and systolic blood pressure (SBP) (HR/SBP) in lieu of using predictors of hemoperitoneum to determine hemodynamically unstable patients.

Materials and Methods

Before the onset of data collection, approval was obtained from the institutional review board to perform a chart review of all patients treated for EP in the present gynaecology department between January 2007 and March 2011. The study population was divided into two groups according to their surgical treatment, laparoscopy or laparotomy. A retrospective chart review was then performed with the use of medical and ultrasound records and the laboratory database. The parameters analyzed were: age, gravidity, parity, gestational week, type of delivery, educational status, menstrual delay, history of EP, sonographic blood acumination in the pouch of Douglas (PoD), the mean size of ectopic focus, β-hCG measurements, preoperative and postoperative hemoglobin (Hb) and hematocrit levels. All charts were reviewed for HR and SBP at the time of the initial emergency department triage, and initial operative intervention, as well as Hb levels, quantity of hemoperitoneum, and the condition of fallopian tube at removal. For each measurement of HR and SBP, a SI was calculated by dividing HR by SBP (normal, 0.5-0.7). The patients with SI > 0.7 were included into the study population.

The statistical analysis was performed by using NCSS (Number Cruncher Statistical System) 2007 Statistical Software, among the two groups, and ruptured and unruptured EP. Statistical analysis included the Pearson’s chi-square test and Mann-Whitney rank test.

Results

One hundred eighty-eight patients were diagnosed with EP during the time period reviewed; 160 of these patients were selected as SI 0.7. There were 111 (69.4%) patients in the laparotomy group and 49 (30.6%) patients in the laparoscopy group. There was no conversion to laparotomy during laparoscopy. The mean age was 33.02 ± 6.31 years in the laparotomy group and 30.24 ± 5.15 years in the laparoscopy group. The gravidity in the laparotomy and laparoscopy groups was 2.91 ± 2.35 years in the laparotomy group and 2.57 ± 1.85, respectively. The mean parity in the laparotomy group was 1.27 ± 1.1 years in the laparoscopy group it was 1.84 ± 1.75, and in the laparoscopy group it was 1.27 ± 1.1 (p = 0.036). The parity was lesser
in the laparoscopy group compared with the laparotomy group. Characteristics of the patients are listed in Table 1. There was no significant difference in the previous type of delivery between the two groups. There was a statistical significance in the history of EP, with a lower level seen in the laparotomy group: five (4.50%) and 15 (30.60%). Preoperative Hb was 9.72 ± 1.6 g/dl in the laparotomy group (p = 0.001) and 10.24 ± 1.9 g/dl in the laparoscopy group (p = 0.0001). There was no significance in preoperative Hb values; the postoperative Hb level was 8.46 ± 1.56 g/dl in the laparotomy group, and 9.37 ± 1.52 g/dl in the laparoscopy group, with lower postoperative levels in the laparotomy group. There was no statistical significance in the mean size of the ectopic mass confirmed by transvaginal ultrasonography, HR, and β-hCG values between the two groups. The sonographic blood accumulation in the PoD was 46.12 ± 21.44 mm in the laparotomy group, and 32.83 ± 19.39 mm in the laparoscopy group (higher in laparotomy group). Preoperative abdominal blood was 582.78 ± 355.29 cc and 398.33 ± 275.94 cc in the laparoscopy group (lower in the laparoscopy group). The preoperative SBP was 94.59 ± 12.19 mmHg in the laparotomy group, and 101.63 ± 9.43 mmHg in the laparoscopy group. There were 88 (79.30%) tubal ruptures in the laparoscopy group, and 29 (59.20%) in the laparoscopy group (such that laparotomy was preferred in the ruptured group). Thirty-nine (35.10%) patients in the laparotomy group had postoperative blood transfusions, and only eight (16.30%) in the laparoscopy group. The mean duration of postoperative hospital stay was 2.37 ± 0.74 days in the laparotomy group and 2 ± 0.84 days in the laparoscopy group (Table 2).

**Discussion**

EP remains the leading cause of death in the first trimester of pregnancy. Approximately one to two percent of all pregnancies in Europe and the United States are ectopic, and in the Western world, tubal EP remains the most common cause of maternal mortality in the first trimester of pregnancy [7]. The occurrence of tubal rupture in EP ranges from 18.0% to 64.5% as reported in previous large population-based studies [8].

The patients in reproductive age tend to be younger and have an excellent physiologic reserve and capacity to accommodate blood loss. Regarding this, patients with significant hemoperitoneum can be misdiagnosed. There is a necessity to use a quantitative predictor to determine in patients with massive hemorrhage. The SI, which is an excellent predictor of hemoperitoneum and 160 patients were found to have an SI above 0.7.

**Table 1. — Characteristics of patients in the laparotomy and laparoscopy groups.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Laparotomy</th>
<th>Laparoscopy</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.02 ± 6.31</td>
<td>30.24 ± 5.15</td>
<td>2.79</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Gravity (kg)</td>
<td>2.91 ± 2.35</td>
<td>2.57 ± 1.85</td>
<td>0.372</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1.84 ± 1.75</td>
<td>1.27 ± 1.1</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Patients with ruptured tubes</td>
<td>88</td>
<td>29</td>
<td>59.20</td>
<td>6.99</td>
</tr>
<tr>
<td>Previous ectopic pregnancy</td>
<td>5</td>
<td>15</td>
<td>30.60</td>
<td>21.19</td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>22</td>
<td>10</td>
<td>20.40</td>
<td>2.25</td>
</tr>
<tr>
<td>VB</td>
<td>57</td>
<td>26</td>
<td>53.10</td>
<td></td>
</tr>
<tr>
<td>C/S</td>
<td>23</td>
<td>12</td>
<td>24.50</td>
<td></td>
</tr>
<tr>
<td>VB + C/S</td>
<td>9</td>
<td>1</td>
<td>2.00</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. — Laboratory, sonographic, preoperative, and postoperative evaluation results of the patients.**

<table>
<thead>
<tr>
<th>Mean size of the ectopic mass (mm)</th>
<th>Laparotomy</th>
<th>Laparoscopy</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.92 ± 17.28</td>
<td>34.55 ± 15.71</td>
<td>1.52</td>
<td>0.132</td>
<td></td>
</tr>
<tr>
<td>β-hCG (IU/l)</td>
<td>5272.66 ± 7474.9</td>
<td>6061.82 ± 10426.72</td>
<td>-1.11</td>
<td>0.268</td>
</tr>
<tr>
<td>Menstrual delay (days)</td>
<td>4.21 ± 1.32</td>
<td>3.71 ± 1.54</td>
<td>2.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Sonographic blood accumulation in the PoD (mm)</td>
<td>46.12 ± 21.44</td>
<td>32.83 ± 19.39</td>
<td>3.47</td>
<td>0.001</td>
</tr>
<tr>
<td>Intra-abdominal free blood volume (cc)</td>
<td>582.78 ± 355.29</td>
<td>398.33 ± 275.94</td>
<td>3.03</td>
<td>0.003</td>
</tr>
<tr>
<td>SBP/mmHg</td>
<td>94.59 ± 12.19</td>
<td>101.63 ± 9.43</td>
<td>-3.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>HR/per min</td>
<td>83.93 ± 8.23</td>
<td>83.08 ± 8.8</td>
<td>0.59</td>
<td>0.558</td>
</tr>
<tr>
<td>Preoperative Hb (g/dl)</td>
<td>9.72 ± 1.6</td>
<td>10.24 ± 1.9</td>
<td>-1.80</td>
<td>0.074</td>
</tr>
<tr>
<td>Postoperative Hb (g/dl)</td>
<td>8.46 ± 1.56</td>
<td>9.37 ± 1.52</td>
<td>-3.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative hematocrit (%)</td>
<td>30 ± 4.77</td>
<td>31.16 ± 5.41</td>
<td>-1.37</td>
<td>0.174</td>
</tr>
<tr>
<td>Postoperative hematocrit (%)</td>
<td>26.14 ± 4.65</td>
<td>28.96 ± 5.1</td>
<td>-3.44</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td>2.37 ± 0.74</td>
<td>2 ± 0.84</td>
<td>2.79</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The management of EP can be expectant, medical, or surgical. The choice depends on clinical circumstances, site of EP, and serum β-hCG levels. In those patients who require surgery, laparoscopy or laparotomy are the choices [11]. In the present study, there were 111 (69.4%) patients in the laparotomy group, and 49 (30.6%) in the laparoscopy group. Compared with laparotomy, the laparoscopic approach has many advantages, including shorter hospital stay, lower cost, decreased morbidity, shorter operation times, less intraoperative blood loss, lower analgesic requirements and less adhesion formation than reported in other gynaecologic operations [12, 13]. In the present study, the authors also had the same results with the literature in the laparoscopy group.

There is a major concern about performing laparoscopy in hemodynamically unstable patients presenting hemoperitoneum. Some studies have compared laparoscopy and laparotomy cases and indicate that hemodynamic stability, diameter of the tubal ectopic pregnancy, and easy visualisation of pelvic organs are the requirements for the laparoscopic surgery [14, 15]. On the other hand, there are...
also studies that report laparoscopy in hypovolemic patients [4, 5, 16].

Concerns regarding the performance of laparoscopy in bleeding patients relate to the creation of pneumoperitoneum and possible delays in bleeding control [2]. Previous studies have concluded that direct cannula insertion is not only safe, but can significantly shorten the time required to insert the cannula into the abdomen to create a pneumoperitoneum, thereby reducing operating time by 2.2 to 4.3 minutes [17, 18]. One limitation in the present retrospective study was the fact that the authors could not obtain the duration of time it took to reach the abdominal cavity in either the laparoscopy or in the laparotomy group, but they used verres insertions to create pneumoperitoneum instead of direct cannula insertion.

Although the major problem with laparoscopy is visualization of the pelvic organs (which are covered by blood), this difficulty can be overcome by using a uterine manipulator to facilitate anteflexing of the uterus and to more easily identify the fallopian tube [19]. Once the bleeding is controlled, evacuation of the blood can subsequently be performed. In their daily practice the authors also use an uterine manipulator when there is a restriction in visualization of the pelvic organs.

The sonographic blood accumulation in the PoD was 46.12 ± 21.44 mm in the laparotomy group and 32.83 ± 20.14 mm in the laparoscopy group. These results show that preoperative sono graphic blood accumulation in the PoD is evidence for the preference for laparoscopy [20, 21].

In conclusion, the SI is a quantitative predictor of massive haemoperitoneum and can be used in patients with ruptured EP. The availability of suitable operative equipment, surgical, and nursing teams, optimal anesthe sia, advanced cardiovascular monitoring, and the ability to convert rapidly to an open procedure if required, as well as advanced laparoscopic surgical skills and experience, all justify operative laparoscopy for the surgical treatment of EP, even in women with elevated SI.

Acknowledgement

The authors would like to thank Mr. Andrew McDonald, PhD for his linguistic help.

References


A comparison of the effect of levonorgestrel IUD with oral medroxyprogesterone acetate on abnormal uterine bleeding with simple endometrial hyperplasia and fertility preservation

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3Yazd Branch, Islamic Azad University, Yazd (Iran)

Summary

Objective: Endometrial hyperplasia is clinically important, because it can lead to abnormal uterine bleeding (AUB) which itself can precede endometrial cancer. Endometrial carcinoma is the most common malignancy of the female genital tract, occurring in about 75%-85% younger, perimenopausal women as endometrial hyperplasia. The treatment is hysterectomy or hormone therapy with progesterone. The aim of this study was, therefore, to compare the effect of levonorgestrel intrauterine device (LNG-IUD) with medroxyprogesterone acetate (MPA) on simple endometrial hyperplasia for fertility preservation. Materials and Methods: Forty women in reproductive age (22-47 years) with AUB with endometrial biopsies confirming simple hyperplasia, were enrolled in this study and then randomly divided into two groups. All patients presented with designed special checklist which was filled with satisfaction. Complete history and physical examination especially blood pressure (BP), body mass index (BMI), breast examination, bimanual vaginal examination, and transvaginal sonography (to measure the thickness of endometrial and exclude the other pathologic lesions) were performed. In the first group, treatment was performed with MPA (20 mg/daily) for ten days and in other group with LNG-IUD was prescribed. After three months, transvaginal sonography and biopsy of endometrium were done. The status of AUB and side-effects of two methods, along with the rate of satisfactory were evaluated. Result: The findings showed the significant differences in the treatment of simple hyperplasia between two groups (LNG-IUD group vs. MPA group) (p < 0.047). Recovery of AUB in the group LNG was enhanced (p < 0.047). Endometrial thickness was reduced in both groups (p < 0.001), but further reduction in LNG group was seen. Also, LNG was tolerated more than MPA. Side-effects of MPA were more and reached significance (p < 0.003). The rate of satisfaction with LNG was higher than MPA and reached significance (p < 0.048). Conclusion: The results of this study show that LNG-IUD is more effective than MPA in treatment of simple endometrial hyperplasia and can be helpful in young women who want to preserve their ferties.

Key words: Endometrial hyperplasia; Levonorgestrel IUD; Medroxyprogesterone acetate (MPA); Abnormal uterine bleeding.

Introduction

Endometrial hyperplasia can lead to endometrial cancer. It can also cause abnormal bleeding or be associated with ovarian tumors secreting estrogen or with hormone therapy. Since 75% to 85% of cases of endometrial cancer occur at an early age and before menopause, it is clinically very important because of fertility preservation [1, 2]. Abnormal uterine bleeding (AUB) is one of the most common clinical problems in the field of gynecology and, according to statistics, includes about 15% of patients visited in clinics [1].

According to patients’ conditions, including age, parity, other clinical conditions, curettage results, and patient’s preference, the type of treatment (hysterectomy vs oral treatment with progesterone) is suggested [1, 2]. One of the methods has been used recently which led to significant results is levonorgestrel intrauterine device (LNG-IUD). Considering the ease of use of this device and associated benefits, such as reducing AUB and its utility as a contraceptive device, in this study, this method was used in endometrial hyperplasia cases suffering from AUB who referred to Shahid Sadoughi Hospital for treatment. The pathology findings were evaluated before and after the usage of LNG-IUD and the results were also compared to the previous methods used to treat AUB.

Methods and Materials

This clinical trial survey was designed as a prospective randomized controlled study. It was performed in 40 patients during the reproductive age (22-47 years) with AUB due to endometrial hyperplasia, who tend to preserve their fertility, and the diagnosis was reached by pathology. Patients were randomly divided into two groups: group one including 20 patients on 20 mg oral medroxyprogesterone acetate (MPA) daily for ten days in each menstrual cycle, for three months and group two including 20 patients with LNG-IUD which releases 20 mcg levonorgestrel per day.

Response to treatment was evaluated three months following treatment using vaginal ultrasound and reviewing the pathology reports. Complications of treatment and patients’ satisfaction were also examined. All data on personal characteristics, type of
AUB, endometrial thickness, and type of treatment were recorded. Complications of treatments were monitored during three months and later on. The endometrial thickness, pathology findings, patients' satisfaction, and completion or failure to fulfill treatment were evaluated. Statistical analysis was performed using SPSS.

Results

Frequency distribution of body mass index (BMI), age, treatment completion, and history of diseases in MPA group is shown in Table 1. According to this Table, the most common range of BMI was about 25-29/9. Seventy-five percent were under 40 years of age. The most common history of disease was obesity and then utility of polycystic ovary. The highest rate of treatment completion in MPA group was 85%.

Frequency distribution of BMI, age, treatment completion, and history of diseases in LNG group is shown in Table 1. According to this Table, the most common range of BMI was again about 25-29/9. The commonest range of age was also under 40 years. The most common history of disease was obesity and the highest rate of treatment completion was 95%.

The menstrual conditions of patients were as bellow:

- Before treatment, in MPA group: ten (50%) cases menometrorrhagia, six (30%) hypermenorrhea, and four (20%) oligomenorrhea (Table 2).
- In LNG group: 16 (80%) menometrorrhagia, three (15%) hypermenorrhea, and one (5%) oligomenorrhea (Table 2).
- After treatment, in MPA group, six (30%) cases menometrorrhagia, four (20%) hypermenorrhea, three (15%) amenorrhea, and seven (35%) cases had normal menstruation (Table 2).
- In LNG group, three (15%) menometrorrhagia, two (10%) amenorrhea, four (20%) oligomenorrhea, one (5%) hypermenorrhea and ten (50%) cases had normal menstruation (Table 2).

Comparing the two groups showed that in the LNG group, menstruation had returned to normal status more than in MPA group (Table 1).

Pathological status after treatment was also evaluated. In MPA group, four (20%) cases observed to be progesterone, two (10%) atrophic, five (25%) proliferative, two (10%) menstrual, four (20%) simple, and one (5%) atypical (Table 3). In LNG group, 11 (55%) cases were reported to be progesterone, three (15%) proliferative, one (5%) simple, four (20%) atrophic and one (5%) secretory. All 16 patients in this group responded to the treatment and were proliferative cases, although response to treatment was reported. The probability of recurrence existed (Table 3). In terms of treatment response and improved pathology results, patients in the LNG group showed significant differences compared to the MPA group (p < 0.047, Tables 3, 4, 5).

Endometrial thickness in both groups was evaluated before and after treatment using vaginal ultrasound. In MPA group, the thickness of endometrium from 13.59 ± 3.59 cm reached 8 ± 3.09 cm which were significantly different. In the LNG group, the endometrial thickness of 15.1 ± 3.65 cm became significantly thinner (7.4 ± 2.41, p < 0.001, Table 4).

In terms of treatment satisfaction (Table 3) in MPA group ten patients (50%) were not satisfied because of untreated AUB or the severity of treatment side-effects. However, in the LNG group, 16 patients (80%) were quite satisfied with only four unsatisfied patients. Therefore, the patients' satisfaction in LNG group was significantly higher than patients treated with MPA (p < 0.047, Table 2).

Table 1. — Pre-treatment frequency distribution of BMI, age, treatment completion, and history of diseases in MPA and LNG groups.

<table>
<thead>
<tr>
<th>Variant</th>
<th>MPA</th>
<th>LNG</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>5</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>25-29/9</td>
<td>8</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>≥ 30</td>
<td>7</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>15</td>
<td>75</td>
<td>12</td>
</tr>
<tr>
<td>≥ 40</td>
<td>5</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>3</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Overweight + PCO</td>
<td>4</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. — Frequency distribution of AUB status, pathology findings, and treatment satisfaction after treatment in LNG and MPA groups.

<table>
<thead>
<tr>
<th>Variant</th>
<th>MPA</th>
<th>LNG</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progestational effect</td>
<td>5</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Proliferative</td>
<td>7</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>Simple</td>
<td>2</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Atrophic</td>
<td>5</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Atypia</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. — Determination of the frequency of pathology findings of the study groups.

<table>
<thead>
<tr>
<th>Variant</th>
<th>MPA</th>
<th>LNG</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not simple Progesterone</td>
<td>5</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Not simple Proliferative</td>
<td>7</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Simple</td>
<td>2</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Atypia</td>
<td>5</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. — Comparison of the mean endometrial thickness before and after treatment in MPA and LNG groups.

<table>
<thead>
<tr>
<th>Endometrial thickness</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p value</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial thickness</td>
<td>15.95 ± 3.59</td>
<td>10 ± 3.09</td>
<td>&lt; 0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>
in the LNG group it was 100%. In the Shaaban et al. study which was performed in 112 patients with simple hyperplasia, 56 treated with LNG and 56 with oral contraceptive pills, they found that after three months the reduction in the endometrial thickness were more in LNG treated patients than in the other patients [8].

Vereide et al. performed a study in 21 and 29 cases on MPA and LNG, respectively. Response to treatment reported was 100% vs 50% in LNG and MPA groups, respectively [9]. Varma et al. also performed another study in 105 simple endometrial hyperplasia cases which showed a response to treatment in 96% of them in one year [10]. It should be noted that in the present study, endometrial thickness decreased significantly in both groups.

Based on a systematic review of the contemporary literature performed by Gunderson et al., endometrial hyperplasia has a significantly higher likelihood of response (66%) to hormonal therapy than grade 1 endometrial carcinoma (48%) [11].

In the present study, one IUD (5%) after first month and the other one after three months (after D&C) were rejected. In a study performed by George et al. in 2011, two (3.6%) IUDs were rejected after three months of study [12]. The Kaplani et al. findings showed IUDs rejection (9.52%), spotting (71.4%), weight gain (30.5%), low back pain (38.3%), and headache (13.3%) [7].

A study performed by George et al. in 2011, 2 (3.6%) IUDs were rejected after three months of study [12]. Kaplani et al. findings showed IUDs rejection (9.52%), spotting (71.4%), weight gain (30.5%), low back pain (38.3%), and headache (13.3%) [7].

In the present study, the most common complication in MPA group was first headache and second weight gain (30%). In a study by Andrew et al. in 2010 in USA, headache was reported as the most common complications (16.3%), then weight gain (5%), and then lower abdominal pain (3.8%) [12, 13].

Conclusion

This study was intended to evaluate the effect of LNG-IUD and the MPA tablets in treatment of AUB caused by simple endometrial hyperplasia. The present findings demonstrated that LNG-IUD, in comparison with MPA, is more effective in treatment of AUB and simple endometrial hyperplasia. This result can help the younger women to preserve their fertilization. Therefore, the authors concluded that LNG-IUD is more effective in decreasing the endometrial thickness with fewer side-effects. It is also more tolerated by patients leading to higher patient satisfaction.

Acknowledgements

The authors did not receive any support on behalf of any company but they thank the Shahid Sadoughi University of Medical Science, Yazd (Iran) who supported this work.
References


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CD34 expression of chorionic villous in pre-eclamptic placenta: an immunohistochemical and ultrastructural study

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1Department of Immunology; 2Department of Histology and Embryology; 3Department of Obstetrics and Diseases; 4Department of Pathology; Dicle University School of Medicine, Diyarbakir (Turkey)

Summary

In this study, pre-eclampsia, proteinuria, and edema associated with hypertension in pregnancy were assessed at the Dicle University School of Medicine Department of Obstetrics and Gynecology Clinic. One group included 20 pre-eclamptic pregnant women with gestational age 20-35 weeks of pregnancy and the same in the control group that included; however, 20 normotensive pregnant women. Histochemistry, immunohistochemistry, and electron microscopy techniques were used. Histopathological examination of syncytiotrophoblast and stromal cells were observed in the increase in hyperplasia and hyalinization. The evaluation immunohistochemical of chorionic villi, placenta, and hematopoietic stem cell markers showed a positive reaction with CD34. Ultrastructural examination showed endoplasmic reticulum dilatation, degeneration of mitochondria in endothelial cells, and capillary vessel edema.

Key words: Pre-eclampsia; CD34; Placenta.

Introduction

Pre-eclampsia is a disorder with widespread vascular endothelial malfunction and occurs after 20 weeks gestation. It is clinically defined by hypertension and proteinuria, with or without pathologic edema. It is widely accepted that deficient trophoblast invasion of the spiral arteries leading to inadequate blood supply to the fetus is the central pathological feature [1, 2]. The development of fetal placental vasculature depends on the actions of angiogenic growth factors and their receptors produced by cells and extracellular matrix in close proximity to the fetal vessels [3]. Maternal endothelial cell dysfunction is a root cause of the peripheral vasoconstriction that characterizes pre-eclampsia and the multiorgan damage that often occurs in severe cases [4]. CD34 monoclonal antibodies are expressed selectively on human hematopoietic progenitor cells, including myeloid and lymphoid progenitors [5]. Fetal CD34+ cells enter the maternal circulation during pregnancy and may persist for decades. These cells are usually depicted as hematopoietic stem/progenitor cells [6].

CD34 expression compared with the pre-eclamptic and normotensive placentas demonstrated the effect of angiogenesis.

Materials and Methods

This study was conducted at the Dicle University Faculty of Medicine Department of Obstetrics and Gynecology clinic and 20 pre-eclamptic pregnant women with gestational age 20-35 weeks of pregnancy and the same as the control group included 20 normotensive pregnant women. The patients were classified according to diastolic blood pressure: normotensive, with diastolic blood pressure between 80 and 90 mmHg; between 90 and above. Immediately after delivery, normal and pathological placentas were transported from the delivery room to the laboratory and, after preliminary gross examination, two series of tissue samples were obtained. The specimens were immersed in 10% buffered formaldehyde. Then, sections of five μm in thickness were cut and made into slides. These were processed for hematoxylin and eosin (H&E) and trichrome masson staining, carried out according to conventional procedures.

Immunohistochemical technique

Immunostaining was carried out using the avidin-biotin-peroxidase complex method. After deparaffinization and dehydration, endogenous peroxidase activity was blocked using three percent hydrogen peroxide in pure methanol for ten minutes at room temperature. The tissues were then treated with 0.01% pepsin in 0.01M HCl at 37°C for 15 minutes. After serum blocking, using two percent bovine serum albumin, the sections were then incubated with the primary antibody for 30 minutes at room temperature. The primary antibody was a mouse monoclonal antibody for low molecular weight cytokeratin at a dilution of one in 40. This was then incubated using the secondary antibody CD34 for 30 minutes.

Table 1. — Expected values, measured values, and SI values of quality control samples.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-eclampsia group (n = 20)</th>
<th>Control group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 24-hour proteinuria</td>
<td>1.6 (± 1.5)</td>
<td></td>
</tr>
<tr>
<td>Mean diastolic blood pressure</td>
<td>123 (± 9.7)</td>
<td>92 (± 1.3)</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>106 (± 9.4)</td>
<td>80 (± 1.9)</td>
</tr>
<tr>
<td>Chorion villi diameters</td>
<td>6,821.55 ± 24,451.84</td>
<td>5,428.42 ± 13,086.36</td>
</tr>
<tr>
<td>Mean 16,300.09, Std 4,412.02</td>
<td>Mean 8,192.06, Std 2,679.8</td>
<td></td>
</tr>
<tr>
<td>Vessel diameters in chorion</td>
<td>Mean 950.96, Std 1,347.82</td>
<td>Mean 135.32, Std 68.79</td>
</tr>
<tr>
<td>Mean 224.32 ± 4,783.02, Std 82.74 ± 330.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Electron microscopy technique

The pieces of tissue were immediately placed in 2.5% buffered glutaraldehyde for four hours, then fixed in OsO₄ for two hours, dehydrated in graded ethanols, and embedded in araldite. Semithin sections one μm thick were cut and stained with methylene blue-azure II for light microscopic examination. Thin 70-nm thick sections were stained with lead citrate-uranyl acetate and examined and photographed under electron microscopy.

Results

Diameters of the villi and fetal vasculature were also estimated by direct measurements. Normotensive and pre-eclamptic placentas were compared statistically and are shown in Table 1. Chorionic villi of 15 randomly selected villus diameter, vessel diameter, and the appreciation of statistics were performed by measuring the distance from the intervillous. Chorionic villi and vessel diameters were increased significantly in the pre-eclamptic group. Histopathologically, villous fibrin deposition outside the area showed an increase in syncytial nodes (Figure 1). The chorionic villi remained unaltered in the control group (Figure 2). Hypertrophy of cytotrophoblast cells, villous congestion, and dilation of blood vessels was also observed. Pre-eclamptic group in another section, stromal cell infiltration, and intervillous area commonly seen in the increase in the erythrocytes. Immunostaining with CD34 in the pre-eclamptic group compared to the control group showed a significant increase in the vessel wall (Figure 3). Ultrastructural examination revealed degeneration of mitochondria in endothelial cells, dilation of endoplasmic reticulum, and an increase of nuclei in the structure of chromatin (Figure 4). In the control group, the ultrastructural appearance of the normal chorionic villi can be seen in Figure 5. CD34 immunoreactivity in tissues from normal and pre-eclamptic pregnancies. Dark brown color of endothelial cells indicated positive staining for CD34 in villous core vessel endothelium (Figure 3). Pre-eclampsia was significantly increased as a result of the activity of CD34 in endothelial cells.
Discussion

In pre-eclampsia there may be vascular endothelial cell damage and dysfunction. Placental pre-eclampsia is characterized by an hypoxic placenta subjected to oxidative stress, while maternal pre-eclampsia arises from the interaction between a normal placenta and a maternal system susceptible or suffering from microvascular diseases, as well as long-term hypertension and/or diabetes [7]. Cytokines, growth factors, and adhesion molecules have been proposed as important mediators for successful placentation, as well as endothelial dysfunction [8]. Vasoconstriction in the development of pre-eclampsia, insufficient spiral artery invasion, trophoblastic vasoactive substances of endothelial origin, and other factors playing a role in the expander have been reported [9-12]. In case of maternal hypertension in pre eclamptic women, fetal vessels react with a general vascular constriction [13], which increases the peripheral resistances.

CD34 marks the endothelial cytoplasm and consequently, the surface of the fetal blood exchange with the maternal medium. Endothelial cell damage causing leakage in the cell membrane protein also commonly leads to a loss of integrity. Indicators of this situation in women with pre-eclampsia are proteinuria and the
development of peripheral and pulmonary edema [14]. Effects of nutrition and metabolic changes in fetal placental vascular bed could be due to the terminal chorionic villi vascularization which plays a very important role in pregnancy. As a result of pre-eclampsia, histopathological changes in villus vessels, as well as degeneration of vascular endothelial cells, and mitochondrial damage were observed. The etiology of pre-eclampsia could be due to decrease of platelets and endothelial cell damage. In the present study, the expression of CD34 in endothelial cells increased in the pre-eclamptic group and influenced the development of blood vessels. The vessel wall, as a result of this change, would be helpful in understanding the etiology of pre-eclampsia. Ultrastructural stage, degeneration of mitochondria to the cytoplasm, and organelles at the level of chromatin explain the increase in endothelial damage. As a result, dysfunction and vascular endothelial cells, in response to the development of intravascular coagulation, are thought to be effective in pre-eclamptic pathology.

References


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Association between mean platelet volume and different phases of menstrual cycle in primary dysmenorrhea

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Department of Obstetric and Gynecology, Dicle University School of Medicine, Diyarbakir (Turkey)

Summary

Purpose: Blood cells play a major role in homeostasis and inflammation. Primary dysmenorrhea (PD) involves the production of prostaglandins and leukotrienes, which cause inflammation in uterine tissue. Aim of this study was to investigate whether there is a relation between complete blood count parameters and PD during the menstrual cycle. Materials and Methods: The study included 41 cases diagnosed as primary dysmenorrhea (mean age, 23.02 ± 3.43 years) and 40 individuals who control subject (mean age, 23.76 ± 3.13 years). Hematologic parameters were measured on menstrual phase (day 1-4), follicular phase (day 9-12), and luteal phase (day 21-23) during menstrual cycle. Results: There were no statistically differences between hematological parameters of two groups except for mean platelet volume (MPV). MPV of PD and control groups at each phase of menstrual cycle were 7.71 vs 8.61 (p = 0.01); 7.66 vs 8.56 (p = 0.005); 7.75 vs 8.53 (p = 0.01), respectively. Conclusion: PD is associated with decreased MPV and platelets may be involved in the inflammatory process of PD. Key words: Primary dysmenorrhea; Complete blood cell count; Mean platelet volume.

Introduction

Primary dysmenorrhea (PD) is defined as a cyclic pain, which begins with menstrual blood flow, or just before, without any evident pathology in pelvic examination. The prevalence of PD is estimated at 25% of women and up to 90% of adolescents [1]. The pathogenesis of PD involves the production of prostaglandins and leukotrienes, both derived from arachidonic acid, which is released as a result of cell destruction in the endometrial tissue due to progesterone withdrawal just prior to menstruation [2]. The inflammation which is caused by the leukotrienes and prostaglandins, creates both uterine cramps and systemic symptoms such as headache, dizziness, nausea, and vomiting. Especially prostaglandin F2 alpha (PGF2 alpha) causes uterine ischemia and pain by producing myometrial contractions and vasoconstriction. In one study, PGF2 alpha levels were four times higher in the endometrial samples of patients with primary dysmenorrhea compared to those of patients with eumenorrhea [2]. In addition to prostaglandins, increased levels of leukotrienes (LTs) and platelet activating factor (PAF) have been detected in the menstrual blood of dysmenorrhea patients. Moreover, no relation between the prostaglandins and the severity of dysmenorrhea has been found; however, a positive correlation has been detected between both LTs and PAF and severity of disease [3].

Blood cells play a role in inflammation. Platelets are activated in a variety of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, arterial thrombosis, and asthma. Leukocytes play a major role in inflammatory processes and platelet–lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) are considered as new markers of systemic inflammation [4,5]. The aim of this study was to investigate whether there is a relation between complete blood count parameters and PD during the menstrual cycle.

Materials and Methods

This study was performed in the Obstetrics and Gynecology Department at Dicle University Faculty of Medicine. This study was approved by the Ethics Committee of the University an informed consent was obtained from all participants in accordance with the Declaration of Helsinki before the study.

The participants of the study were selected from women admitted to the present gynecology clinic with symptoms of dysmenorrhea and for a routine control. The diagnostic criterion for primary dysmenorrhea was cyclic lower abdominal pain, beginning with or just before menstruation and abating within the first few days of menstruation and not related to any pathological condition. Patients who did not have regular menstrual cycles during the previous six months, required medication for any reason, had chronic diseases, smoked or drank alcohol, with diagnosed endometriosis, had identifiable pelvic pathology, or had experienced dysmenorrhea associated with an intrauterine device were excluded from this study. All study participants were informed about the study.

The study population consisted of 41 women with primary dysmenorrhea and a control group of 40 healthy women. Medical history of each participant was reviewed and all participants underwent a physical examination, including height and weight measurements, and pelvic ultrasonographic examination. Blood samples were obtained from all participants during each phase of one menstrual cycle: between the first and the fourth day of the cycle for the menstrual phase, between the ninth and the 12th day, the 21st and the 23rd day of the cycle for the follicular phase and between the 21st and the 23rd day of the cycle for the luteal phase according to ultrasonographic examination. During the menstrual phase, hormonal eval-
ulation (follicle stimulating hormone, luteinising hormone, prolactin, and thyroid stimulating hormone) was performed. Blood samples were drawn from the antecubital vein between 8:00 a.m. and 10:00 a.m. after an overnight fasting period. Blood samples were collected in tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA). All measurements were performed immediately after vein puncture to prevent in vitro platelet activation. The complete blood count was measured and hormone measurements were evaluated.

Statistical Analysis
Data were analyzed using the Statistical Package for Social Sciences version 15.0 (SPSS for Windows 15.0). Normality of continuous data was determined by Kolmogorov-Smirnov test. Parametric and non-parametric tests were conducted with independent Student t test, Mann-Whitney U test, and the Chi square test. Data were expressed as the mean ± standard deviation (SD). A two-tailed \( p < 0.05 \) was considered statistically significant for all comparisons.

Results
The mean age of the 41 patients with dysmenorrhea was 23.02 ± 3.43 years, and the mean age of the 40 patients in the control group was 23.76 ± 3.13 years (\( p = 0.35 \)). Baseline demographic and laboratory characteristics of the patients in both groups are outlined in Table 1. Comparative analysis of the hematological parameters of study population and controls are displayed in Table 2. During all phases of the menstrual cycle, the mean platelet volume (MPV) was significantly lower in patients with PD (7.71 ± 1.17 vs 8.61 ± 1.79 (\( p = 0.01 \)) in menstrual phase; 7.66 ± 0.81 vs 8.56 ± 1.75 (\( p = 0.005 \)) in follicular phase; 7.75 ± 1.05 vs 8.53 ± 1.46 (\( p = 0.01 \)) in luteal phase). No significant differences were found between the two groups for any of the other hematological parameters.

Discussion
In the present study, when hematological parameters were evaluated for patients with PD, MPV was found to be significantly lower, compared to the control group, in all three phases of menstrual cycle. No significant differences were detected for other hematologic parameters. Platelets are essential blood cell components, which play a major role in hemostasis, inflammation and tissue regeneration. Platelets are activated in vascular and inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, atherosclerotic thrombosis, myocardial infarction, asthma, and transplant rejection [6-9]. MPV, which is an indicator of platelet activation and a component of the complete blood cell count (CBC), is inexpensive and is commonly used. MPV has been shown to increase in diseases associated with arterial thrombosis and in conditions associated with high cardiovascular risk, such as hypercholesterolemia, hypertension, and diabetes [8,10-13]. Larger platelets have more active substrates, metabolically and enzymatically, and more alpha granules [7,8]. They express and release more prothrombotic and vasoconstrictor factors, such as thromboxane A2, P-selectin, platelet-derived growth factor, and glycoprotein IIb-IIIa [14]. Vasoconstriction, adhesion, aggregation, and thrombosis occur in some diseases associated with high MPV due to the increased levels of prothrombotic and vasoconstrictor factors [15]. Unlike diseases with atherothrombosis, MPV has been reported to be lower in chronic inflammatory diseases. Kisaçik et al. have shown that MPV is significantly lower in ankylosing spondylitis and rheumatoid arthritis patients with active disease as compared to con-

<table>
<thead>
<tr>
<th>Table 1. — Baseline characteristics.</th>
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<tr>
<td></td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>FSH</td>
</tr>
<tr>
<td>LH</td>
</tr>
<tr>
<td>PRL</td>
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<td>TSH</td>
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</table>

Data are expressed as mean ± SD. (NS) non-significant.

<table>
<thead>
<tr>
<th>Table 2. — Hematological indices.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>WBC 1</td>
</tr>
<tr>
<td>WBC 2</td>
</tr>
<tr>
<td>WBC 3</td>
</tr>
<tr>
<td>NEU 1</td>
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<tr>
<td>NEU 2</td>
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<tr>
<td>NEU 3</td>
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<tr>
<td>LYM 1</td>
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<tr>
<td>LYM 2</td>
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<td>LYM 3</td>
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<tr>
<td>PLT 1</td>
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<td>PLT 2</td>
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<td>PLT 3</td>
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<td>MPV 1</td>
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<td>RDW 1</td>
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<td>RDW 2</td>
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<tr>
<td>P/L 2</td>
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<tr>
<td>P/L 3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. WBC: white blood cell; NEU: neutrophil; LYM: lymphocyte; PLT: platelet; MPV: mean platelet volume; RDW: red blood cell distribution width; N/L: neutrophil-to-lymphocyte ratio; P/L: platelet-to-lymphocyte ratio; 1: values of menstrual phase; 2: values of follicular phase; 3: values of luteal phase.
trols [16]. Similarly, Kapsoritakis et al. reported that MPV levels were significantly decreased in active inflammatory bowel disease, a finding that was well-correlated with the extent of the disease [7]. Gasparyan et al. found that MPV levels gradually increased in patients with rheumatoid arthritis, culminating in a significant difference at the end of the three months of anti-TNF therapy [17]. Decreased MPV levels have also been shown in inflammatory diseases in several other studies [18-20]. In addition to chronic inflammation, MPV was reported to be lower in acute inflammatory conditions, including acute attacks of chronic inflammatory diseases [20-22].

The cause of lower MPV in acute and chronic inflammatory conditions is still not entirely known. Increased production of acute phase reactants and pro-inflammatory cytokines due to inflammation may affect megakaryopoiesis resulting in the development of smaller platelets [20,23]. Another possible explanation for low MPV could be increased consumption of larger platelets at the inflammatory site [17]. Platelets that are structurally larger are more active and can release more pro-inflammatory and thrombotic agents [7,8]. Danese et al. speculated that reduced MPV could be due to the consumption or sequestration of large activated platelets in the intestinal vasculature of patients with Crohn’s disease and ulcerative colitis [24]. It is possible that the increased demand at the inflammatory site could result in increased consumption of larger platelets, leaving mostly platelets of smaller volume in systemic circulation.

Abnormal uterine activity with myometrial vasoconstriction and inflammation resulting from production of leukotrienes and prostaglandins are implicated in the etiology of primary dysmenorrhea [25]. PGF2 alpha is the most implicated agent in this process. Prostaglandin levels in the endometrial fluids of patients with dysmenorrhea were high and correlated with the severity of the pain [26]. Lundstrom demonstrated that intruterine administration of PGF2 alpha during the secretory phase of the menstrual cycle increased uterine contractility [27]. However, some patients with dysmenorrhea do not derive any benefit from non-steroidal anti-inflammatory medications, which act as anti-PG agents, suggesting the existence of other non-PG pathways in the etiology. High leukotriene (LT) infiltration has been observed in the endometriums of patients who did not respond to PG antagonists [3,28]. Rees et al. demonstrated increased LT levels in uterine tissue samples obtained after hysterectomies of patients who had dysmenorrhea [28]. Nigan et al. showed a close correlation between menstrual flow LT-C4/D4 levels and the severity of dysmenorrhea in patients who responded poorly to therapy with prostaglandin synthetase inhibitors [3]. Despite the many potential explanations, the pathogenesis of primary dysmenorrhea is still poorly understood. Recent evidence also links nitric oxide and vasopressin with dysmenorrhea pathogenesis [29,30].

In the present study, MPV levels of patients with PD were lower compared to the control group during all menstrual cycle phases. The authors believe that platelets may be involved in inflammatory process of PD. In addition to controlling thrombosis and hemostasis, platelets have role in inflammatory processes. The changes in megakaryopoiesis due to inflammation related to PD may have suppressed the size of the platelets. The fact that MPV was found to be low throughout the entire menstrual cycle suggests that the inflammation is not limited to the menstrual phase but continues as chronic inflammation in patients with PD. Alternatively, increased consumption of larger, metabolically active platelets in the uterine tissue in which inflammation occurs could explain the low MPV levels. The authors suggest that not only mediators released from the endometrial cells but also vasoactive substrates released from the large platelets in inflammation site might be causing uterine ischemia and pain via vasoconstriction in patients with PD.

The limitations of this study are that being relatively small number of study subjects and cross-sectional one-center study design that limited the authors’ ability to infer a casual association between MPV levels and PD, and may not be a real index of the general population.

**Conclusion**

In conclusion, the present findings imply that platelets may be involved in the inflammatory process for PD. Although the mechanism causing MPV change has not been fully explored, the authors suggest that low MPV values might indicate inflammation in patients with PD. On the other hand, low MPV in all phases of the menstrual cycle suggests that PD can be a chronic inflammatory process, which needs to be tested with larger scale studies.

**References**


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Mild increases in serum FSH in late follicular phase increases the risk of the luteinized unruptured follicle: case report

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Summary

**Purpose:** To find a case that will support the concept that mild elevation of serum follicle stimulating hormone (FSH) levels in late follicular phase may interfere with the release of the oocyte from the follicle. **Materials and Methods:** Oocyte release was determined in a woman in a complete natural cycle vs cycles where a boost of very low dose FSH was given, human chorionic gonadotropin (hCG) 10,000 units or leuprolide acetate 1 mg every 12 hours x two doses was given in FSH stimulated cycles. **Results:** Oocyte release occurred in all natural cycles vs none of the mildly stimulated cycles. **Conclusions:** Mild changes in the FSH level in the late luteal phase may affect the production of certain FSH dependent factors needed to release the oocyte.

Key words: Luteinized unruptured follicle syndrome; Gonadotropin; Human chorionic gonadotropin; Leuprolide acetate; Natural cycle.

Introduction

In any given menstrual cycle, a woman can develop a dominant follicle, generate a luteinizing hormone (LH) surge from a corpus luteum, and have spontaneous menses two weeks after the induction of progesterone (P) secretion and yet technically not ovulate because the oocyte was not extruded from the follicle [1]. This is known as the luteinized unruptured follicle (LUF) [2].

A global review of the world literature concluded that although LUF does exist as a clinical phenomenon in natural cycles, but from a clinical standpoint it probably does not constitute a syndrome [3]. However, in women treated with gonadotropin and human chorionic gonadotropin (hCG), data demonstrated 91% of 128 woman who presented evidence of oocyte release in cycle one demonstrated a definite release in cycle two; only 6% of 16 women who failed to release an oocyte in cycle one showed evidence of release in cycle two [4].

Thus the possibility exists that the use of FSH drugs creating a change in the normal LH-FSH-estradiol (E2) relationships during the follicular phase may cause a greater chance of the various chemical and cytokines produced to negatively influence oocyte release from the follicle [5].

A case is presented demonstrating this hypothesized phenomenon in one individual, i.e., oocyte release with natural cycles, but LUF even with very mild FSH stimulation.

Case Report

The patient is a 40-year-old woman presenting with primary infertility. She had a history of diminished oocyte reserve with her highest serum FSH at 31 mIU/ml.

Evaluating natural cycles in her first cycle of evaluation, she attained a mature follicle with her peak serum E2 of 282 pg/ml on a cycle day 8 with a 22.9 mm follicle and the follicle completely collapsed two days later to < 10 mm, thus showing evidence of oocyte release. She had similar findings in cycle 2, which was completely natural.

In cycle three her day 4 serum FSH was 27.9 mIU/ml with a serum E2 of 48 pg/ml. She wanted to be more aggressive, so on cycle day 10 she was boosted with 150 IU exogenous FSH (Gonal-F®) and 1 mg of Ganirelix. Her serum E2 initially dropped to 163 pg/ml on day 11 from 242 on day 10. Serum P remained low at 0.6 ng/ml, but eventually she reached a 333 pg/ml serum E2 with an 18, 14, and 12 mm follicle. The LH rose from 4.3 mIU/ml to 24.6 mIU/ml, therefore 10,000 units of hCG was given. The 12 mm follicle increased to 21.7 mm and the 18 mm follicle increased to 37.5 mm in average diameter and the 14 mm remained about the same three days later - indicative of LUF.

For cycle four she tried a natural cycle again and attained a 19.1 mm follicle with a serum E2 of 311 pg/ml. She was given leuprolide acetate in lieu of hCG 1 mg every 12 hours x two doses, and demonstrated oocyte release.

For cycle five she failed to attain a mature follicle, so it was decided to boost with FSH again. In cycle six she attained an 18.2 mm follicle on day 16 with a serum E2 of 436 pg/ml, P of 0.4 ng/ml, LH of 6 mIU/ml, and an FSH of 15.8 mIU/ml. Following leuprolide acetate 1 mg every 12 hours x two doses, the 18.2 mm follicle increased to a 20.2 mm and serum P rose to 3.3 ng/ml, indicative of non-release again.

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Discussion

One question that arises about LUF is whether one can be sure that merely failing to demonstrate sonographic follicle collapse is definitely indicative of oocyte release. Support that sonography can indeed detect oocyte release from the follicle is the fact that in one study, the pregnancy rate in the first and second gonadotropin stimulated cycle, the pregnancy rate was 13.5% and 15.7% when oocyte release was demonstrated, but was zero in both cycles when LUF was diagnosed [4].

One study found that LUF occurred in about 5% of natural cycles [5]. Yet given 35 more cycles in these same women, LUF only occurred in one (about 3%) [5]. Others claim that LUF may occur in about 7% of natural cycles [6]. The incidence of LUF may increase significantly if one evaluates LUF in natural cycles of women with unexplained infertility [7].

The woman described in the case report consistently released her oocytes in natural cycles but failed all FSH stimulated cycles although at most she received a tiny boost from the late follicular phase and despite receiving 10,000 units of hCG. A previous publication found that leuprolide acetate, a gonadotropin releasing hormone agonist (GnRH-a), by causing release of endogenous LH and FSH, had a high success rate in advancing oocyte release in women failing to release with FSH stimulation and hCG [8]. Indeed the woman did release following leuprolide acetate injection in a natural cycle (though it may not have been needed), but failed when given just a mild boost of FSH despite leuprolide acetate.

This case demonstrated that LUF is not from failure to generate an LH surge but possibly failure to generate a biologically active LH surge. The case strongly suggests that increasing FSH even mildly in the late follicular phase causes some change in the factors that are needed for oocyte release [5].

This case report suggests that if oocyte release occurs in natural cycles, one cannot assume that oocyte release will occur in subsequent cycles, even if only a boost of low-dose FSH is given to merely help ensure adequate follicular maturation. Some assume there is no downside to improve follicular maturation with a small dosage of FSH. This case shows that the LUF syndrome is one potential negative effect of mild FSH stimulation.

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An autopsy case of acute aortic dissection during postpartum period

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Summary
Background: Aortic dissection in young women without Marfan disease is related in most instances to pregnancy. This is a potentially catastrophic occurrence. Case: An autopsy case of acute aortic dissection type B (Stanford classification), clinically undiagnosed during late puerperium period in a young woman with no discernible risk factors (e.g. family history and signs of connective tissue diseases) is presented. Autopsy with ancillary investigations revealed that knowledge of this albeit relatively rare complication of postpartum may assist the clinician in earlier diagnosis and referral of patients for surgical treatment. Conclusion: This case is presented to raise awareness and review the literature for the critical care of postpartum patients.

Key words: Descending thoracic aortic dissection; Postpartum; Autopsy.

Introduction
Acute aortic dissection occurring during pregnancy and peri- and postpartum periods is a rare but recognized phenomenon [1-4]. The results can be devastating for both mother and fetus with reported mortality of one percent per hour for the first 48 hours [5, 6]. Most reported cases are associated with connective tissue disease (e.g., Marfan’s syndrome), systemic hypertension, and congenital heart disease (e.g. coarctation and bicuspid aortic valve) [5, 6]. Aortic dissection during puerperium is rarely reported in literature [1, 7, 8]. The authors describe the case of an acute type B aortic dissection (Stanford classification) occurring in an apparently fit and healthy postpartum woman with no discernible risk factors.

Case Report
A previously healthy 29-year-old Japanese woman (gravida 2, para 2), 155 cm, 55 kg was urgently transported to the medical center for evaluation and treatment of loss of consciousness 19 days after a full-term Cesarean delivery. She suddenly developed severe back pain for several minutes, then collapse and unresponsiveness. On presentation, the woman was in cardiac arrest and underwent aggressive resuscitation and emergency standard cervical intubation and mechanical ventilation. Computed tomography (CT) with angiographic contrast did not detect alcohol, common illicit, or prescribed drugs or pesticides. The death was attributed to its main branches were essentially intact. Postmortem toxicological screening of blood did not detect alcohol, common illicit, or prescribed drugs or pesticides. The death was attributed to hemothorax due to ruptured dissecting descending thoracic aorta.

Discussion
Aortic dissection is rare in young women but, when it does occur, is often associated with pregnancy [1-3]. During pregnancy, the highest incidence is in the later months, thus leading to speculation that the hemodynamic alterations of pregnancy may play a role. There is little in the literature regarding postpartum onset of aortic dissection including rupture of aneurysm, as in the present case. [1, 7, 8]. Investigators have found that the aortic media in rabbits [9] and humans [10] changes morphologically and biochemically during pregnancy. In particular, the reticulum becomes fragmented, the elastica attenuates with loss of corrugation, and there is a decrease in the amount of acid mucopolysaccharides. These changes probably render the aorta more vulnerable to dissection in pregnancy. In support of this, similar changes were found in the aortas of pregnant women who had had aortic dissections [11]. How-

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ever, other investigators have not found differences between the aortas of pregnant and non-pregnant women [12]. The condition is also described in systemic hypertension and is associated with the use of crack cocaine [7]. The patient’s serological studies for exogenous pathogens and antibody assay did not yield any positive findings.

In the present case, the decedent was a young non-Marfan woman, who underwent a lower segmental Cesarean delivery without any operative or postoperative complications. She suddenly developed severe back pain for several minutes, and then collapsed and lost consciousness on the 19th day of puerperium. Subsequent complete pathological autopsy along with ancillary investigations revealed that she died due to hemothorax from ruptured dissecting descending thoracic aorta. She had no prior history of chronic hypertension. Most cases in the literature focus on patients that are at risk for aortic dissection and discuss appropriate surgical intervention prior to acute complications. The condition needs to be identified and promptly addressed because mortality increases if surgical treatment is delayed. The diagnosis of acute aortic dissection should be considered if circulatory collapse and chest or back pain suddenly occur during the peri- and postpartum periods.

References


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Immature malignant sacrococcygeal teratoma: case report and review of the literature

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Summary

Immature malignant sacrococcygeal teratoma (SCT) is a rare tumor, deriving from the three germinal layers and is found in the sacrococcygeal region. It is the most frequent site of teratomas in the fetus. A nut-brown, solid tumor with cystic areas with a ten-cm diameter is reported in the sacrococcygeal region of a female fetus of 23 weeks and with a weight of 308 g. The ultrasound and pathology evaluations revealed characteristics of an immature malignant SCT. The incidence of this tumor type is one in 35,000 to 40,000 live births and females are four times more likely to be affected than males. Sacrococcygeal and cervical teratomas can be diagnosed by prenatal ultrasound and magnetic resonance imaging (MRI). Teratomas are considered an interesting field for research.

Key words: Teratoma; Fetus; Malignant; Sacrococcygeal.

Introduction

Teratomas are tumors that consist of multiple organ and tissue components resembling the derivatives of all three germinal layers of the embryonic disk. In rare cases, not all the three germinal layers can be determined [1]. The components of a teratoma may be quite different from near tissues and may be dissimilar [2].

The most common germ cell tumor diagnosed in neonates and fetuses [3] belongs to a class of tumors known as non-seminomatous germ cell tumors (NSGCT). All these tumors originate from the abnormal development of embryonal and germ cells. The type of pluripotent cell defines the location of the teratoma in the body [4].

Teratomas are observed in many sites at birth, more frequently in the sacrococcygeal and presacral regions and in the neck and are most commonly diagnosed in the fetus [3]. It is also reported that such tumors can be found in the pericardium and oropharynx. They can cause uterine enlargement, polyhydramnios, hydrops fetalis, and tumor mass [5].

There are IV types of sacrococcygeal tumors: a) type I: predominantly external, b) type II: tumors have significant external and intrapelvic components, c) type III: small external component with the majority of the lesions extending intrapelvically and intra-abdominally, d) type IV: tumor occupy the presacral space and have no external component.

Case Report

The authors present a case of a female fetus 23w that was born in the Department of Obstetrics and Gynecology of the University General Hospital of Alexandroupolis and received by the Laboratory of Histology-Embryology of Medical School of Democritus University of Thrace for further examinations. Its weight was 308 g and the measurements of the basic embryonic growth parameters fell within the normal range for the respective age of gestation.

Ultrasound examination showed a large hypervascular exophytic mass with cystic and solid components in the sacrococcygeal region. Gross examination showed a nut-brown solid tumor with cystic areas of maximum diameter of ten cm (Figure 1). Microscopically, the tumor had the histological characteristics of an immature malignant sacrococcygeal teratoma (SCT) and it was constituted of a heterogeneous collection of elements of the three germ cell layers, not arranged in organoid fashion. This variant included islands of poorly-formed cartilage, loose mesenchyme (muscle bundles), clusters of squamous epithelium, neuroblasts (neural tissue, brain substance), structures reminiscent of thyroid gland, bronchial epithelium, and bits of intestinal wall, all embedded within a fibrous gray-white solid matrix (Figures 2 and 3). All the elements were undifferentiated.

Furthermore, the molecular detection for human parvovirus B19-possible factor for hydrops fetalis by polymerase chain reaction (PCR) was negative (Figure 4). The method was performed using the PCR kit Parvovirus B19 according to the manufacturer’s instructions.

Interestingly, this stillborn derived from a twin pregnancy of which the other embryo survived.

Discussion

In the present article, the authors report a case of an immature malignant SCT. In general, a mature teratoma is benign and is usually found in females, while an immature teratoma is typically malignant and is commonly found in males. Although, it is reported that in some cases, tumor immaturity does not appear to be a measurement for malignancy in congenital teratomas [5].

Factors usually associated with benign lesions include: early (neonatal) presentation, female patient, cystic composition, and the presence of large areas of calcification or ossification. Factors associated with malignant lesions in-
clude: clinical presentation beyond infancy, male patient, presacral location, solid composition (particularly with areas of hemorrhage or necrosis), and lack of calcification.

A clinicopathologic study of 22 fetal and neonatal tumors by Heerema-McKenney et al., reports that in the majority, congenital immature teratomas that require in utero intervention present small foci of conventional yolk sac tumors and are more commonly immature at variance to those that are dissected postnatally. Furthermore, they may present hepatic differentiation and immature endodermal glands, as it is known that teratomas are composed of recognizable tissues of ectodermal, mesodermal, and endodermal origin, in any combination [5].

Germ cell line teratomas are usually present in adult men and women, fetuses, and children. The embryonal ones are most often found in fetuses, in babies at birth, and in young children. Teratomas may also be found in non-humans as reported by Catone et al. [6].

SCT is diagnosed in one out of 40,000 fetuses. According to the current world population birthrate, this means 1,800 per year. Adding to these, the number of teratomas diagnosed in other locations of the body and SCT that are diagnosed later in life increase the incidence rate to 10,000 cases per year [7].

An immature teratoma that is benign has a much higher risk to become malignant and requires closer follow-up [8]. These cases may be difficult to correctly diagnose. As sacrococcygeal and cervical teratomas spread from the fetal body into the surrounding amniotic fluid, they can be detected by prenatal ultrasound examination. Additional diagnostic methods may include prenatal magnetic resonance imaging (MRI) of the uterus which is more informative for those teratomas within the fetal body that are less clearly seen with ultrasound [9]. Tumors, however, affecting fetuses and newborns differ from those found in older children and adults, which means that the therapeutic and diagnostic challenges are different, including surgical resection and chemotherapy.
Generally, teratomas comprise the most common fetal tumors and cause high morbidity because they can also develop hydrops fetalis or premature delivery [5]. In the case of the present reported embryo, the PCR amplification for human parvovirus B19, which is a common agent causing hydrops fetalis [10], was negative.

In association with other reported cases, the authors agree that teratomas should be specifically assessed, aiming for a prenatal prognosis, diagnosis, and treatment that increases the chance for survival of the infected embryos.

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Laparoscopic surgery for ectopic pregnancy within a cesarean scar

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Summary

Background: Ectopic pregnancy in a previous cesarean section scar is rarely reported, and is very difficult to manage. There are only 12 cases to date that have been successfully managed under laparoscopy. Cases: Two females were admitted to this Hospital and were suspected to suffer from ectopic pregnancy from a previous cesarean section scar under the impression of Doppler ultrasound. Conservative regimen was first applied to one patient but failed to demonstrate enough efficacy. The beta-human chorionic gonadotropin (β-HCG) of the other patient was extremely high, therefore conservative treatment was not considered. Then laparoscopic surgery was performed for the two patients and not only confirmed the diagnosis, but also successfully removed the ectopic gestational products. Surgery under laparoscopy was uneventful. Total surgical time was 80 minutes, blood loss was only about 100 ml, and blood transfusion was not necessary. Conclusion: It seems that for a skilled surgeon, laparoscopy may be a sound and reasonable technique to diagnose and resect ectopic pregnancy within a cesarean scar.

Key words: Laparoscopic surgery; Ectopic pregnancy; Cesarean scar.

Introduction

A pregnancy implanted within the uterine scar of a previous cesarean scar was first reported in 1978 by Larsen and Solomon in South Africa [1], and since then, more and more cases are reported [2-5]. Patients suffering from cesarean scar pregnancy are at high risk of vaginal bleeding, uterine rupture, and hysterectomy [6]. Although conservative therapy methods such as systemic and local methotrexate, potassium chloride, and hyperosmolar glucose have been successful, only surgical resection has access to remove the ectopic pregnancy and simultaneously repair the defect considering that a uterine dehiscence will accompany cesarean scar implantation, potentially affecting future pregnancy [6]. So in this report the authors piloted laparoscopic resection technique of ectopic pregnancy within a cesarean scar.

Case one

A 28-year-old female, gravida 3, para 1, abortus 2, was admitted to this Hospital on May 10, 2011 with amenorrhea for 43 days requiring termination of pregnancy. She had not experienced any vaginal bleeding, abdominal pain, or other uncomfortable complaints. Her last menstrual period (LMP) was March 28, 2011. Urine pregnancy test was positive after 37 days of amenorrhea. She underwent an uneventful term transverse lower segment cesarean operation five years prior. Endovaginal Doppler sonographic scan suggested ectopic pregnancy within a cesarean scar (Figure 1) and serum beta-human chorionic gonadotropin (β-HCG) was 18,341 mIU/ml on the admission day.

Conservative therapy was first applied to this patient with single dose of 1.8 mg trichosanthin intramuscular injection. Serum β-HCG decreased to 6,218 mIU/ml six days after trichosanthin intramuscular injection but rebounded to 26,359 mIU/ml three days later (Figure 2). The treatment regimen was then converted to methotrexate (MTX) intramuscular injection. Sixty-seven mg of methotrexate was intramuscularly injected four times every other day. During MTX injection, serum β-HCG improved to as high as 46,755 mIU/ml (two days after MTX injection), but declined to 46,450 mIU/ml (five days after MTX injection) and 31,368 mIU/ml (nine days after MTX injection) at the end of treatment (Figure 2). Alanine transaminase (ALT) was slightly elevated to 52 IU/l and aspartate transaminase (AST) was normal during methotrexate injection. The patient refused to undergo conservative therapy or expectant management and demanded surgery treatment.

Given that total laparoscopic hysterectomy has been a routine procedure in this Hospital and 12 such cases have been treated under laparoscopy in Taiwan, Hong Kong, and Singapore, therefore laparoscopic management was arranged for this patient on her 66th day of pregnancy. Laparoscopic surgery was performed under general endotracheal anesthesia and the patient was placed in the 15 degree Trendelenburg position on the operating table. Before commencing surgery, an indwelling Foley catheter was inserted to keep the bladder empty and monitor urine output continuously during and after the procedure. A Veress needle was inserted through a small incision just inferior to the umbilicus. Pneumoperitoneum was created by insufflating carbon dioxide at a maximal pressure of 13 mm Hg. After the Veress needle was removed, an operative five-mm trocar was inserted into the abdomen. A laparoscope with an attached camera was inserted through the cannula to visualize the intra-abdominal organs. Two additional five-mm (right side) and ten-mm (left side) trocars were inserted at the level of the anterior superior iliac spine, lateral to the epigastric blood vessels. The uterus was retroverted and as large as pregnancy 50 days in size, and the surface of cesarean scar showed violet blue and a bulging mass measuring three cm in diameter protruding from serosa of the cesarean scar (Figure 3). The cesarean scar adhered to the bladder and the right pelvic wall. Bilateral adnexae were normal. There was no free fluid in the pouch of Douglas. The adhesions were carefully and completely removed. The previous cesarean section scar was resected and the gestational sac was shown. The amniotic fluid and...
Figure 1. — Endovaginal sonography of the gestational sac in the anterior part of the uterine isthmus, just located in the previous cesarean section scar. CDFI shows rich blood flow signals surrounding the gestational sac and the sac edge is only two to three mm from the uterine serosa.

Figure 2. — Serum $\beta$-hCG was 18,341 mIU/ml on the admission day; single dose of 1.8 mg trichosanthin im on May 11; MTX im qod four times from May 21 to 27; surgery on June 2.

Figure 3. — A: The uterus is as large as pregnancy at 50 days and bilateral adnexae are normal. B) A bulging mass measuring three cm in diameter protruding from serosa of the cesarean scar and the cesarean scar adhering to bladder and the right pelvic wall can be seen. C) The amniotic fluid and chorionic villi are seen in the sac and the gestational tissue was removed using spoon-shaped forceps. D) One layer of continuous endoscopic sutures along the affected uterine wall was made with 1-0 Ethicon.

Figure 4. — Pathological examination revealing the chorionic villi and decidual tissue of the submittal specimen and also recognized villous interstitial edema and slightly trophoblastic hyperplasia.

Figure 5. — Transvaginal ultrasound suggesting villus-like echoes in the anterior part of the uterine isthmus, just located in the previous cesarean section scar with an area of about 37 × 29 × 40 mm. CDFI showing rich blood flow signals surrounding the gestational sac and the sac edge only two mm from the uterine serosa.
chorionic villi were seen in the sac and the gestational tissue was removed using spoon-shaped forceps. About $3 \times 2 \times 2$ cm of bright red tissue in total was submitted for rapid and routine pathological diagnosis. Homeostasis was achieved using bipolar forceps at 30W. One layer of continuous laparoscopic sutures along the affected uterine wall was made with 1-0 Ethicon (Figure 3). The total operative time was 80 min, blood loss was only about 100 ml, and blood transfusion was not necessary. The rapid pathological examination during laparoscopic surgery and the routine pathological examination after the surgery both confirmed the chorionic villi and decidual tissue of the submitted specimen and also recognized villous interstitial edema and slightly trophoblastic hyperplasia (Figure 4). Serum $\beta$-HCG drastically reduced to as low as 541.4 mIU/ml on the fifth day after laparoscopic surgery and serum progesterone was 0.488 ng/ml at the same day. Although recovery was uneventful, taking into account that the first laparoscopic management of an ectopic pregnancy within a cesarean scar that was performed in mainland China, the patient was discharged on the seventh postoperative day for the sake of prudence.

Case two

A 31-year-old female, gravida 2, para 1, abortus 1, was admitted to this Hospital on January 10, 2012 with amenorrhea for 51 days requiring termination of pregnancy. She had not experienced any vaginal bleeding, abdominal pain, or other uncomfortable complaints. Her LMP was November 20, 2011. Urine pregnancy test showed positive after 40 days of amenorrhea. She underwent an uneventful term transverse lower segment cesarean operation eight years prior. Endovaginal Doppler sonographic scan suggested ectopic pregnancy within a cesarean scar.
tinidazole was intravenous infused for only three days and the
was 134 mg/l. The origin of fever was unknown. Etimicin and

trophil granulocytes was 81.5%, and C-reactive protein (CRP)

section scar was resected and the gestational sac was shown. The
were removed carefully and completely. The previous cesarean

There was no free fluid in the pouch of Douglas. The adhesions
adhered to the right pelvic wall. Bilateral adnexae were normal.

in size, and the surface of the cesarean scar showed violet blue
and a bulging mass measuring five cm in diameter protruding
from serosa of the cesarean scar (Figure 6). The cesarean scar
adhered to the right pelvic wall. Bilateral adnexae were normal.

was as high as 100,363 mIU/ml on the admission day.

Considering that the extremely high level of β-HCG and had
successfully managed the first ectopic pregnancy within cesarean
scar with laparoscopic surgery, the authors directly performed la-
paroscopic surgery for this patient on January 12, 2012 before
conservative regimen was applied. The surgical procedure was
similar as in the first patient.

The uterus was antevverted and as large as pregnancy 50 days
in size, and the surface of the cesarean scar showed violet blue
and a bulging mass measuring five cm in diameter protruding
from serosa of the cesarean scar (Figure 6). The cesarean scar
adhered to the right pelvic wall. Bilateral adnexae were normal.

There was no free fluid in the pouch of Douglas. The adhesions
were removed carefully and completely. The previous cesarean
section scar was resected and the gestational sac was shown. The
amniotic fluid, chorionic villi, and embryo were seen in the sac
and the gestational tissue was removed using spoon-shaped for-
ceps. About 4 × 3 × 2 cm of bright red tissue in total was sub-
mitted for routine pathological diagnosis. A rapid pathological
examination was not prescribed for this patient. Homeostasis was
achieved using bipolar forceps at 30W. One layer of continuous
laparoscopic sutures along the affected uterine wall was made
with 1-0 Ethicon (Figure 6). The total operative time was 80 min,
blood loss was only about 200 ml, and blood transfusion was not
necessary. The routine postoperative pathological examination
confirmed the embryo, chorionic villi, and decidual tissue of the
submittal specimen and also recognized villous interstitial edema.

(Figure 7), and serum β-HCG was as high as 100,363 mIU/ml on
the admission day.

Figure 7. — Pathological examination revealing the chorionic
villi and decidual tissue of the submittal specimen and also rec-
ognized villous interstitial edema.

Considering that the extremely high level of β-HCG and had
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in size, and the surface of the cesarean scar showed violet blue
and a bulging mass measuring five cm in diameter protruding
from serosa of the cesarean scar (Figure 6). The cesarean scar
adhered to the right pelvic wall. Bilateral adnexae were normal.

There was no free fluid in the pouch of Douglas. The adhesions
were removed carefully and completely. The previous cesarean
section scar was resected and the gestational sac was shown. The
amniotic fluid, chorionic villi, and embryo were seen in the sac
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blood loss was only about 200 ml, and blood transfusion was not
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confirmed the embryo, chorionic villi, and decidual tissue of the
submittal specimen and also recognized villous interstitial edema.

(Figure 7), and serum β-HCG was as high as 100,363 mIU/ml on
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Considering that the extremely high level of β-HCG and had
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Discussion
Ectopic pregnancy within a cesarean scar is a very rare
pregnant phenomenon, although cesarean section delivery
is a very common procedure worldwide. Precisely because
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ple research is infeasible and there are only some case re-
ports available. There are only 12 cases to date that have
been successfully managed under laparoscopy, among these
cases, ten were performed in Taiwan [7-9], one in Hong
Kong [10], and one in Singapore [3]. The natural history
of such a condition is unknown. Therefore no universal

Figure 8. — Serum β-HCG was 1,003,631 mIU/ml on the
admission day; laparoscopic surgery was performed on January
12, 2012; 75 mg MTX im and 150 mg mifepristone per os on
January 17. 50 mg MTX im on February 3.

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Laparoscopic surgery for ectopic pregnancy within cesarean scar

It is possible for the patient to undergo serious maternal morbidity and hysterectomy and thus lose subsequent fertility. Therefore early diagnosis establishment for a patient suffering ectopic pregnancy within a cesarean section scar is imperative and urgent. Doppler sonography is reliable to establish early diagnosis before surgery intervention and thus enable treatment options, can avoid uterine rupture and hemorrhage, and thereby preserve the uterus. In both cases, Doppler ultrasound revealed an empty uterine cavity, an empty cervical canal, and development of the gestational sac in the anterior part of the uterine isthmus, just located in the previous cesarean section scar. Ultrasound also suggested the volume of the gestational sac, 30 × 23 × 16 mm and 37 × 36 × 26 mm, respectively. Color Doppler flow imaging (CDFI) showed rich blood flow signals surrounding the gestational sac and the sac edge was only two to three mm from the uterine serosa. Healthy myometrium between the bladder and the gestational sac was absent under sonographic scan (Figures 1 and 5), hence early diagnosis was established with ultrasound in both cases and was confirmed later by laparoscopy.

Laparoscopic surgery not only confirms diagnosis, but also simultaneously removes the ectopic gestational products. As far as the authors’ knowledge is concerned, they prefer laparoscopic surgery to laparotomy for a patient with a high index of cesarean scar pregnancy suspicion and early diagnosis with transvaginal sonography. If a life-threatening situation occurs, laparoscopy is not available, or the surgeon is not very skilled at minimally invasive surgery (MIS), laparotomy is considered to be better. Overall the authors recommend surgical intervention other than nonsurgical treatment to manage ectopic pregnancy within a cesarean scar, especially for patients with very high serum β-HCG, considering that a uterine dehiscence will accompany cesarean scar implantation, potentially affecting future pregnancy, and only surgical management is able to remove the ectopic pregnancy and repair the defect simultaneously. If the serum β-HCG delays to normal after surgery, MTX intramuscular injection is recommended.

References


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Heterotopic pregnancy diagnosed before the onset of severe symptoms: case report

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Summary
Background: A heterotopic pregnancy (HP) is an extremely rare disease that represents the simultaneous occurrence of two or more implantation sites in the uterus and extraterus. Early diagnosis of HP is difficult because of the presence of an intrauterine pregnancy (IUP). In most cases, a precise diagnosis was made after symptoms develop through the rupture or bleeding of the ectopic pregnancy (EP). The authors present a case that was successfully diagnosed as an undemonstrative HP. Case: A 24-year-old multiparous woman became pregnant after taking clomiphene citrate. At ten weeks of pregnancy, an ultrasonography revealed gestational sacs containing fetuses in the uterus and the right adnexal region, respectively. The patient was diagnosed as having a HP and an emergency right tubal resection was performed. The IUP progressed normally and the fetus was delivered at 37 weeks of pregnancy. Discussion: Even if a gestational sac can be confirmed in the uterus, a careful ultrasonographic examination should always be considered to determine the presence of a concurrent extraterine pregnancy.

Key words: Heterotopic pregnancy; Ectopic pregnancy; Ultrasonography.

Introduction
Heterotopic pregnancy (HP) is an uncommon clinical entity and a diagnosis of HP remains challenging. A rupture of ectopic pregnancy (EP) causes catastrophic consequence due to massive bleeding. In the majority of cases, the diagnoses can be made only after the onset of lower abdominal pain and/or hypovolemic shock that develops suddenly. The authors report a case of asymptomatic HP which was successfully diagnosed.

Materials and Methods
A 24-year-old woman, 3 gravida, 1 para, 2 spontaneous abortion, became pregnant. The reminder of the past history and family history were not contributory. The patient had undergone the induction of ovulation with clomiphene citrate for her ovulatory disturbance. At her antecedent clinic, a normal pregnancy had been diagnosed. At ten weeks and two days after her last menstrual period, a transvaginal ultrasonography (TVUS) revealed a viable intrauterine pregnancy (IUP) and the presence of right tubal EP simultaneously. The patient was transferred to this hospital for further examination of HP. On admission, blood pressure was 104/70 mmHg, and heart rate was 72 beats/min. Body temperature was 37.4°C. The patient did not complain of any lower abdominal pain or abnormal genital bleeding. Laboratory profile showed that red cell counts were 453×10^4/μl, hemoglobin 12.3 μg/dl, and hematocrit 37.3%. Chlamydia IgA antibody was negative, whereas the IgG antibody was positive. Pelvic examination showed that the uterus was enlarged up to the size of a goose egg. The patient complained of no tenderness in the uterus and bilateral adnexal regions. TVUS revealed a 35 mm-sized, viable fetus corresponding to ten weeks in the normal uterine cavity, as well as one more 30 mm-sized fetus without fetal heart movement in the right adnexal region (Figure 1). There was no ascites in the cul-de-sac. Magnetic resonance imaging (MRI) of the pelvis clearly displayed a gestational sac with a high intensity in the intrauterine cavity and a cystic sac-like structure in the right adnexal region on T2-weighted image (Figure 2).

On the basis of these imaging technologies, she was diagnosed as having a HP. The patient was informed of the possibility of a life-threatening complication accompanied by the rupture of EP. She underwent an emergency laparotomy under spinal anesthesia. Laparotomy revealed the bulging at the right ampulla of the fallopian tube, confirming a right tubal pregnancy. Resection of the right tube was performed (Figure 3). No severe adhesion was detected in the abdomen, and intraoperative blood loss was minimal. Postoperatively, 50 mg progesterone was injected intramuscularly every day as a luteal support in order to maintain the intrauterine normal pregnancy. Her postoperative course was uneventful with no sign of threatened abortion and was discharged from the hospital eight days postoperatively.

Figure 1. — Simultaneous extraurine tubal pregnancy (arrowhead) and intrauterine normal pregnancy (arrow) seen on abdominal ultrasonography at ten weeks of pregnancy.
Thereafter, she was transferred back to her antecedent clinic. She was admitted again for five weeks from 29 to 34 weeks of gestation in this clinic under the risk of a preterm delivery and received continuous intravenous administration of ritodrine hydrochloride. At the 37th week and four days of pregnancy, she went into labor spontaneously and delivered a female baby with a birth weight of 2,748 g. An Apgar score was 8 at one minute and 9 at five minutes. The patient and her baby had uneventful postpartum courses.

**Discussion**

HP is an extremely rare event with its occurrence being one per 30,000 pregnancies [1]. Both assisted reproductive technologies including in vitro fertilization and embryo transfer (IVF-ET) which insert several fertilized ova into the uterine cavity, and the induction of ovulation are reported to increase the incidence of HP up to one percent [2].

The risk factors causing HP are similar with those causing single EP and are as follows: tubal factors such as unilateral tubal occlusion, a past history of ectopic pregnancy, hydrosalpinx, and the pelvic inflammatory factor such as previous intrapelvic operation including myomectomy, intrauterine device (IUD), and a past history of therapy for infertility [3]. In this case, the patient showed a positive chlamydia IgG antibody, suggesting that the past history of pelvic inflammatory disease could be one of the reasons to cause HP, whereas the use of ovulation-inducing agents seemed also to be the reason to cause this disease.

It remains a clinical challenge to diagnose unexpressed HP at an asymptomatic status. Tal et al. reported that almost 70% of HP were detected between five and eight weeks of pregnancy and that 20% were detected between nine and ten weeks of pregnancy [2]. The remaining ten percent were detected after 11 weeks of pregnancy [2].

The main symptoms of the HP include abdominal pain, signs of peritoneal irritation, adnexal tumors, and uterine swelling. Among them, the most frequent symptom to detect and diagnose this disease is abdominal pain, which occurs in 72% of patients, followed by 51% of abnormal genital bleeding, 44% of bleeding in the peritoneal cavity, and development of hypovolemic shock after the rupture of HP [4]. In most cases, HP can be diagnosed only after the patients become symptomatic, while this patient was fortunately diagnosed at an asymptomatic status.

The EP portion in the case of HP is different from that of the single EP, which often lacks abnormal genital bleeding. It is suggested that because an intrauterine normal pregnancy occupies the intrauterine cavity, bleeding accompanied by the rupture of tubal pregnancy does not flow into the vagina, but into the intra-abdominal space via the fallopian tubes or through the point of bleeding. Furthermore, the EP generates genital bleeding due to unstable secretion of human chorionic gonadotropin (hCG). In contrast, this bleeding happens less frequently in HP, since the normal IUP can maintain the secretion of hCG [5].

TVUS is useful to diagnose this disease. The definite diagnosis of HP can be made by the simultaneous detection of EP and normal IUP. However, such cases are very rare. Unlike single EP, the presence of normal IUP may make it
more difficult to detect another EP portion in the HP. The authors employed MRI as a complementary tool for the diagnosis of the HP in addition to TVUS. MRI was found to be useful for the evaluation of the location of an extrauterine gestational sac (GS), as was TVUS.

Van Dam et al. reported that successful rates to diagnose HP correctly with the detections of GS or embryo preoperatively was 14% and that 62% of the cases did not show any abnormal findings [5]. Barrenetxea et al. demonstrated that almost 74% of HPs were diagnosed after laparoscopic surgery or open laparotomy, while only 26% could be diagnosed correctly preoperatively [3]. On the other hand, ovarian swelling due to ovarian hyperstimulation syndrome (OHSS) may make it difficult to detect the EP portion around the fallopian tubes, and OHSS also makes it difficult to distinguish between pooling ascites and bleeding in the peritoneal cavity, which leads to a misdiagnosis of this disease. However, even in such a case, culdocentesis may be useful to distinguish between ascites and bleeding in the pelvis, and MRI can distinguish between tubal pregnancy and ovarian swelling in the case of OHSS. Tamai et al. reported that MRI provided a better delineation when ultrasonographic findings in EP were indeterminate and that the key MRI features of EP typically appeared as a cystic sac-like structure, frequently associated with surrounding acute hematoma showing a distinct low intensity on T2-weighted image [6].

Surgery is the mainstay for HP. Especially in the case of tubal pregnancy, tubal resectionectomy is the most common therapy for this disease. On the other hand, in the case of single EP, injection of methotrexate (MTX) into the muscle could be one of the choices for effective therapy. However, in the case of the HP, the MTX injection could not be the choice, considering that MTX has a toxic effect on the intrauterine fetus. Transvaginal local injections of potassium chloride (KCl) or hyperosmolar glucose have been reported for the less invasive and conservative treatment of HP. These therapies are adapted for patients who demonstrate severe abdominal symptoms or instability of vital signs, but do not show intra-abdominal bleeding. However, regarding KCI injection, its safety with respect to the fetus in uterus has not yet been proved. Goldstein et al. reviewed and analyzed eleven cases who underwent transvaginal KCl injection therapy, and reported that six in eleven cases (55%) followed hydrosalpinx or acute abdomen, and salpingectomies were finally performed [7]. On the other hand, Jefferey reported two cases that had achieved a successful result after transvaginal local injection of high-dose glucose [8]. More accumulation of cases is expected to warrant the safety of KCl therapy.

The reports regarding the prognosis of the intrauterine fetus are rare. Rizk et al. reported that only one case among the ten cases of the HP caused abortion after therapy [9], while Rojansky et al. reported that 70% of the patients could succeed with living babies [4]. According to the report of Clayton et al., IUP in the case of the HP had about 2.1 times higher risk of abortion compared with normal single pregnancy, however they could not find any significant difference in the following pregnancy risk between the HP patients who passed the risk of abortion and normal pregnancy [10].

In conclusion, early diagnosis and early therapy for HP may offer a better prognosis for HP patients who overcome the abortion risk at the early stage of pregnancy. The authors experienced the patient who was successfully diagnosed as having an undemonstrative HP. Although rare, an awareness of HP is important to prevent a delayed diagnosis and determine a prompt and adequate treatment option at the asymptomatic stage, particularly when the patient has conceived with artificial reproductive technologies. A careful ultrasonographic survey for the presence of concurrent EP is important, even if normal pregnancy is confirmed in the uterus.

References

Second-trimester miscarriage and umbilical cord knot. Case report and review of the literature

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Summary

Purpose of investigation: The present study presents a case with an umbilical cord knot along with extensive literature review. Materials and Methods: Presentation of a rare case of second-trimester abortion which was attributed to a tight umbilical cord knot. Furthermore the authors reviewed the literature from 1952 to 2012 in order to compare impact of knots on intrapartum and perinatal outcomes. Results: Four large retrospective studies assessed several predisposing factors. Long umbilical cords, male embryos, and multiparity were correlated with knots in three of these studies. Data regarding perinatal effects of true knots from three studies were summarized and compared. Conclusion: Umbilical cord true knots during the second trimester could be a very rare cause of abortion. The presence of knots during the third trimester and labor do not seem to be associated with increased perinatal and intrapartum morbidity and mortality, although there is still some controversy in the literature on this topic.

Key words: Knot; Umbilical cord; Cord knot; Pregnancy outcome; Miscarriage; Abortion.

Introduction

To present a second-trimester miscarriage where a true knot was the more probable cause of fetal demise and to review the literature on umbilical cord knots. Objective of the present review was to gather and compare the data on important aspects of knotted pregnancies, such as predisposing factors, ultrasound diagnosis, and effects of knots on pregnancy outcome.

A review of the literature took place on Medline Pubmed, and Embase from 1952 to April 2011. The words included in the search were: “umbilical cord knots” and “true knots”. From 1952 to date the search yielded 64 articles. The authors included in this review only large retrospective studies, interesting case reports, and general articles. Hence, data from nine retrospective studies, nine case reports, and four general articles were studied. An attempt was made to compare the results of retrospective studies, the aspects of the studies were assessed with similar parameters.

Case Report

A 34-year-old woman, gravida 2 para 1, had a second-trimester miscarriage at 16+1 weeks of gestation. Her first pregnancy, at the age of 30, was terminated at 32 weeks of gestation (preterm birth history). She had been diagnosed with an intrauterine growth restriction (IUGR) embryo and the pregnancy was terminated at the 32nd week, when she delivered by cesarean section a female baby weighing 1,450 g. Due to this incident, she had been diagnosed with an inherited thrombophilia. Her remaining medical and family histories were clear. She was a non-smoker with a 22 body mass index (BMI) score.

At the 16th week of gestation she was submitted to a routine ultrasound scan where no fetal heart function was detected and an arrest of fetal development was documented. Thus a second-trimester miscarriage was diagnosed.

The following day she was introduced to the Hospital and received 400 mg of vaginal misoprostole and a vacuum aspiration completed the emptying of the uterus several hours later. At the time of abortion a tight knot was noted in the umbilical cord (Figure 1), while the fetus and the placenta were morphologically normal.

Her postoperative recovery was uneventful and she was discharged home the next day. Both the dead embryo and the placenta were histopathologically examined. No anatomical malformations were found in the organs of the dead female fetus. The placenta weighted 65 g with no anomalies found.

The only pathological finding was a true knot of the umbilical cord at 7.6 cm distance from the cord’s placental outgrowth. The knot of the umbilical cord was found with venous distention at the knot’s location (placental side) and a mural thrombosis in the umbilical vein.

Discussion

True umbilical cord knots occur in approximately one percent of singleton pregnancies. While single umbilical artery is the more frequent abnormality of the umbilical cord, true knots represent approximately four percent of cord complications [1]. In a recent retrospective study of gross cord abnormalities, a much higher incidence (20.6%) of knots was found among single cord abnormalities [2].

A study of spontaneous abortion specimens by Javert and Barton found 35% (104 out of 297) of the analyzed cords to be abnormal. The percentage of knotted cords among these abnormal cords was 0.9% (one out of 104). The afore-
mentioned study showed the rare presence of umbilical cord knots associated to fetal demise that occurred until the 20th week of gestation [3]. A knot is formed when the embryo passes through a loop of the umbilical cord. This is apparently easier for a small embryo or when the cord is long enough. Conditions that ease fetal movements (e.g. long cords, more amniotic fluid) or provide a larger uterus (multiparity), could be contributing factors to knot formation. Long umbilical cords and polyhydramnios had been correlated with true knots [2, 4-7].

Several factors are considered to predispose to knot formation. Table 1 summarizes the results of four retrospective studies [1, 4, 5, 8] which assessed the correlation between various factors and knot presence in a large series of births. In three out of four studies male sex appears as a predisposing factor in knot formation. The fourth did not assess this parameter. By combining the fact that three out of four studies discuss long umbilical cords as a predisposing factor and the well-documented fact in the literature that male fetuses have longer cords [7, 9], an explanation could be postulated for this gender differentiation among knotted fetuses. Male fetuses due to their longer umbilical cords, which allows greater mobility, may form knots more easily than female fetuses. Also, three out of four studies [4, 5, 9] agree on multiparity as a predisposing factor. The larger uterus in this case, which enables fetal mobility, may explain this finding.

An interesting conclusion is that none of these four large studies found any correlation among small fetuses and knots. Although theoretically speaking small fetuses could pass easier through cord loops, giving rise to knots, this assumption was not confirmed by either of the studies. Furthermore, it appears that the presence of knots is not associated with uteroplacental insufficiency. Previous miscarriages and maternal age were considered predisposing factors in two studies [5, 9]. Only one study (5) correlated polyhydramnios with umbilical knots. Herskovitz et al. were the first who assessed amniocentesis and chronic hypertension and found a statistical significant correlation. The same researchers did not find any correlation between mild or severe pregnancy-induced hypertension and umbilical cord knots. In the present case, there was none of the above predisposing factors.

Monochorionic monoamniotic twins, although frequently referred to as high-risk population for knots, were either excluded or not assessed in these studies.

Several opinions have been expressed on the gestational age at which knot formation takes place. Blickstein et al. [4] proposed the period between nine and 28 weeks as the more probable for knot formation.

The prenatal diagnosis of knots through ultrasound examination is rare and difficult. A specific sonographic finding has been reported by Ramón y Cajal and Martinez [10], and called it the “hanging noose sign”. In this sign, by using power Doppler imaging, a transverse section of the umbilical cord is surrounded by a loop of umbilical cord. The use of 3D/4D imaging may confirm these sonographic signs, as has been mentioned by several reports [11-13].

Another specific sonographic feature is the “four-leaf clover”. Two parts of the umbilical cord crossing each other may give rise to the sonographic appearance of this sign, which however lacks specificity and cannot contribute to the differential diagnosis between true and false knots [14, 15].

Variation of the umbilical vein can be differentiated from a knot by ultrasound, due to the lack of cord’s tortuosity at the level of the knot [16].

Three large retrospective studies [4, 8, 17] assessed the risks for fetal demise due to umbilical cord knots, by comparing knotted and unknotted pregnancies. Fetal death rates and other perinatal parameters from these studies are summarized in Table 2. Fetuses with true umbilical cords were found to be at a four- to ten-fold increased risk of stillbirth [8, 17].

As for the mode of delivery, cesarean section rates were found to be either similar [17] or unexpectedly lower [8] in the knotted pregnancies. Findings concern-
Obstet/ 1.22% 3.7% Unknotted: 4.8% suspicious


2000/ Acta Obstet 216 6 24 2,304 8 1,310 ?Gynecol Scand/ 1% 2.7% 11.1% 10.6% 3.71% 6.9%

Sornes et al./

2002/ Am J 288 4 34 3,881 34 1,377 FHR among knotted/

Blickstein et al./ Knotted: 12,9% pathologic

Sorner et al. [17] found equal Apgar scores, while Airas et al. [9] recorded lower Apgar Scores at one min in the knotted group.

In a study that compared umbilical arterial and venous blood gas values (without FHR tracings recorded), the authors reported similar results between the knotted and the control population [18]. They concluded that umbilical cord knots lack clinical significance since no alterations were found on umbilical artery acidemia (lower pH values on the umbilical artery of the knotted pregnancies, but with no statistical significance). Therefore, perhaps the “degree” of tightness of the knot may play a key role, and together with the cord diameter, can represent the parameters which will increase the risk of fetal demise in a knotted pregnancy, which otherwise (without extreme knot-tightening and a decreased cord diameter) has no increased risk for negative outcomes [18]. Small umbilical cord diameter in knotted cords has been associated with an increased venous perfusion pressure in a study by Chasnoff and Fletcher that included fifty umbilical cords [19].

In an interesting paper by De Felice et al. [20], all infants with knots lacked inferior labial frenulum. Congenital oral mucosal changes (increased complexity and destructed randomness of oral vascular networks) were also recorded in these infants with an umbilical cord knot history. It has been speculated by the authors that a possible lower degree of extracellular matrix impairment may explain these findings [20].

As for any correlation between IUGR embryos and true knots, only a few case reports found a correlation between knots and IUGR fetuses [21, 22]. In none of the large retrospective studies that were reviewed was there any association among true knots and IUGR fetuses.

Table 2. — Correlation of umbilical cord knot with perinatal and intrapartum outcome.

<table>
<thead>
<tr>
<th>Study</th>
<th>Knotted pregnancies/ percentage</th>
<th>Fetal death in knotted pregnancies/ percentage</th>
<th>Cesarian section in knotted pregnancies/ percentage</th>
<th>Cesarian section in unknotted pregnancies</th>
<th>Vaginal operative deliveries in knotted pregnancies</th>
<th>Vaginal operative deliveries in unknotted pregnancies</th>
<th>Fetal heart rates pathologic/suspicious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airas et al. 2002/ Am J Obstet Gynecol 23,215 deliveries</td>
<td>288</td>
<td>1.25%</td>
<td>14%</td>
<td>24</td>
<td>3,881</td>
<td>14%</td>
<td>Similar rates of pathologic FHR among knotted/ unknotted (16.7% vs 15.9%)</td>
</tr>
<tr>
<td>Sornes et al. 2000/ Acta Obstet Gynecol Scand 22,012 deliveries</td>
<td>216</td>
<td>1%</td>
<td>11%</td>
<td>34</td>
<td>2,304</td>
<td>8</td>
<td>? (similar rates of pathologic or suspicious)</td>
</tr>
<tr>
<td>Blickstein et al. 1987/Int J Gynaecol Obstet 4,650 deliveries</td>
<td>57</td>
<td>1.22%</td>
<td>2%</td>
<td>9.4%</td>
<td>?</td>
<td>?</td>
<td>Knotted: 12.9% pathologic or suspicious</td>
</tr>
<tr>
<td>Unknotted: 12.9% pathologic (unknotted)</td>
<td>3%</td>
<td>3.7%</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Unknotted: 4.8% suspicious</td>
</tr>
</tbody>
</table>

*: not assessed in the study. FHR: fetal heart rate.

Conclusion

In conclusion, umbilical cord true knots during the second trimester could be a very rare cause of abortion. During the third trimester, knots seem to be a rare event and main predisposing factors for their formation are considered male sex and long umbilical cords. In addition, the presence of umbilical cords during the third trimester and labor do not seem to be associated with increased perinatal and intrapartum morbidity and mortality, although there is still some controversy on this topic in the literature. The use of ultrasonography could be helpful in specific cases.

References


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Urethral sex in a woman with previously undiagnosed Mayer-Rokitansky-Küster-Hauser syndrome

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Summary

**Purpose:** To report a case of urethral sex in a woman with previously undiagnosed Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. **Materials and Methods:** A 32-year-old woman presented with severe pain, dysuria, and mild hematuria after each intercourse. Secondary sexual characteristics were normal. Vaginal and rectal examinations revealed an absent vagina and uterus. Further investigations showed a normal hormonal profile, a 46 XX karyotype, and a normal intravenous pyelography. Pelvic ultrasonography and magnetic resonance imaging (MRI) confirmed the absence of a uterus and the presence of bilateral ovaries. **Results:** A diagnosis of MRKH syndrome was made and the patient underwent a modified Vecchietti operation for the creation of a new vagina. The urethral meatus was noticeably dilated. Postoperatively, the vaginal length was six to seven cm. Long-term follow-up revealed that she was able to have normal and satisfactory vaginal intercourse without any problems. **Conclusion:** Urethral intercourse is documented here for the first time in a case of misdiagnosed MRKH syndrome.

Key words: Urethra; Sex; Mayer-Rokitansky-Küster-Hauser syndrome.

Introduction

A review of the published literature shows that urethral sex has never before been reported. A unique case of urethral intercourse in a woman diagnosed with Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is presented.

Materials and Methods

This was an apparently healthy 32-year-old woman who sought medical advice for severe pain, dysuria, and mild hematuria after each intercourse. She had no history of urinary incontinence. At time of presentation, she had been married for two years to a 65-year-old man. Intercourse was infrequent and occurring twice every month. She was seen by other physicians more than ten years prior for primary amenorrhea and was reassured. She had never had any menses. On examination, her weight was 111 kg and height was 168 cm. Secondary sexual characteristics were normal. Vaginal and rectal examinations revealed an absent vagina (Figure 1) and uterus. Investigations showed a normal hormonal profile, a 46 XX karyotype, and normal intravenous pyelography. Pelvic ultrasonography and magnetic resonance imaging (MRI) confirmed the absence of the uterus and the presence of bilateral ovaries. A diagnosis of MRKH syndrome was made and she underwent a modified laparoscopic Vecchietti operation for the creation of a new vagina as previously reported [1]. The urethral meatus was noticeably dilated (Figure 2).

Results

After five postoperative days, the vaginal length was six to seven cm. Vaginal dilators were used after discharge from the hospital. She was able to have vaginal intercourse three weeks after the surgery. Long-term follow-up revealed a normal and satisfactory sexual relationship.

Discussion

MRKH syndrome is the second most frequent cause of one in 1,500 to one in 4,000 female births [2]. It is associated with normal ovarian function, normal female karyotype, and presence of secondary sexual characteristics. The syndrome is due to congenital aplasia of the Müllerian ducts. Congenital anomalies of the upper urinary tract may occur in 30%-40% of cases. Structural anomalies of the vagina and urogenital system are often challenging to diagnose and treat [3]. This is illustrated in the present case, where the diagnosis of MRKH syndrome was missed when the patient first complained of primary amenorrhea. Women with MRKH syndrome may also present with concerns of dyspareunia or the inability to have intercourse. However, in this cultural context, sexual issues are rarely revealed and discussed. In addition, sexual ignorance, as in the current case, is another contributing factor for the missed diagnosis. With respect to the treatment of MRKH syndrome, many non-surgical and surgical approaches exist. The aim is to create a neovagina of adequate size to allow normal sexual intercourse. Gradual dilatation with the use of dilators by the patient was described by Frank in 1938 [4]. Surgical corrections include a list of different techniques described in the literature [5]. The McIndoe procedure uses skin graft to cover a mold inserted into a surgically-created space between the bladder and rectum [6]. Recent evidence suggests that the modified laparoscopic Vecchietti operation is simple, safe, and effective [7]. In conclusion, urethral sex is documented for the first time in a case of previously undiagnosed MRKH syndrome.

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Antepartum embolization in management of labor induction in placenta previa

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Summary
The authors present a case of a 29-year-old woman, gravid 2 para 1, who experienced complete placenta previa and underwent vaginal delivery, after performing antepartum uterine artery embolization and rivanol amniotic injection due to contraindication of obstetric surgery. In this case, treatment was successful despite thromboembolism. Hypercoagulability in pregnancy needs to be addressed.

Key words: Uterine artery embolization; Placenta previa; Induction; Medical abortion; Antepartum embolization.

Introduction
Placenta previa poses a high-risk for massive hemorrhage, from the antenatal period until after cesarean section. This condition increases the risk of maternal and neonatal mortality and morbidity [1]. Therefore, vaginal delivery in the third trimester is not recommended for pregnant women with complete placenta previa. This places physicians with a dilemma when patients with placenta previa request termination of pregnancy due to complications in the third trimester. The authors present a case of complete placenta previa who had vaginal delivery with the antepartum uterine artery embolization (UAE).

Case Report
A 29-year-old woman (gravid 2, para 0) presented to this department at 29 weeks of gestation seeking termination of pregnancy. She had a complete placenta previa (Figure 1) and severe malformation of fetus (single atrium and ventricle). On admission, her laboratory tests were normal.

The patient underwent UAE to prevent hemorrhage during the process of labor and delivery. The procedure was performed with a 5Fr Cobra-catheter via a femoral artery. Embolization of the right and left uterine arteries was preferentially carried out with pledgets of absorbable gelatine sponge (Figure 2). Due to enlarged uterus, it took 3.5 hours to complete the embolization, during which bilateral femoral arterial punctures were normal.

Twenty minutes after the embolization, rivanol (ethacridine, 100 mg/4 ml) amniotic injection was given. The patient complained of mild pain on her left leg five hours after embolization, but the physical examination was normal at that time. On the first day of post-embolization, the pain worsened. During physical examination, skin temperature of left leg was low and the pulsation of the dorsal artery of foot could not be palpated. A color Doppler was performed and a thrombus was found.

After consultation with vascular surgeons, an emergency embolectomy was performed. A 15-mm thrombus was removed from the left femoral artery. During surgery two units of packed red blood cells were transfused. After the procedure, intravenous heparin sodium (100 mg/250 ml saline) was infused over 24 hours.

During this anticoagulant therapy, the prothrombin time (PT) was in normal range, D-dimer was positive, activated partial prothrombin time (APTT), and thrombin time (TT) were extended to more than 120 sec.

On first postoperative day of thrombectomy, the patient was febrile with a rise in temperature to 38.9°C. She was given cefazolin sodium (one gm Bid) to control the fever.

Uterine contractions began after 29 hours of rivanol amniotic injection. The cervix was not dilated even after eight hours of contractions. Hence, intravenous oxytocin infusion (0.5%) was initiated. When the patient was in labor, inj heparin sodium infusion was stopped. There was sudden vaginal bleeding of about 150 ml when the cervix was dilated to one cm. On vaginal examination, spongy tissue was felt. No special treatment was given except for keen monitoring. No further hemorrhage occurred. After full dilation of cervix, the fetus was expelled within 15 minutes.

Following spontaneous vaginal delivery, the placenta failed to expel within 30 minutes. Manual removal of placenta was performed. During the procedure, most of the placenta was removed and some parts were adherent to the uterine wall which lead to vaginal bleeding (around 600 ml). A balloon tamponade was inflated in the lower uterine segment to control the bleeding. Blood coagulation profile returned to normal 12 hours after the delivery.

Figure 1. — Ultrasoundography depicting placenta previa. BL: bladder; CX: cervix; Pt: placenta.
After two days of the delivery, ultrasonography (USG) depicted a mixed echo mass about 56 x 32 x 18 mm in size in the lower segment of the uterus, and its borderline was clear without abnormal vascular activity. The retained placenta was removed with uterine curettage and about 150 ml blood was lost. On fourth day, the patient was in stable condition with normal lab tests and ultrasonography. Menstruation resumed two months after the surgery.

**Discussion**

Despite the rarity to terminate pregnancy in the third trimester, there are circumstances where such practice is indicated for those with complications such as maternal medical indications, stillbirths, and fetal defects. Medical termination of mid-trimester pregnancy with placenta previa has also been reported [2-4]. In an optimal setting, patients at high-risk for hemorrhage are referred to tertiary care centres where multidisciplinary teams are prepared to care for and deal with known potential complications [5]. This poses a prominent challenge in developing countries, where a sophisticated peripartum and maternal care system is not in place. Arterial embolization is a safe and effective treatment for persistent post-partum hemorrhage that is unresponsive to conservative management [6].

The arterial embolization in this case was performed in anticipation of antepartum and postpartum bleeding [2, 3]. The authors managed this case of 29 weeks of gestation with complete placenta previa by antepartum embolization followed by vaginal delivery. Uterine artery embolization blocks the main blood supply to the placenta, which prevents bleeding by preventing rupture of vessels during uterine contraction. From this experience, the authors conclude that cesarean section can be avoided by antepartum embolization in women.

**Figure 2.** — Contrast images of the right femoral arterial punctures before (A) and after right uterine artery embolization (B). Contrast images of the left femoral arterial punctures before (C) and after left uterine artery embolization (D).
with placenta previa in third trimester. Medline research (English language; 1966-2012; search terms: “uterine artery embolization”, “placenta previa”, “induction”, and “antepartum embolization”), showed that this is the first report conducting antepartum embolization to manage labor induction for patient with placenta previa in the third trimester.

Patients in late pregnancy with hypercoagulability are prone to thromboembolic events. Thromboembolic occurrence has been reported in embolization therapy [7, 8]. Femoral artery thrombus occurred in this case, although the exact cause is unclear. However, this may be resulted from the pre-existing hypercoagulability together with the vascular damage incurred by repeated puncture in UAE. With increased pelvic vessel pressure caused by the enlarged uterus, catheterization may be a challenge and multiple attempts may be required. Therefore, it is crucial to be cautious about thromboembolism and take immediate intervention to prevent the occurrence of such complications.

Although data are scanty, antepartum UAE may serve as another option for those patients with complete placenta previa who require to terminate pregnancy with medical complications in late second and even third trimesters of pregnancy.

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References

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Simultaneous dermoid cyst and endometriosis in the same ovary: a case report

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Summary
The authors present a case of a 33-year-old infertile woman with coincidental dermoid cyst and ovarian endometriosis in the same ovary. She was admitted to the Clinic because of cystic tumor of the left adnexa. Transvaginal ultrasound (TVUS) examination found a bilocular tumor of complex structure on the left ovary. Video-laparoscopy was also performed. On the left ovary, two adjacent cystic formations were found. Laparoscopic ovarian cystectomy was performed and a surgical specimen was sent for histopathologic analysis. The diagnosis was a dermoid cyst and ovarian endometriosis. Without complications, the patient was released from the hospital. The patient was treated with an analogue of gonadotropin releasing hormone (GnRH) for three months as a preparation for in vitro fertilization (IVF).

Key words: Dermoid cyst; Endometriosis; Laparoscopy; Infertility; IVF.

Introduction
Ovarian dermoid cysts or mature cystic teratomas are considered to arise from the primordial germ cells of the yolk sac on their way to the primitive gonads [1, 2]. More than 80% of mature cystic teratomas with complete differentiation occur during the reproductive years [3, 4]. Dermoid cysts occur approximately in 43% to 70% of benign ovarian masses, and are present in 15% of all ovarian tumors. In both instances, mature cystic teratomas occur in only 10% of cases [5]. During the reproductive years, dermoid cysts are frequent causes of ovarian torsion. Typical ultrasound examination of the dermoid cysts shows the presence of focal or diffuse hyperechoic echoes. Such hyperechoic echoes are a consequence of the presence of hair, teeth, and body fat [6]. The origin of dermoid cysts in the ovary are explained with several theories such as: abnormal blastomere morphology with low egg viability, vanishing and conjoined twins, and consequent of the parthenogenesis [7]. A collision tumor represents the coexistence of two histologically distinct tumors in the same organ. The coexistence of different tumors in the same ovary is a rare finding. The most common collision tumors in the ovary are composed of teratoma and cystadenoma [8]. Endometriosis is a female health disorder that occurs when the endometrial tissue grows outside the uterine cavity and myometrium in other areas of the body [9]. The endometrial tissue implants are called endometriomas. Women with endometriosis typically have endometriomas on the ovaries and on the peritoneal area of the cul-de-sac. Endometriomas may rarely occur in other organs such as in skin scars in the lungs, and elsewhere. Endometriosis leads to painful menstrual periods and pain during sexual intercourse before and during menstrual bleeding. This process often leads to irregular bleeding and to infertility in these women. Usually, with these symptoms the diagnosis of endometriosis is suspected. Transvaginal ultrasound (TVUS) examination has the ability to make a nearly correct diagnosis. On ultrasound (US), endometriomas are homogeneous and well-delimited structures, with low to moderate density contents, and with hyperechoic capsules [10]. In the presence of an avascular calcified solid or mixed components, endometriomas with differential diagnosis include: dermoid cyst, hemorrhagic follicular cyst, corpus luteum, carcinoma, and other cystic tumors of the ovary [11]. The estimated prevalence of endometriosis is 10% to 20% in reproductive-aged women. In infertile women, the prevalence of endometriosis is 20% to 50%. Endometriosis in the ovary is the most common, followed by broad ligament, peritoneum of the cul-de-sac, and on uterosacral ligaments [12].

Case Report
A 33-year-old patient, nulliparous, was admitted to the clinic because of cystic tumor of the left adnexa. The cyst was detected on pelvic US examination by a gynecologist at the Health Center. The patient complained of occasional pain in the left adnexa. She had very painful periods and her menstrual cycles were regular. She had menarche at the age of 14 years. She did not have regular gynecological check-ups. The last check-up examination was performed three years prior. Laboratory blood tests performed were normal. The values of CA-125 tumor marker were elevated at 48 mIU/ml. The values of Ca was 19.9, and both HE 4 and Roma index were normal. A vaginal gynecological examination revealed a uterus normal in size and with solid consistency. In the area of the left adnexa examined, the cystic formation was insensitive to palpation. The right adnexa was free and insensitive. TVUS revealed in the left adnexal region, the existence of a bilocular tumor of complex structure on the left ovary. Video-laparoscopy was also performed. On the left ovary, two adjacent cystic formations were found. Laparoscopic ovarian cystectomy was performed and a surgical specimen was sent for histopathologic analysis. The diagnosis was a dermoid cyst and ovarian endometriosis. Without complications, the patient was released from the hospital. The patient was treated with an analogue of gonadotropin releasing hormone (GnRH) for three months as a preparation for in vitro fertilization (IVF).
and US, it was concluded that the patient most likely had a dermoid cyst of the left ovary. The patient was submitted to video-laparoscopy. Two cystic adjacent formations were found in the left ovary with adhesions of the peritoneum pouch of Douglas. The fluid from the pouch of Douglas was aspirated and peritoneal washings were sent for cytological analysis. LCS scissors i.e. using Ultracision Harmonic Scalpel combined with hydro-dissection performed the ovarian cystectomy and released the left ovary. Both cystic formations of the left ovary were removed intact and as a whole without opening the capsule. The specimen was placed within an Endobag and removed from the pelvis. The ovarian defects were closed with 2-0 polydioxanon sutures using intracorporeal knot tying. The contralateral ovary was the control. Intraoperatively the authors routinely wash out the abdominal cavity with saline solution. Removed cystic formations were sent to the histopathological analysis. Without complications, the patient was released from hospital. The final histopathological finding were: mature cystic teratoma of the ovary and endometriosis of the ovary. These are shown in Figures 1 and 2, respectively. Cytological findings of the fluid from the pouch of Douglas and peritoneal lavage were normal. The patient was treated with gonadotropin releasing hormone (GnRH) analogue, triptorelin 3.75 mg for three months as a preparation for in vitro fertilization (IVF). A month after the last injection, the patient re-established her menstrual cycle which was not painful. Control pelvic findings were also normal.

Discussion

Up to 25% of ovarian tumors originate from germ cells. Dermoid cysts are the most frequently germinoma during the reproductive years and are present in 15% of all ovarian tumors, usually occurring in one ovary, and most of them are mature cystic teratomas [4, 5]. The clinical characteristics of a dermoid cyst is that it is a most frequently isolated adnexal tumor. The US image of cystic teratoma appears as well-circumscribed area with fields of focal or diffuse hyper-echogenicity associated with posterior acoustic shadow with or without a cystic component [1, 13]. The presence of ovarian dermoid may cause complications such as rupture, infection, and malignant degeneration. Secondary malignant transformation of ovarian teratomas is a rare event, but is described to occur with an incidence of 1% to 2%. Squamous-cell carcinoma accounts for 80% of secondary malignant transformation of ovarian teratomas [14]. Because of possible listed complications, teratomas should be surgically removed. Dermoid early coexists with other types of ovarian tumors [5].

Incidence of endometriosis is from 10% to 20% for the general female population. The diagnosis of endometriosis is based on visual US inspection presents with circular homogeneous and clearly delimited structures from the parenchyma with hypoechoic “tissue” of low-level echo-density contents without papillary proliferation, with values of tumor markers, especially CA 125 and CA 19-9, and based on visual observations during diagnostic laparoscopy [1, 15]. Diagnostic laparoscopy is the gold standard for evaluating an adnexal mass. Histopathological examination confirmed the diagnosis of endometriosis [1]. The presence of endometrioma in the pelvic cavity is closely associated with local pelvic inflammatory process, resulting in the development of numerous adhesions to the surrounding organs. Incidence of endometriosis is from 20% to 50% in infertile women [1, 12]. The treatment of adnexal endometriosis depends on the desire to give birth, patient’s age, US examination findings, and on combination of levels of serum CA-125 with positive clinical findings [16, 17]. Operative laparoscopy, with its advantages of less postoperative adhesion formation, lower postoperative pain, rare complications, and acceptable appearance of scars on the skin, compared with open laparotomy represents an important surgical technique in the treatment of infertility [18]. Due to the accumulation of calcium in the endometrioma, differential diagnosis with US is required as it can resemble a cystic teratoma [1]. The coexistence of endometriosis and dermoid cysts in the same ovary is a rare occurrence [12]. In published literature, regarding collision endometrioma and teratoma in the ovary, the authors found until now only a few previous reports and all these described this entity in young women who underwent laparoscopy [19, 20]. The patient described in this study was a 33-year-old infertile woman, who was admitted to the Clinic due to bilocular cystic tumor of the left adnexa. This entity is the first such case published.
in Serbia. TVUS examination revealed in the left adnexal region the existence of the bilocular complex, 8.5 x 7.5 x 8.0 cm in diameter. Given this US, the desire of the patient to give birth, tumor size, and studies which indicated a significantly higher risk of a neoplasm existing in ovarian cysts greater than five cm, the authors opted to perform video-laparoscopy. Ultracision harmonic scalpel combined with hydro-dissection performed the ovarian cystectomy and released the left ovary. The ovarian defects were closed with 2.0 polydioxanone sutures using intracorporeal knot tying. The contralateral ovary was the control. The removed cystic formations were sent for histopathological analysis. Histopathology examination showed an ovarian benign tumor composed of endometriosis and coexisting mature teratoma tissue. As a number of studies have shown that dermoid cysts can be successfully removed laparoscopically, the authors decided to remove it using US harmonic scalpel technique [13]. Regarding classical laparoscopic cystectomy for dermoid cysts, it was reported that spillage had occurred in 88% [21]. As suggested by several articles, in order to prevent spillage of dermoidal contents avoiding potential risk of chemical peritonitis, or excess adhesion formation, the authors used an Endobag [5, 17, 22]. Chemical granulomatous peritonitis is the major complication after laparoscopic dermoid cystectomy [12, 17]. In order to prevent this intraoperative complication, the authors routinely wash out the abdominal cavity with saline solution to ensure appropriate removal of all particles, and to check hemostasis after surgery. Without complications, the patient was released from hospital. In preparation for IVF the patient was advised to receive a GnRH analogue for the next three months.

Conclusion

A dermoid cyst collision with ovarian endometriosis in the same ovary as a separate entity is rarely seen. All benign collisions of dermoid cyst with ovarian endometriosis can be successfully removed through video-assisted laparoscopy. In order to prevent spillage of dermoidal contents or excess adhesion formation after surgery, the authors suggest using an Endobag and routinely wash out the abdominal cavity.

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Congenital disorder of true cyclopia with polydactyly: case report and review of the literature

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Summary

Cyclopia is a rare type of holoprosencephaly and a congenital disorder characterized by the failure of the embryonic forebrain to properly divide the orbits of the eye into two cavities (the embryonic forebrain is normally responsible for inducing the development of the orbits). As a result a birth defect in which there is only one eye is developed. This eye is centrally placed in the area normally occupied by the root of the nose. As a rule, there is a missing nose or a non-functioning nose in the form of a proboscis (a tubular appendage) located above the central eye. In this report the macroscopic, radiographic, and immunohistochemical findings of a case of true cyclopia in a female fetus are described. Cyclopia is a lethal condition that is associated with dramatic symmetric deformities of the nose, skull, orbits, and brain.

Key words: Cyclopia; Proboscis; Polydactyly; Holoprosencephaly; Sonic Hedgehog (SHH).

Introduction

The term “cyclopia” derives from Cyclops, the one-eyed giants of Greek mythology, who had a single round eye in the center of the forehead. The word “cyclops” itself comes from the Greek terms “kyklos” (circle) + “ops” (eye) [1].

Generally, cyclopia occurs in the second week of gestation [2] and can be caused by chromosomal abnormalities [3-5] (most frequently it is trisomy 13 and trisomy 21) [6, 7] as well as gene mutations [8]. The Sonic Hedgehog (SHH) molecular pathway, holds a central role, especially SHH protein, the inhibition of which stops brain’s division into two distinct hemispheres, resulting in one optic lobe and one olfactory lobe, and consequently in one eye [9, 10]. Also, certain toxins can cause cyclopia, a good example is cyclopamine or 2-deoxyjervine, a teratogenic alkaloid toxin that is found in wild plants [11].

Approximately 1.05 in 100,000 births are identified as cyclopians; including stillbirths [12]. Expecting alcoholic and drug-addicted mothers have a high rate of having a cyclopic fetus, most likely due to high amounts of toxins entering the body [13]. The sex distribution shows a female predominance [14].

Case Report

The authors present a rare case of true cyclopia in a female fetus that was born on July 2004 in the Department of Obstetrics and Gynecology of the University General Hospital of Alexandroupolis and received by the Laboratory of Histology-Embryology of Medical School of Democritus University of Thrace for further examinations.

The fetus was stillborn after 32 weeks of gestation and weighed 1,444 gr. There were orbital malformations - true cyclopia - single median eye in single median orbit (Figure 1) and associated brain malformations, namely alobar holoprosencephaly. There was a non-functioning nose in the form of proboscis measuring 15 mm in length and 10 mm in diameter with a single orifice above the central eye (Figure 2). The radiographic findings included fusion of the thalami with resultant absence of the third ventricle. The cerebrum was presented as a “pancake-like” mass of tissue located anteriorly in the skull. A single large ventricle was found posteriorly. There was an absence of the interhemispheric fissure, falx cerebri, and corpus callosum. The outer appearance of the rest of the body was normal, except for the existence of an extra finger in both the above limbs (polydactyly). In the placenta, many necrotic points were found, along with interspersed hemorrhagic areas and severe calcinosis, whereas the embryonic membranes presented acute inflammation.

Immunohistochemical study was carried out to define the expression of SHH in brain tissue of this case using the polyclonal antibody SHH according to the manufacturer’s instructions. The immunohistochemistry was applied with vectastain, ABC kit. As a positive control, the authors used normal brain tissue originating from an embryo at the same gestational age. The results show that the SHH staining was absent in the present case, in contrast to the normal one (Figure 3).

Discussion

The positioning of the eyes contributes significantly to facial proportions [7]. The migration of the eyes from their fetal lateral location toward the median plane determines to a great extent the “character” of the face, varying from a “wide-eyed” hyperteloric appearance to a narrow “foxy” hypoteloric appearance [7]. In cyclopia, the fronto-nasal prominence is missing, failing to descend to the maxillary...
level, which accounts for the converged eyes, absent or displaced nose, and hypoplasia or aplasia of the premaxilla and incisor teeth [7]. Cyclopia is the most severe malformation of holoprosencephaly with a single median orbit that may be anophthalmic, monophthalmic, or synophthalmic [7]. There is no nose, but a variable proboscis is usually present [7]. It must be due to failure in the development of the ethmoid. A remnant of the missing ethmoid may be the proboscis above the eye [13, 15]. This failure in ethmoid bones development results in extensive consequences in facial development and malformations of the entire middle and upper face. Proboscis structure is represented by a “tube-like” cartilage with a central canal which is lined with squamous epithelium, as well as respiratory and olfactory mucosa [13, 15]. It is interesting to mention that olfactory fibers pass from the proboscis into the extradural space of the ethmoidal notch forming a collection of tissue similar to the inferior layer of the normal olfactory bulb [13]. The location of proboscis indicates the failed horizontal separation of orbital and olfactory anlagen, due to rostral shift of the olfactory placodes, consequent upon separation of the terminal notochord from the oropharyngeal membrane [7, 8]. The proboscis represents the anterosuperior part of the normal nasal cavity developed in the absence of median components. As a conclusion, cyclops face constitutes a model for the study of the development of the normal face [13]. Also complete absence of the pituitary gland has been sometimes described in the cyclopic infant [7]. Radiographic findings in other cases showed hypoplasia of frontal, ethmoidal, sphenoid, maxillary, and zygomatic bones and absence of nasal, vomerine, and lacrimal bones [9]. Cyclopia may be associated with holoprosencephaly, the most common developmental defect of the forebrain with an incidence of 1:250 during embryogenesis [1]. However, the observed cases are less frequent, due to intrauterine lethality, one case in 100,000 stillbirths. Seventy-five percent are of the cyclop fetuses born alive and die within a few minutes [9]. The cyclopia proportion in female and male fetuses is 2:1 [11]. A relation to the multiple genes have been implicated in ventral forebrain induction [1]. During normal differentiation of the cephalic midline structures, there is increased expression of *pax-2* gene and inhibition of *pax-6* gene from the notochord [7]. Inappropriate expression of these genes may result in cyclopia [7]. Mutation of the *SHH* gene has also been implicated in the formation of cyclopia [4, 6]. Other genes that play a role in the formation of these defects include genes that code the hedgehog signal transduction proteins patched (Ptc) and smoothened (Smo) [10], as well proteins of the gli family that are related with polydactyly and ZIC2 and SIX3 [4, 6].
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