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Editorial Office (M. Critelli):
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CLINICAL AND EXPERIMENTAL OBSTETRICS AND GYNECOLOGY (ISSN 0390-6663) publishes original work, preferably brief reports, in the fields of Gynecology, Obstetrics, Fetal Medicine, Gynecological Endocrinology and related subjects. (Fertility and Sterility, Menopause, Uro-gynecology, Ultrasound in Obstetrics and Gynecology, Sexually Transmitted Diseases, Reproductive Biological Section). The Journal is covered by INDEX MEDICUS, MEDLINE, EMBASE/Excerpta Medica, PUD MED.

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Low hypoosmotic swelling test scores correlate better with lower percent motility than any other abnormal semen parameters

J.H. Check, D. Kramer, W. Hourani, A. Bollendorf
The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology
Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if any single abnormal semen parameter is associated with low hypoosmotic swelling (HOS) test scores. Methods: A retrospective review evaluating males with single sperm defects of sperm concentration, % progressive motility, morphology using strict criteria, and antisperm antibodies. The percentage of these males with HOS test scores < 50% was then determined. Results: By far the abnormal semen parameter most associated with a subnormal HOS test was poor motility. Conclusions: Though we believe that the simple inexpensive HOS test should be performed routinely when performing semen analysis, it is especially important to evaluate in males with poor motility since simple intrauterine insemination will not allow pregnancies.

Key words: Hypoosmotic swelling test; Motility; Antisperm antibodies; Sperm concentration.

Introduction

Despite years of investigating normal and abnormal semen parameters, standard semen parameters have failed to reliably identify the subfertile male with the exception of extremely poor sperm concentration or motility [1]. A test that is very reliable in identifying the subnormal male is a low hypoosmotic swelling (HOS) test score < 50% [2]. In contrast to most other subnormal semen parameters which inhibit fertilization of the oocyte, sperm with a subnormal HOS test allows normal fertilization of the oocytes but creates normal appearing embryos that do not implant [3-10].

A subnormal HOS test can exist as the sole subnormal semen parameter or co-exist with other abnormalities [2]. The present study evaluated single semen parameter abnormalities of sperm concentration, motility, and morphology using strict criteria or antisperm antibodies to determine if any one of these abnormalities is more associated with low HOS scores than other defects.

Materials and Methods

A 10-year retrospective review of semen analyses was performed. Males with single sperm parameter defects were identified as follows: concentration < 20 x 10^6/ml, progressive motility < 50%, morphology using strict criteria < 4%, antisperm antibodies > 50%

The frequency of associated low HOS test scores < 50% with each of these single sperm defects was then determined. Motility defects were further subdivided into four motility percentages and subnormal HOS test frequency was then determined according to these subdivisions.

Results

Table 1 shows that subnormal percent motility was the category that was most associated with a subnormal HOS test score. An abnormal HOS test score was found in 14.2% (63/443) of males with % motility < 50% vs 2.7% (23/831) of males with the combination single defects of concentration, morphology or antisperm antibodies (p < 0.0001, chi-square analysis).

In fact low HOS test scores were found in 25.8% (29/112) of males whose motility was < 40% (Table 2). However even in the 40-49.9% range of motility there were twice as many having low HOS test scores compared to all the other abnormal sperm categories combined.

Discussion

Based on the fact that intracytoplasmic sperm injection corrects the implantation disorder associated with low HOS test scores it has been hypothesized that the infertility problem is caused by the transfer of a toxic factor that impairs the functional integrity of the sperm membrane to the zona pellucida by the supernumerary sperm that attach which in turn become incorporated in the embryo membrane. These events thus lead to this toxic factor impairing the functional integrity of the embryo membrane and thus impair the embryo from implanting [10]. Support for this hypothesis was provided by showing that the avoidance of zona pellucida contact by performing ICSI markedly improves pregnancy rates [11, 12].

Based on the fact that treatment of the sperm with the protein digestive enzyme chymotrypsin can improve the HOS test score and achieve pregnancies, it has been hypothesized that the toxic factor is a protein and may be provided by the ejaculatory ducts rather than in the testes [11, 13, 14].

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Original Articles

Reproductive Biology Section

Low hypoosmotic swelling test scores correlate better with lower percent motility than any other abnormal semen parameters

J.H. Check, D. Kramer, W. Hourani, A. Bollendorf
The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology
Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

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Key words: Hypoosmotic swelling test; Motility; Antisperm antibodies; Sperm concentration.
The data from this study suggest that this hypothesized toxic protein may also impair motility but not sperm concentration. Moreover these data suggest that this toxic protein is not an antisperm antibody.

### Table 1. — Frequency of low hypoosmotic swelling tests with other single defects in semen parameters.

<table>
<thead>
<tr>
<th>Abnormal semen parameters</th>
<th>Males No.</th>
<th>Males No. with low HOS test scores</th>
<th>% of males with low HOS test scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low concentration</td>
<td>212</td>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>Low % progressive motility</td>
<td>443</td>
<td>63</td>
<td>14.22</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5%</td>
<td>487</td>
<td>12</td>
<td>4.52</td>
</tr>
<tr>
<td>&lt; 2%</td>
<td>57</td>
<td>2</td>
<td>3.51</td>
</tr>
<tr>
<td>Antisperm antibodies</td>
<td>132</td>
<td>7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

### Table 2. — Frequency of low hypoosmotic swelling test scores according to the degree of motility impairment.

<table>
<thead>
<tr>
<th>% of sperm with progressive motility</th>
<th>Males No.</th>
<th>Males No. with low HOS test scores</th>
<th>% of males with low HOS test scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19.9%</td>
<td>9</td>
<td>3</td>
<td>33.30</td>
</tr>
<tr>
<td>20.0-29.9%</td>
<td>20</td>
<td>6</td>
<td>30.00</td>
</tr>
<tr>
<td>30.0-39.9%</td>
<td>83</td>
<td>20</td>
<td>24.10</td>
</tr>
<tr>
<td>43.0-49.9%</td>
<td>331</td>
<td>34</td>
<td>10.27</td>
</tr>
</tbody>
</table>

The data from this study suggest that this hypothesized toxic protein may also impair motility but not sperm concentration. Moreover these data suggest that this toxic protein is not an antisperm antibody.

### References

Pregnancy rates following frozen embryo transfer (ET) in women failing to conceive despite fresh ET in women using low dosage follicle stimulating hormone (FSH) protocol for follicular maturation of several eggs

J. Check, E. Dix, D. Check, D. Summers-Chase, D. Horwath

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine the pregnancy rate following frozen embryo transfer using embryos derived from low dosage follicle stimulating hormone (FSH) stimulation protocols in women aged ≤ 42 who did not have diminished egg reserve as evidenced by a day 3 serum FSH ≤ 12 mIU/ml. Methods: A retrospective review was performed evaluating pregnancy rates on frozen embryo transfers from women who usually had diminished egg reserve and thus used no more than 150 IU of FSH. The pregnancy rates were calculated on the first frozen embryo transfer of women failing to successfully conceive on the fresh embryo transfer. Results: The clinical and live delivered pregnancy rates per transfer were 33.3% (14/42) and 23.8%. The implantation rate was 20.0%. Thirty-one percent of the transfers were in women aged 40-42. Conclusions: These data show that despite the fact that with minimal stimulation protocols, the remaining frozen embryos are of lesser quality because of de-selection, nevertheless, it is worth transferring these embryos.

Key words: Minimal stimulation; Frozen embryo transfer.

Introduction

There are data demonstrating good pregnancy rates in women up to age 42 undergoing in vitro fertilization (IVF) and fresh embryo transfer (ET) despite having diminished egg reserve as manifested by an elevated day 3 serum follicle stimulating hormone (FSH) [1-3].

A good live delivery rate has been reported despite the transfer of only one embryo in this group of women with a paucity of remaining follicles [2, 3].

Live deliveries following IVF-ET and fresh embryo transfer have even occurred in women in apparent premature menopause where ovulation was achieved by restoring down regulated FSH receptors in the granulosa-theca cells of the follicle using ethinyl estradiol [4, 5].

Very good live delivery rates have been found in women with normal embryo reserve using low dosage gonadotropin stimulation controlled ovarian hyperstimulation (COH) protocols [6].

Women with diminished egg reserve are not likely to have leftover frozen embryos. However, it would not be unusual to have embryos to freeze following embryo transfer of fresh embryos in women with normal egg reserve using low-dose gonadotropin stimulation.

The number of cryopreserved embryos would be likely to be less when using low-dose gonadotropin protocols vs high-dose FSH in women with normal egg reserve.

Materials and Methods

Retrospective review of frozen embryo transfer cycles in women using minimal or low dose gonadotropin stimulation protocol.

By low dosage it is meant that they did not use more than 150 IU of exogenous FSH unless a gonadotropin releasing hormone (GnRH) antagonist was used (cetrorelix or ganirelix with a 14 mm diameter follicle) when the dosage could be increased by 75 IU.

Women were aged ≤ 42. The day 3 serum FSH was ≤ 12 mIU/ml. Pregnancy rates on first frozen-thawed embryo transfer after a failed fresh transfer were determined. Thus the embryos were de-selected. If all embryos were frozen and no fresh ET occurred from that oocyte retrieval, the woman was excluded from the study.

The same woman could be used more than one time as long as the second batch of frozen embryos resulted from another de-selected fresh embryo transfer. There were no minimal criteria for embryo morphology to allow embryo transfer except a minimum of four blastomeres. No embryo was excluded because of severe fragmentation.

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Results
Forty-two frozen-thawed embryo transfers were evaluated. Twenty-eight women were aged ≤ 39, and 14 were aged 40–42 at the time of egg retrieval. The clinical pregnancy rate per transfer was 33.3% (14/42). The live delivery rate was 23.8% (10/42). The implantation rate was 20.0%. The average number of embryos transferred was 2.5.

Discussion
These 42 frozen embryo transfer cycles were taken from the 141 women with normal egg reserve having low-dose gonadotropin stimulation who did not achieve a live pregnancy. Some proceeded to another fresh transfer so they did not proceed with a frozen embryo transfer. There were 31 such patients.

Frozen embryos were available in 42 of the remaining 110 women (37.2%) despite the low dosage of gonadotropins used for stimulation. These data show that in spite of the fact that with minimal stimulation protocols the remaining embryos have lower morphology scores because of de-selection, nevertheless, it is worth transferring these embryos before proceeding to another IVF-ET cycle.

References

Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
ICSI outcome of patients with severe oligospermia vs non-obstructive azoospermia

B. Demir¹, I.I. Arikan², G. Bozdağ³, I. Esinler³, L. Karakoc Sokmensuer⁴, S. Gunalp⁴

¹Department of Gynecology and Obstetrics, Ergani State Hospital, Diyarbakir
²Departments of Gynecology and Obstetrics, Zonguldak Karaelmas University, Faculty of Medicine, Zonguldak
³Department of Obstetrics and Gynecology, Hacettepe University, Faculty of Medicine, Ankara
⁴Department of Histology and Embryology, Hacettepe University, Faculty of Medicine, Ankara (Turkey)

Summary

Objective: To compare the results of intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) cycles in men with severe oligospermia or non-obstructive azoospermia. Materials and Methods: This study included 91 ICSI cycles performed due to male factor infertility. Patients are divided into two groups according to spermatozoa. Group 1 consisted of 38 cycles in which sperm was obtained from testicles (cases with non-obstructive azoospermia). In Group 2, 53 consecutive cycles were included in which ejaculated sperm was available for ICSI in spite of severe oligospermia (<100,000/ml). Fertilization, embryo quality and clinical pregnancy rates were compared between the groups. Results: Although, the female age and mean number of oocytes retrieved were similar among the two groups, fertilization rate was significantly lower in the non-obstructive azoospermia (34.6%) group compared to group in which patients underwent ICSI with ejaculate spermatozoa (55.3%) (p < 0.05). However, there were no differences regarding mean number of available grade 1 embryos on day 3 and pregnancy rate between the two groups. Conclusion: Testicular sperm from non-obstructive azoospermia patients had significantly lower fertilization rates than the ejaculated spermatozoa from severe oligospermia patients in ICSI cycles. However, it did not bring about improved pregnancy rate.

Key words: Non-obstructive azoospermia; Intracytoplasmic sperm injection; Oligozoospermia.

Introduction

After it was first reported by Palermo et al. in 2002 intracytoplasmic sperm injection (ICSI) has become the preferred technique for fertilization in assisted reproductive technologies (ART) not only in severe male factor infertility as azoospermia or oligoasthenoteratozoospermia but also in unexplained and tubal factor infertility [1-4]. Characteristics of male patients as obstructive (OA) or nonobstructive azoospermia (NOA); semen parameters indicating number, motility and morphology and the sperm source as fresh ejaculate or surgically removed are the conflicting factors affecting treatment outcomes in ICSI cycles, and the debate has continued these topics for two decades. Some reports demonstrated improved fertilization and pregnancy rates with ejaculated or testicular sperm in obstructive azoospermia according to testicular sperm from nonobstructive azoospermia [5-14] while others showed comparable results [15-19].

In the current study, we aimed to understand whether the source of sperm differs in means of fertilization rate, embryo quality and pregnancy outcome in ICSI cycles. Therefore, ICSI cycles in men with severe oligozoospermia and non-obstructive azoospermia were compared in which sperm was obtained with either ejaculation or testicular biopsy, respectively.

Materials and Methods

We retrospectively analyzed the records of patients who underwent ICSI at the Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Fertility and Reproductive Endocrinology between July 2001 and January 2010. Before the beginning of data collection, institutional review board approval was obtained.

Patients are divided into two groups according to spermatozoa source for ICSI: 1) sperm was successfully obtained from testicles (cases with non-obstructive azoospermia and normal peripheral karyotype) (group 1; n = 38), and 2) ejaculated (severe oligozoospermia: <100,000/ml) (group 2; n = 53).

Preoperative evaluation included a complete history and physical examination. In both groups, neither preoperative hormonal treatment nor a diagnostic biopsy was planned. In group 1, testicular sperm extraction (TESE) was performed under local anesthesia by widely opening the testes in an equatorial plane, one day prior the day of oocyte retrieval. Microdissection was carried out with examination of the seminiferous tubules using an operating microscope (Carl Zeiss, OPMI Pico Surgical Microscope) at x 20 magnification. Enlarged seminiferous tubules were selected, removed and evaluated by an embryologist. Each sample was mechanically cut and dispersed in 1 ml of G-IVF (Vitrolife, Kungsbacka, Sweden) supplemented with 10% HSA (Vitrolife, Kungsbacka, Sweden) in a petri dish (Falcon Plastics, Becton-Dickinson). Each specimen was evaluated under phase-contrast microscope at x 200 magnification. If intact spermatozoa were noted, the procedure was terminated. If no sperm were seen, microdissection of additional areas of testicular parenchyma was carried out and additional samples were taken. After dissection, tunica albuginea was closed with 5-0 polypropylene. The tissue was collected in a sterile conic tube (Falcon Plastics, Becton-Dickinson). Following washing
with the gradient method (Isolate sperm separation medium, Irvine Scientific, Santa Ana, CA, USA), the prepared sample was incubated at 37°C with 5% CO₂. In the morning of scheduled oocyte retrieval day, the sample was transferred into a petri dish (Falcon Plastics, Becton-Dickinson) and covered with oil (Vitrolife, Kungsbacka, Sweden) for identification and collection of spermatozooa.

All patients underwent controlled ovarian hyperstimulation (COH) using luteal-long leuprolide acetate (LA; Lucrin; Abbott, Cedex, Istanbul, Turkey) and recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) using the step-down protocol. The starting dose of gonadotropin was determined based on the woman’s age, body mass index (BMI) and antral follicle count at baseline transvaginal ultrasonography (TVS). Ovarian response was monitored with frequent serum estradiol (E2) measurements and TVS, as described previously. The criterion for hCG (Pregnyl; Organon, Istanbul, Turkey) administration was the presence of two or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration. Semen samples of the male patients with oligozoospermia were collected by masturbation after two to seven days of sexual abstinence on the day of egg retrieval.

The most morphologically normal motile spermatozoa were selected for ICSI. Where all sperm had morphological defects, sperm with fully developed tails and grossly normal heads were injected. The presence of fertilization was evaluated by examining oocytes 12-17h after injection for the presence of distinct pronuclei and two polar bodies.

Embryos were graded on day 3 according to a 1-4 scoring system (with 1 being the best), which was based on fragmentation, cell symmetry and blastomere number [20]. The embryos with even blastomeres and no fragmentation were graded as grade 1, the embryos with even blastomeres and < 20% fragmentation as grade 2a, the embryos with uneven blastomeres and no fragmentation as grade 2b, the embryos with uneven blastomeres and < 20% fragmentation as grade 2ab. The embryos with 20-50% fragmentation and > 50% fragmentation were graded as the grade 3 and 4 embryos, respectively. Grades 1-3 were considered as transferable embryos.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at TVS. Statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL). The normal distribution of the variables was tested with Kolmogorov-Smirnov. Parametric and numeric variables were compared with Independent samples T-test. The χ² test was used to analyze nominal variables in the form of frequency tables: p values of < .05 or less were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise.

Results

Female age, body mass index (BMI), duration of infertility and basal antral follicle count were comparable among the two groups (Table 1). The COH performance of the two groups also revealed similar mean number of metaphase-II oocytes retrieved (Table 2). However, fertilization rate was significantly lower in the non-obstructive azoospermia (34.6%) group when compared to the group in which patients underwent ICSI with ejaculate spermatozoa (55.3%) (p < 0.05, Table 2).

In group 1, 51 of 53 cycles reached embryo transfer (ET); however, only 18 of 38 cycles succeeded in reaching ET. There was no difference according to ovarian response to stimulation, embryo quality and clinical pregnancy/ET between the ejaculated and non-obstructive azoospermia groups (Table 2). However, it is noteworthy to mention that pregnancy rate seems to be higher when ejaculated sperm is available, but no statistical significance was reached probably due to the small study group.

Discussion

According to results of our study, testicular sperm from non-obstructive azoospermia patients had worse fertilization rates than the ejaculated spermatozoa from severe oligospermia patients. The outcomes according to developing embryo quality and clinical pregnancy rates were comparable between the two groups.

Verza et al. have evaluated the effect of severity of sperm abnormality to treatment outcomes in ejaculated sperm in ICSI cycles and compared the ICSI results of obstructive and non-obstructive azoospermia patients [12]. Non-obstructive azoospermia patients had the worst results according to fertilization, embryo quality and clinical pregnancy rates among the all other groups. Miscarriage rates were comparable between the groups. Furthermore, they reported that fertilization rate decreased with the increasing severity of sperm abnormalities in patients of the ejaculated sperm group. We could only detect a decrease in the fertilization rate which is concor-
ICSI outcome of patients with severe oligospermia vs non-obstructive azoospermia

B. DEMIR, M.D.

Address reprint requests to:
B. DEMIR, M.D.
83, 3114. Beykoz Mah. 282. Sokak
Peyas Mah. 282. Sokak
B. DEMIR, M.D.
Diyarbakir 3114. Beykoz Mah. 282. Sokak

References


Effect of sperm morphology on clinical outcome parameters in ICSI cycles

B. Demir1, L.I. Arikan1, G. Bozdag1, I. Esiner1, L. Karakoc Sokmensuer1, S. Gunal1

1Department of Gynecology and Obstetrics, Ergani State Hospital, Diyarbakir
2Department of Gynecology and Obstetrics, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak
3Department of Obstetrics and Gynecology, Faculty of Medicine, Hacettepe University, Ankara
4Department of Histology & Embryology, Faculty of Medicine, Hacettepe University, Ankara (Turkey)

Summary
Objective: To assess the effect of isolated teratozoospermia with a normal sperm count and total motility by means of the fertilization rates, embryo quality and clinical pregnancy rate only in ICSI cycles. Materials and Methods: We retrospectively analyzed the records of patients who underwent ICSI at Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Fertility and Reproductive Endocrinology between July 2001 and January 2010. Only patients with normal sperm count and total motility were recruited. The remaining cycles were further divided into two groups according to their sperm morphology with respect to Kruger’s strict criteria. In Group 1, 537 consecutive cycles were enrolled whose sperm morphology was < 4%. In Group 2, 118 cycles were identified with a morphology of ≥ 4%. Results: A total of 655 ICSI cycles were included in the final analysis. The fertilization rates were 72.0% and 70.8% in Groups 1 and 2, respectively. There were no differences regarding embryo quality, clinical pregnancy and implantation rates between the two groups. Conclusion: Our data suggest that detection of morphology defect has no value in the prediction of fertilization, embryo quality and clinical pregnancy in ICSI cycles.

Key words: Sperm morphology; Intracytoplasmic sperm injection; Fertilization.

Introduction
Decreased pregnancy rates in spontaneous [1-5] and in vitro fertilization (IVF) [6-11] cycles due to poor fertilization because of sperm morphological abnormalities have been demonstrated by several studies in the literature after it was first claimed by Kruger et al. in 1986 [6] when they published a new classification of sperm morphology.

After the introduction of intracytoplasmic sperm injection (ICSI) by Palermo et al. in 1992, a new gate of hope was opened for such patients because the fertilization defect was attributed to be the cause of decreased fertility in men with isolated teratozoospermia [12, 13]. Despite this new technique for fertilization, conflicting results continued to be published as some demonstrated improved outcomes with increased fertilization and clinical pregnancy rates [14-20], while others reported comparable results [21-24].

Most of the published data above compared conventional IVF to ICSI as the fertilization technique. The aim of this study was to assess the effect of isolated teratozoospermia with a normal sperm count and total motility by means of fertilization rates, embryo quality and clinical pregnancy rate only in ICSI cycles.

Materials and Methods
We retrospectively analyzed the records of patients who underwent ICSI at the Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Fertility and Reproductive Endocrinology between July 2001 and January 2010. Before the beginning of data collection, institutional review board approval was obtained. Only patients with normal sperm count and total motility were recruited. The normal sperm count was accepted as ≥ 20 million/ml and a total motility of ≥ 50%. The remaining cycles were further divided into two groups according to their sperm morphology with respect to Kruger’s strict criteria [25].

In Group 1, 537 consecutive cycles were enrolled whose sperm morphology were < 4%. In Group 2, 118 cycles were identified with a morphology of ≥ 4%. All patients underwent controlled ovarian hyperstimulation (COH) using luteal-long leuprolide acetate (LA; Lucrin; Abbott, Cedex, Istanbul, Turkey) and recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) using the step-down protocol. The starting dose of gonadotropin was determined based on the woman’s age, body mass index (BMI) and antral follicle count at baseline transvaginal ultrasonography (TVS). Ovarian response was monitored with frequent serum estradiol (E2) measurements and TVS. The criterion for hCG (Pregnyl; Organon, Istanbul, Turkey) administration was the presence of two or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using a vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration. Semen samples of the male patients were collected by masturbation after two to seven days of sexual abstinence on the day of egg retrieval.

ICSI was performed for all metaphase II oocytes, as described by Van Steirteghem et al. [26]. Spermatozoa were selected for injection based on motility. The presence of fertilization was evaluated by examining oocytes 12-17 h after injection for the presence of distinct two pronuclei and two polar bodies [27]. Embryos were graded on day 3 according to a 1-4 scoring system (with 1 being the best), which was based on fragmentation, cell symmetry and blastomere number. The embryos with even blastomeres and no fragmentation were...
grated as grade 1, the embryos with even blastomers and < 20% fragmentation as grade 2a, the embryos with uneven blastomers and no fragmentation as grade 2b, the embryos with uneven blastomers and < 20% fragmentation as grade 2 ab. The embryos with 20-50% fragmentation and > 50% fragmentation were graded as the grade 3 and 4 embryos, respectively [28]. Grades 1-3 were considered as transferable embryos. All the procedures of embryo transfer were performed with soft catheter under TVS. The luteal phase was supported by daily vaginal progesterone suppositories (Crinione, Serono, Istanbul, Turkey) starting one day after oocyte pick-up.

Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at TVS.

Statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL). Normal distribution of the variables was tested with Kolmogorov-Smirnov. Parametric and numeric variables were compared with the independent samples T test. The χ² test was used to analyze nominal variables in the form of frequency tables; p values of 0.05 or less were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise.

Results

A total of 655 ICSI cycles were included in the final analysis. The baseline characteristics of patients and demographic features were comparable between the two groups (Table 1). The controlled ovarian hyperstimulation performance was also similar. The fertilization rates were 72.0% and 70.8% in Groups 1 and 2, respectively (Table 2). There were no differences regarding embryo quality, clinical pregnancy and implantation rates between the two groups (Table 2).

Discussion

Fertilization rate was similar among couples with severe teratozoospermia and normal sperm morphology according to our study (Table 2). The baseline characteristics and ovarian response of the patients were comparable between the groups. Both groups with or without severe teratozoospermia had similar results according to embryo quality and clinical pregnancy rates.

Lundin et al. demonstrated the adverse effect of sperm morphology defect on fertilization and pregnancy rates in conventional IVF cycles and the improvement effect of ICSI in these patients [14]. They offered ICSI for patients with poor sperm morphology to improve fertilization. Similar to our study, they could not show any adverse effect of sperm morphology defect severity to fertilization in the ICSI cycle itself.

Osowa et al. reported similar fertilization and pregnancy results in both conventional IVF and ICSI cycles [17]. However, the adverse effect of severity of teratozoospermia on fertilization was seen in the conventional IVF cycles even though the total fertilization rate was comparable with the ICSI cycles. The similar total fertilization rate may be due to the small percentage of patients with severe teratozoospermia in the conventional IVF group. Again similar to the report by Lundin et al. [14], in our study there was no significant difference in the fertilization rate according to severity of teratozoospermia among the patients who underwent ICSI cycles.

To evaluate the effect of isolated teratozoospermia on assisted reproductive technologies success, Keegan et al. compared conventional IVF with ICSI cycles and demonstrated that there was no effect of teratozoospermia on fertilization and pregnancy rates either in conventional IVF or ICSI cycles [23]. They concluded that isolated teratozoospermia did not adversely affect the outcomes of ART. They showed similar rates of fertilization and pregnancy in all patients who underwent conventional IVF or ICSI cycles either with severe teratozoospermia or normal sperm morphology. Furthermore to search in more detail, they analyzed 17 cycles with half of the retrieved oocytes used in conventional IVF and the rest in ICSI cycles. Again no difference in the fertilization rate was found between the groups.

In a retrospective comparison of pregnancy outcome following conventional oocyte insemination vs ICSI for isolated abnormalities in sperm morphology using strict criteria, Check et al. reported significantly lower pregnancy rates with ICSI and suggested the zona pellucida can do a better job of sperm selection [29].

Similar to our study French et al. evaluated effects of severe teratozoospermia only in the ICSI cycles and could not demonstrate any adverse effect on the outcomes even in the subgroup analysis of teratozoospermia [24].

### Table 1. — Baseline characteristics of patients.

<table>
<thead>
<tr>
<th></th>
<th>Normal sperm morphology (Group 1, n = 537, %)</th>
<th>Normal sperm morphology (Group 2, n = 118, %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (years)</td>
<td>32.3 ± 4.5</td>
<td>33.3 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Male age (years)</td>
<td>36 ± 5.3</td>
<td>36.6 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 17.6</td>
<td>24.9 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (mo)</td>
<td>92.3 ± 61.2</td>
<td>86.6 ± 60</td>
<td>NS</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>10.7 ± 5.9</td>
<td>9.3 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol level on the day of hCG administration (pg/ml)</td>
<td>2407.5 ± 1461.2</td>
<td>2341.4 ± 1671</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NS:** not significant.

All values are expressed as mean ± SD.

### Table 1. — Embryological data and pregnancy outcome according to sperm morphology.

<table>
<thead>
<tr>
<th></th>
<th>Normal sperm morphology (Group 1, n = 537, %)</th>
<th>Normal sperm morphology (Group 2, n = 118, %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocyte-cumulus complexes retrieved</td>
<td>11.8 ± 6.5</td>
<td>11.3 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of metaphase II oocytes</td>
<td>10.1 ± 5.7</td>
<td>9.4 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>72.0</td>
<td>70.8</td>
<td>NS</td>
</tr>
<tr>
<td>No. of available grade 1 embryo on Day 3</td>
<td>0.8 ± 1.3</td>
<td>1.0 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos having ≥ 7 blastomers on Day 3</td>
<td>3.8 ± 3.4</td>
<td>3.6 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of transferred embryo on Day 3</td>
<td>3.0 ± 1.1</td>
<td>3.2 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy/embryo transfer (%)</td>
<td>53.7</td>
<td>53.5</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>19.4</td>
<td>19.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NS:** not significant.

All values are expressed as mean ± SD.
It is clear from the literature that severity of teratozoospermia does not affect ART outcomes in ICSI cycles and no effect surpasses in the comparison of outcomes between conventional IVF and ICSI cycles.

Our data suggest that detection of morphology defect has no value in the prediction of fertilization, embryo quality and clinical pregnancy in the ICSI cycles.

References


Pregnancy outcome following in vitro fertilization-embryo transfer according to the percentage of metaphase II oocytes retrieved

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden
Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology
Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if the presence of a lower percentage of metaphase II eggs during oocyte retrieval leads to a lower fertilization rate of these metaphase II eggs since they may be more likely to be not quite fully mature, and to determine if transfer of embryos made from these eggs leads to lower pregnancy and implantation rates. Methods: Fertilization and pregnancy rates determined according to deciles of percent of metaphase II eggs beginning with < 30%. Results: Though there was no difference in fertilization rates when comparing those with < 60% metaphase II eggs vs ≥ 60%, there were significantly higher clinical and live delivered pregnancy rates and implantation rates when there were ≥ 60% of the eggs retrieved that were metaphase II. Conclusions: An inferior pregnancy outcome with a lower percentage of metaphase II eggs despite similar fertilization rates is consistent with the hypothesis that subtle full maturation defects may result in pregnancy failure despite embryo transfer.

Key words: Metaphase II; Pregnancy rates; In vitro fertilization embryo transfer.

Introduction

One theoretical cause of infertility is the possibility of oocyte retrieval before the final process of maturation has occurred despite the presence of what appears to be metaphase II eggs. The possibility exists that when the total percentage of metaphase II eggs retrieved is lower, then the greater the chances are that the eggs are not fully mature as compared to women with a larger percentage of metaphase II eggs.

During stimulated in vitro fertilization (IVF) cycles, careful monitoring is needed to ensure maximum numbers of mature oocytes at collection. Despite following protocols, variability in number of mature metaphase II oocytes retrieved does occur [1]. Fewer mature oocytes adversely affects pregnancy rates in two ways: 1) there may be fewer embryos formed because of less metaphase II eggs and thus less top embryos to choose from for transfer, and 2) affecting cytoplasmic maturation by not synchronizing with nuclear maturity, compromising those oocytes that have achieved the metaphase II stage [2].

The present study was conducted to determine if there exists a metaphase II egg percentage number which can detect lower pregnancy rates.

Materials and Methods

A retrospective cohort analysis was conducted on all IVF-embryo-transfer cycles where there were at least five eggs retrieved and at least two embryos transferred. Women were all aged ≤ 39. Donor egg recipients and gestational carriers were excluded.

Pregnancy and implantation rates were sorted into deciles of % metaphase II oocytes, beginning with < 30%.

Results

Pregnancy and implantation rates based on percentage of mature eggs retrieved are given in Table 1. The clinical pregnancy rate (ultrasound evidence of pregnancy at 8 weeks) for < 60% metaphase II eggs was 31.8% (77/242) as compared to 46.2% (915/1979) when there was ≥ 60% metaphase II eggs (p < 0.001).

The live/delivered pregnancy rate for < 60% was 28.5% (69/242) versus 41.6% (824/1979) for ≥ 60% (p < 0.001).

Implantation rates were also significantly lower with lower percentage of metaphase II eggs (16.5%, 119/722 vs 22.9%, 1438/6275) (p < 0.001).

Discussion

A lower percentage of mature eggs does not lead to a lower fertilization rate. A lower percentage of mature eggs does adversely affect both pregnancy and implantation rates.

The stimulation protocols were combined in the study. However, when separately examining agonist and antagonist protocols, it was found that only 9% of the agonist cycles produced less than 60% metaphase II oocytes as compared to 14% of the antagonist cycles.

These lower pregnancy and implantation rates are hypothesized to be related to a subtle defect in full maturation of apparent metaphase II eggs that are likely to be present in a higher percentage when the percentage of metaphase II eggs out of the total retrieved is < 60%.
Table 1. — Pregnancy outcome according to the percentage of the eggs retrieved that were at the metaphase II stage of meiosis.

<table>
<thead>
<tr>
<th>% Mature eggs retrieved</th>
<th>&lt; 30</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
<th>≥ 90</th>
</tr>
</thead>
<tbody>
<tr>
<td># transfers ≥ 2ET</td>
<td>19</td>
<td>27</td>
<td>52</td>
<td>144</td>
<td>259</td>
<td>343</td>
<td>576</td>
<td>801</td>
</tr>
<tr>
<td>Average age</td>
<td>34.2</td>
<td>34.0</td>
<td>33.4</td>
<td>33.5</td>
<td>33.6</td>
<td>33.4</td>
<td>33.6</td>
<td>33.6</td>
</tr>
<tr>
<td># eggs retrieved</td>
<td>358</td>
<td>426</td>
<td>1257</td>
<td>3273</td>
<td>5900</td>
<td>9280</td>
<td>12058</td>
<td>15751</td>
</tr>
<tr>
<td># M retrieved</td>
<td>53</td>
<td>146</td>
<td>556</td>
<td>1793</td>
<td>3840</td>
<td>6941</td>
<td>10199</td>
<td>15180</td>
</tr>
<tr>
<td>% fertilized</td>
<td>63.7</td>
<td>66.3</td>
<td>62.0</td>
<td>60.9</td>
<td>66.3</td>
<td>63.8</td>
<td>63.8</td>
<td>68.6</td>
</tr>
<tr>
<td># clinical preg.</td>
<td>6</td>
<td>13</td>
<td>13</td>
<td>45</td>
<td>115</td>
<td>147</td>
<td>271</td>
<td>382</td>
</tr>
<tr>
<td>% clinical preg./trans.</td>
<td>31.6</td>
<td>48.1</td>
<td>25.0</td>
<td>31.3</td>
<td>44.4</td>
<td>42.9</td>
<td>47.0</td>
<td>47.7</td>
</tr>
<tr>
<td># viable</td>
<td>5</td>
<td>11</td>
<td>13</td>
<td>40</td>
<td>101</td>
<td>140</td>
<td>238</td>
<td>345</td>
</tr>
<tr>
<td>% viable/trans.</td>
<td>26.3</td>
<td>40.7</td>
<td>25.0</td>
<td>27.8</td>
<td>39.0</td>
<td>40.8</td>
<td>41.3</td>
<td>43.1</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>11.3</td>
<td>23.3</td>
<td>12.9</td>
<td>17.1</td>
<td>22.5</td>
<td>20.1</td>
<td>23.5</td>
<td>23.8</td>
</tr>
</tbody>
</table>

ET: embryos transferred; M: mature eggs.

References


Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
General Section

Increased lectin-like oxidized LDL receptor-1 expression in the placentas of women with intrahepatic cholestasis during pregnancy

Y. Yin, Q.Y. Zhu, S.J. Ren, D.M. Wang
Department of Obstetrics, First Affiliated Hospital of Xinjiang Medical University, Urumqi (China)

Summary

**Background:** Intrahepatic cholestasis of pregnancy (ICP) is a reversible cholestatic liver disease of undefined etiology and pathogenesis. It is likely associated with dyslipidemia [1]. However, the explanation for the role of dyslipidemia is not clear. We hypothesized that an increased oxidized low-density lipoprotein (oxLDL) and lectin-like oxidized LDL receptor-1 (LOX-1) may play a crucial role in the development of the disease. Thus, the aim of this study was to investigate the plasma oxLDL level and LOX-1 expression in placentas from women with ICP.

**Methodology:** The plasma oxLDL level and LOX-1 expression were detected in 94 intrahepatic cholestasis of pregnancy patients (ICP group) and 94 healthy pregnant women (control group). The placental LOX-1 expression was detected by Western blotting; the plasma oxLDL was measured by enzyme-linked immunosorbent assay.

**Results:** The placental LOX-1 expression in the ICP group was higher than that in the control group (p < 0.05), whereas the plasma oxLDL did not differ significantly between the patients with ICP and healthy pregnant women. **Conclusions:** LOX-1 may play a crucial role in the pathophysiological processes of ICP caused by over-apoptosis of trophocytes. Moreover, LOX-1 could be a potential target for therapeutic intervention.

**Key words:** Cholestasis; Pregnancy; OxLDL, LOX-1.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disorder in pregnancy that adversely affects maternal well being and fetal outcome. The etiology of ICP is complex and not fully understood, but it is likely associated with dyslipidemia [1]. However, no explanation for the role of dyslipidemia involvement in ICP has been offered. It is our aim here to supply the missing explanation. Small dense low density lipoprotein (LDL) was more susceptible to oxidation, resulting in the generation of oxidized LDL (oxLDL) [2]. Oxidative modification of LDL alters its biological properties, resulting in stronger cytotoxicity, so that it damages vascular endothelial cells and induces cell over-apoptosis. OxLDL can bind to the lectin-like oxidized LDL receptor-1 (LOX-1) on endothelial cells. LOX-1 is a type II membrane protein cell surface receptor identified in endothelial cells, vascular smooth muscle cells, and monocyte macrophages. Recent studies have reported that oxLDL and LOX-1 were strongly related to various cell apoptoses [3]. However, no study has shown whether oxLDL and LOX-1 are associated with ICP. We hypothesized that there is an increased plasma oxLDL level and LOX-1 expression in placentas of women with ICP. We further hypothesized that these factors would contribute to over-apoptosis of trophocytes. Thus, the aim of this study was to investigate plasma oxLDL levels and LOX-1 expression in placentas from women with ICP.

Material and Methods

**Participants**

Ninety-four women with ICP and 94 age- and gestational-week-matched healthy pregnant women (controls) were recruited prospectively from the patients in our hospital from January 2009 to April 2010. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China). It was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants. The clinical diagnosis of ICP was confirmed using values as reported [4]. On detection of abnormal liver function, possible alternative causes of liver disease were sought by testing for Epstein Barr virus, cytomegalovirus, hepatitis A, hepatitis B, and hepatitis C serology and by performing liver ultrasonography. Patients with hypertension, diabetes, nephritis, immune system disease and hyperthyreosis causing dyslipidemia were excluded. The characteristics of these subjects are described in Table 1.

**Clinical assessments**

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Blood samples were obtained under fasting conditions from the median cubital vein and immediately centrifuged at 2000 g for 20 min and then stored at –80°C. Plasma lipid profiles and liver function were tested by a selective inhibition colorimetric assay using a direct double precipitation method (ABX Diagnostics, Montpellier, France). The measurement of plasma OxLDL was done by an enzyme-linked immunosorbent assay (ELISA) method [5] (OxLDL, ELISA Kit, USA).

**Placental LOX-1 expression**

Placenta biopsies were obtained within 30 min after delivery and then snap frozen in liquid nitrogen and stored at –80°C. Western blotting was for the detection of LOX-1. The cells were
lysed by a 4°C pretreated cell lysis solution, and the total protein level was determined by the Comassie blue method. The samples with 30 μg total protein each were applied for SDS-PAGE and were electro-transferred to the nitrocellular membrane (NC) membrane. In addition, 5% lipid-free milk powder was used for blocking for two hours. Anti-rat goat LOX-1 (1:200) was added, and the membrane was incubated at 4°C overnight. After washing, the anti-goat and anti-rabbit IgG antibody linked with horseradish peroxidase (HRP) were added for incubation for one hour, and the exposed film was later developed. Using β-actin as the internal control, the relative protein expression levels were determined quantitatively using an imaging analyzing system to scan the absorbance of specific bands.

**Statistical analysis**

Values are expressed as means ± SD. Comparison of two groups was conducted using the Student’s t-test. All statistical analyses were completed using SPSS software version 16.0 (SPSS, Chicago, IL, USA); statistical significance was established as *p < 0.05*.

**Results**

**Clinical characteristics**

In the ICP group, the mean maternal age was 29 ± 4 years and the median gestation week was 37.4 ± 3.7 weeks (range 32-40 weeks). Compared to the control group (28 ± 3 years, 38 ± 1.6 weeks), there were no significant differences (Table 1). Plasma lipid profiles and liver function of ICP women were significantly greater than for the control group (*p < 0.05*). The level of plasma oxLDL in the ICP group was slightly higher than the control group (Table 1), but there was no significant difference between them.

**Placenta LOX-1 expression**

LOX-1 expression was significantly increased in placentas from women with ICP (1.3898 ± 0.2961 pg/ml; *p < 0.05*) in comparison with the control group (0.9726 ± 0.2168 pg/ml) (Figure 1).

**Discussion**

Intrahepatic cholestasis in pregnancy (ICP) is a pregnancy-specific liver disorder characterized by maternal pruritus in the third trimester, raised serum bile acids, and increased rates of adverse fetal outcomes. The etiology of ICP is complex and not fully understood, but it is likely associated with dyslipidemia, which may contribute to the pathogenesis of the disease. The elevation of LDL cholesterol and reduction of high-density lipoprotein cholesterol before clinical diagnosis may prove to be a useful biomarker for the early identification of ICP [1]. LDLs are more susceptible to oxidation, and oxidative modification of LDL alters its biological properties, resulting in chemotaxis of monocytes or T lymphocytes in addition to the modulation of growth factors and cytokine production from endothelial cells, smooth muscle cells, and macrophages [6]. Stimulation of the endogenous suicide cell death pathway by oxLDL in endothelial cells may be one cause of endothelial injury. In vitro studies have also demonstrated that oxLDL has cytotoxic effects on endothelial cells [7]. One study demonstrates that oxLDL induced dysfunction of the mitochondrial membrane potential, leading to cytochrome C release into the cytosol, and thereby stimulated apoptosis of human endothelial cells [8]. We hypothesized that there is an increased plasma oxLDL level and LOX-1 expression in placentas from women with ICP. We further hypothesized that these factors would contribute to the over-apoptosis of trophocytes. To our knowledge, however, there has been no report in the literature about oxLDL and LOX-1 levels. Table 1. — Clinical characteristics and serum oxLDL in controls and ICP patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ICP (n = 94)</th>
<th>Control (n = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 4</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>37.4 ± 3.7</td>
<td>38 ± 1.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.6 ± 3.3</td>
<td>27.9 ± 3.6</td>
</tr>
<tr>
<td>Ox-LDL (ng/l)</td>
<td>24.7 ± 10.90</td>
<td>22.87 ± 10.84</td>
</tr>
<tr>
<td>*TC (mmol/l)</td>
<td>6.51 ± 0.72</td>
<td>5.05 ± 1.19</td>
</tr>
<tr>
<td>*TG (mmol/l)</td>
<td>3.11 ± 0.19</td>
<td>2.32 ± 0.28</td>
</tr>
<tr>
<td>*LDL-C (mmol/l)</td>
<td>3.69 ± 1.47</td>
<td>3.24 ± 0.85</td>
</tr>
<tr>
<td>*HDL-C (mmol/l)</td>
<td>1.53 ± 0.18</td>
<td>2.23 ± 0.12</td>
</tr>
<tr>
<td>*DBIL (μmol/l)</td>
<td>16.7 ± 9.4</td>
<td>5.8 ± 2.5</td>
</tr>
<tr>
<td>*TBIL (μmol/l)</td>
<td>26.8 ± 11.3</td>
<td>14.4 ± 4.5</td>
</tr>
<tr>
<td>*AST (U/l)</td>
<td>167.0 ± 54.0</td>
<td>38.3 ± 12.0</td>
</tr>
<tr>
<td>*ALT (U/l)</td>
<td>133.0 ± 41.0</td>
<td>31.0 ± 13.0</td>
</tr>
<tr>
<td>*γ-GT (U/l)</td>
<td>78.3 ± 22.4</td>
<td>41.6 ± 16.8</td>
</tr>
<tr>
<td>*TBA (μmol/l)</td>
<td>45.7 ± 27.8</td>
<td>12.3 ± 5.1</td>
</tr>
</tbody>
</table>

Values presented are mean ± SD.

TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density; Ox-LDL = oxidized low-density lipoprotein; DBIL = direct bilirubin; TBIL = total bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; γ-GT = γ-glutamyl transpeptidase; TBA = total bile acid.

*p < 0.05 versus controls.
Increased lectin-like oxidized LDL receptor-1 expression in the placentas of women with intrahepatic cholestasis during pregnancy

section

Increased lectin-like oxidized LDL receptor-1 expression in the placentas of women with intrahepatic cholestasis during pregnancy was higher in the ICP group than that in the control group \( (p < 0.05) \). Accordingly, it is believed that placental LOX-1 may be a potential key marker for ICP. LOX-1 promoter expression can be induced by oxLDL, fluid shear stress, Ang II, proinflammatory cytokines, lipopolysaccharide, phosphoryl 12- myristate 13-acetate (PMA), oxidants, heparin-binding epidermal growth factor (HB-EGF) and others [14]. Nevertheless, the mechanism of placental over-expression of LOX-1 in ICP is uncertain.

LOX-1 may mediate over-apoptosis in VSMCs of placentas of ICP. Apoptosis is regulated by various apoptosis-related proteins. Bcl-2 acts as an anti-death factor preventing the release of cytochrome c (cyt c) and other apoptogenic factors from the mitochondria [15]. In contrast, Bax reduces mitochondrial membrane potential and thereby causes cyt c release and caspase activation, leading to apoptosis. Some experimental evidence has suggested that LOX-1 may mediate oxLDL-induced apoptosis through an increase in the Bax to Bcl-2 ratio in vivo [16]. Our previous study indicated that the placental dysfunction of ICP may be closely related to apoptosis, abnormal over-expression of p53, Bax and low expression of Bcl-2 on placental tissue as the main reason for placental over-apoptosis [17]. Therefore, LOX-1 may play a crucial role in the pathophysiological processes of ICP caused by over-apoptosis of trophocytes. Moreover, as over-apoptosis of placental trophocytes is related to the LOX-1 pathway, LOX-1 could be a potential target for therapeutic intervention.

References


Address reprint requests to:
D.M. WANG, M.D., Ph.D.
Department of Obstetrics
First Affiliated Hospital of Xinjiang
Medical University
Urumqi (China)
e-mail: karenwdm000@126.com
Effect of pravastatin on endothelial function and endothelial progenitor cells in healthy postmenopausal women

G. Paradisi¹, M. Bracaglia¹, F. Basile¹, S. D’Ippolito¹, F. Di Nicuolo¹, F. Ianniello¹, L. Quaglizzi¹, L. Donati¹, A. Labianca¹, C. Di Cesare¹, M. Viggiano¹, A. Biaggi², C. De Waure³, F. Andreotti³, N. Di Simone¹, A. Caruso¹

¹Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart; ²San Pietro Fatebenefratelli Hospital; ³Epidemiology and Biostatistics Unit, HTA Unit, Institute of Hygiene, Catholic University of the Sacred Heart; ⁴Department of Cardiovascular Medicine, Catholic University of the Sacred Heart, Rome (Italy)

Summary

Purpose: Coronary heart disease is the leading cause of morbidity and mortality in postmenopausal women. Among statins, pravastatin has been shown to significantly reduce fatal and non-fatal cardiovascular events in primary and secondary prevention trials. The aim of the present research was to investigate whether treatment with pravastatin can modify some indices of cardiovascular risk in healthy postmenopausal women such as significant reductions in total and LDL cholesterol and triglyceride levels.

Methods: 20 patients were randomized in double-blind fashion to treatment for eight weeks with either pravastatin 40 mg/day or placebo, and subsequently, after one-week wash-out, crossed-over to the alternative treatment (placebo or pravastatin) for the following eight weeks. We performed clinical and laboratory investigations, before and at the end of each treatment period, to evaluate patient response to the treatment with pravastatin. Results: After eight weeks pravastatin therapy reduced the median low density lipoprotein (LDL) and total cholesterol (p < 0.01 in both cases). In contrast, insulin level and insulin sensitivity did not show any difference with regard to values observed after placebo treatment. The absolute number of endothelial progenitor cells-colon-forming unit (EPC-CFU) was significantly increased by pravastatin treatment (30.6% increase, p < 0.05) and the number of senescent cells was significantly decreased. However pravastatin did not increase tube-like formation by EPC and did not improve endothelial function. Conclusions: Despite beneficial effect on lipids and EPC, short term pravastatin does not seem to improve other cardiovascular risk factors, at least in healthy postmenopausal women.

Key words: Menopause; Endothelial function; Pravastatin; Endothelial progenitor cells; Cardiovascular risk factors; Insulin resistance.

Introduction

Coronary heart disease (CHD) represents one of the main causes of death in Western countries [1]. In particular CHD is the leading cause of morbidity and mortality among postmenopausal women [2]. Women have much less coronary atherosclerosis than men, especially those in younger age groups. The lower coronary atherosclerosis is likely a function of both lower premenopausal risk factors and the effects of estrogen on the arterial wall [3]. The beneficial effects of statins in treating hypercholesterolemic subjects have been well established. Among statins, pravastatin has been shown to significantly reduce fatal and non-fatal cardiovascular events in primary and secondary prevention trials [4, 5]. Accumulating evidence indicates that the cardiovascular protection of pravastatin therapy is not confined solely to its anti-lipemic effects. Other potential effects such as inhibition of inflammation and improvement of insulin sensitivity have also been suggested [6]. It is well known that long-term therapy with pravastatin might reduce levels of CRP and prevent cardiovascular risk in high-risk subjects [7]. In contrast, the effect of statins on insulin resistance is controversial and poorly studied in non diabetic subjects [8]. Pravastatin not only reduces serum lipids, but also improves the glucose metabolism, including insulin resistance, in dyslipidemic patients [9]. Pravastatin use may be an effective approach in the treatment of metabolic syndrome with impaired glucose tolerance (IGT) by its advantageous effects on insulin resistance [10]. The question of whether therapy with statins decreases cardiovascular-related mortality rates along with a better quality of life in postmenopausal women remains to be investigated [11]. According to recent data [12, 13], cardiovascular risk factors as age, sex, hypertension, diabetes, smoking, positive family history of CHD and LDL cholesterol levels, are inversely related to the number of circulating endothelial progenitor cells (EPCs). EPCs may contribute to repair areas of initial vascular damage caused by these risk factors [12, 13]. There is evidence that chronic exposure to increased plasma cholesterol levels might also oppose the repair of lipoprotein-mediated endothelial injury, possibly by reducing the availability and function of circulating endothelial progenitors [14]. The number of circulating EPC can be assessed in vitro, either as number of colony formations [13] or by functional assays [15]. Statins are known to increase circulating EPC in patients with stable coronary artery disease [16], as well as restore endothelial function [17], and prevent ischemic vascular disease [18, 19], and diabetes [20]. Statins, therefore, could be useful in the primary prevention of vascular disease in postmenopausal women [21], a population at increased cardiovascular risk. The aim of the
present study was to investigate whether treatment with pravastatin could modify some indices of cardiovascular risk in healthy postmenopausal women. To this end we evaluated, in a double-blind, placebo-controlled, randomized, cross-over study, the effect of pravastatin on endothelial function, insulin-resistance, lipid profile, inflammatory response and number and function of circulating EPCs.

Materials and Methods

Study design

Informed consent was obtained from each subject before the study. The subjects were randomized in double-blind fashion to treatment for eight weeks with either pravastatin 40 mg/day or placebo, and after one-week wash-out, crossed-over to the alternative treatment (placebo or pravastatin) for eight weeks. Clinical and laboratory investigations were performed before and at the end of each treatment period. At each time-point a 50 ml blood sample was used for the biochemical and functional evaluations outlined below. The study was conducted at the Department of Obstetrics and Gynecology at the Catholic University in Rome.

Subjects

We selected 25 patients for the study in our Divisional outpatient menopause center. Of these, 20 patients that fulfilled the following inclusion criteria: spontaneous menopause for a period of ≥ 1 year, body mass index ≤ 30 kg/m², and serum LDL cholesterol < 190 mg/dl participated to the study. None of these patients had serum LDL cholesterol > 160 mg/dl or > 2 risk factors (as defined by NCEP III guidelines) [22]; neoplastic diseases, surgical menopause, past or current hormone replacement therapy, ischemic vascular disease, diabetes mellitus, liver, renal or respiratory insufficiency, treatment with antidepressant drugs, ACE-inhibitors, AT-receptor antagonists, beta-blockers, calcium-antagonists and non-steroidal anti-inflammatory drugs.

Analytical methods

Measurement of endothelial function

Ultrasound evaluation of endothelium-dependent and endothelium-independent arterial dilatation was performed as follows [23, 24]. Brachial artery diameter was measured by B mode ultrasound image, by the use of a 7.5 MHz linear array transducer and a standard ESAOTE AU 570 A system. In all patients, scans were obtained with the subject at rest, after inflation of a pneumatic tourniquet placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mmHg for 4-5 min, and 3-4 min after the administration of sublingual nitroglycerin spray (400 µm). The brachial-artery diameter was expressed as a percentage of the average diameter of the artery in two resting control scans (considered as 100%). The velocity of arterial flow was measured with a pulsed Doppler signal. For the reactive hyperemia scan, measurements of diameter were taken 50–60 sec after deflation of the cuff. The vessel diameter in scans obtained after reactive hyperemia [flow-mediated dilatation (FMD)] and the administration of nitroglycerin (nitrate-induced dilatation NID) was expressed as a percentage of the average diameter of the artery in the two resting (or control) scans (considered 100%). Reactive hyperemia was calculated as the maximal flow recorded in the first 15 sec after cuff deflation divided by the flow during the first resting (baseline) scan.

Measurement of body composition

The bioelectrical impedance was evaluated using a tetrapolar impedance plethysmograph (Soft Tissue Analyzer, Akern Bioresearch, Florence, Italy) to estimate body composition according to Lukaski [25]. The percentage of body fat, fat free mass, and total body water were assessed by specific software (Bodygram, Akern Bioresearch, Italy). The patients, free of any conductive materials, were asked to lie on a bed. The electrodes were placed in the middle of the dorsum of the hands and feet proximal to the metacarpal-phalangeal metatarsal-phalangeal joints respectively, and also medi ally between the distal prominences of the radius and the ulna and between the medial and lateral malleolus at the ankle. An excitation current of 800 mA, AC, at 50 Khz was introduced at the distal electrodes and the voltage drop across the patient was detected by the proximal electrodes.

Biochemical and functional evaluation

- **Serum:** glucose, insulin, total HDL and LDL-cholesterol, triglycerides, creatinine, blood urea nitrogen, AST, ALT, CK, total bilirubin, C-reactive protein. **Whole blood:** full blood count [26]. Plasma glucose levels were measured by the glucose oxidase method (Beckam, USA). Total cholesterol and triglyceride concentration were determined by an enzymatic assay, high-density lipoprotein cholesterol (HDL-C) concentrations were determined after precipitation of chylomicrons, very-low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) (Boehringer, Germany). All blood tests were performed in fasting subjects.

**HOMA insulin resistance**

\[
\text{Insulin resistance (HOMA IR) =} \frac{\text{fasting insulin (µU/ml)}}{\text{fasting glucose (mmol/l)}} \times 22.5
\]

Evaluation of circulating EPCs by colony formation

EPCs were isolated from 12 ml of citrate/dextran anticoagulated peripheral blood as follows: peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation (FICOLL, Sigma-Aldrich, USA). Recovered cells were washed twice with phosphate buffered saline (PBS, Sigma) and plated (5 x 10⁶ cells/well) on dishes coated with human fibronectin (Sigma) in EndoCult medium (Stem Cell Technologies). After 48h hours, non adherent cells were harvested and replated (1x 106 cells/well) in fibronectin-coated 24-well plates in EndoCult medium. This step removes monocytes and mature endothelial cells. Growth medium was changed every three days. EPCs were characterized by the appearance of a spindle-shaped morphology and their ability to develop CFUs (colony forming units). EPC-CFU were composed of a central core of predominantly spherical cells surrounded by sprouting elongated cells. Numbers of colonies were counted seven days after plating.

Measurement of senescence

In each colony, β-galactosidase activity was evaluated by a commercial Kit (Cell Signalling) designed to histochemically detect β-galactosidase activity at pH 6, a known characteristic of senescent cells. Briefly, the cells were fixed in fixative solu-
tion for 10-15 min at room temperature and incubated with staining solution overnight at 37°C. Senescent cells were checked under an optical microscope (200 x total magnification) for development of blue color. The absolute numbers of β-galactosidase-positive cells were counted out of 100 cells.

Functional evaluation of EPC by tube assay

An in vitro angiogenesis assay was used to examine the ability of EPCs to differentiate into capillary-like tube structures.

Early EPCs (cells obtained from colonies after 1 week of culture) were labelled with fluorescent Dil-Ac-LDL (2x 10⁴) and co-plated with human umbilical vein cells (HUVEC, 4x 10⁴) in matrigel coated wells (96 well plates) with endothelial cell culture medium (EGM-2) at 37°C for 24 hours. Incorporation of fluorescence-labeled EPC into the tube-like structure formed by HUVEC was examined under a fluorescence microscope (Carl Zeiss).

Late EPCs (cells obtained from colonies cultured for 3 weeks that show a better differentiation capability) [27] were added into matrigel coated wells (96 wells plates) with endothelial cell culture medium (EGM-2, Cambrex), while HUVEC were placed in other wells as controls, and incubated at 37°C for 48-72 hours. The tube formation was observed using an inverted phase optical microscope (Olimpus IX50).

Capillary-like tube structure was defined as a structure exhibiting a length 4 times its width. Images were acquired with a digital camera (Nikon) and quantified by Photoshop software measuring the number and the total length of the tubules in each well.

Statistical analysis

Sample size computation

Sample size was computed in relation to total cholesterol and flow-mediated vasodilatation (FMD). Power was set at 80% and alpha at 0.05. The difference in means was assumed approximately 60 mg/dl and standard deviation (SD) was set at 35 mg/dl for cholesterol level while it was considered respectively 5 and 3% for FMD [24]. In both cases minimal sample size was five pairs.

Descriptive statistics were used to evaluate median and interquartile range (IQR: 75th percentile - 25th percentile) of each parameter. Univariate analysis was carried out applying a non parametric test for paired samples (Wilcoxon signed rank test) because of the limited number of patients in each group. The purpose of univariate analysis was to find out differences between the eight weeks of pravastatin treatment and the eight weeks of placebo. The significance level was set at $p \leq 0.05$ and statistical analysis was performed using SPSS version 12.00 for Windows.

Results

Baseline characteristics

As expected by study design, all patients had normal characteristics without any risk factors (Table 1).
PBMCs developed an elongated spindle-shaped morphology and produced colonies (EPC-Colony Forming Unit, (EPC-Colony Forming Unit, Figure 2)). The absolute number of EPC-Colony Forming Unit (EPC-CFU) was significantly affected by treatment with pravastatin (40 mg/day) as compared to baseline or placebo group patients (30.6% increase, p < 0.05, Figure 1B).

The number of senescent cells was significantly decreased in EPCs obtained from patients treated with pravastatin with respect to baseline or placebo groups (Figure 2).

To test the endothelial function of EPCs an in vitro angiogenesis assay was carried out. Early EPCs co-cultured with differentiated endothelial cells (HUVEC) were incorporated into tube-like structures but alone failed to form tubules in matrigel three-dimensional cultures (data not shown). EPCs cultured for 21 days (late EPCs) led to successful capillary formation on matrigel. Nevertheless, there were no significant differences in the number or length of tube-like structures among cells obtained from pravastatin treated patients and the placebo group (Figure 3 A,B).

**Discussion**

Eight weeks of pravastatin treatment in healthy postmenopausal women was able to reduce serum lipids and increase the count of EPC colonies but did not improve the endothelial function, or insulin sensitivity.

The beneficial effect of pravastatin in preventing cardiovascular disease in peri- and postmenopausal hyperlipidemic women is described in the literature [5]. There

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### Table 1. — Patient characteristics (n = 20).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median IQR Median IQR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.50 52 - 62 10</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.00 156 - 168 12</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.50 61 - 76 15</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.65 24 - 30 6</td>
<td></td>
</tr>
<tr>
<td>Years from menopause</td>
<td>6.00 3 - 11 8</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>130.00 110 - 140 30</td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>80.00 70 - 90 20</td>
<td></td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>77.00 69 - 88 9</td>
<td></td>
</tr>
<tr>
<td>Insulinemia (mU/l)</td>
<td>6.35 5 - 9 4</td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>1.22 1 - 2 1</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>66.50 54 - 76 12</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>137.00 119 - 163 44</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>213.50 202 - 252 53</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>104.00 67 - 120 53</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.90 1 - 1 0</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST) (U/l)</td>
<td>21.00 17 - 24 7</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT) (U/l)</td>
<td>21.50 18 - 29 9</td>
<td></td>
</tr>
<tr>
<td>Creatinine kinase (CK) (U/l)</td>
<td>85.00 62 - 121 39</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.70 1 - 1 0</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>56.25 41.25 70</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>137.00 119 - 163 44</td>
<td></td>
</tr>
<tr>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>56.25 41.25 70</td>
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</tbody>
</table>

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### Table 2. — Pravastatin (after 8 weeks) versus placebo (after 8 weeks). Median, interquartile range (IQR), p-value.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Placebo Median IQR</th>
<th>Pravastatin Median IQR</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Systolic pressure (mmHg)</td>
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<td></td>
<td></td>
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<tr>
<td>Diastolic pressure (mmHg)</td>
<td>80 14 78 15 0.42</td>
<td></td>
<td></td>
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<tr>
<td>Glycemia (mg/dl)</td>
<td>77.00 24.50 81.00 14.25 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulinemia (mU/l)</td>
<td>6.35 3.37 7.21 5.28 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>1.28 0.90 1.51 0.78 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>66.00 17.85 67.00 19.25 0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>137.00 51.35 103.50 55.25 &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>215.00 64.85 189.00 56.25 &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>101.00 71.75 94.00 49.25 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.85 0.18 0.80 0.10 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>21.50 6.00 20.50 6.75 0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>18.00 15.50 21.00 4.75 0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (U/l)</td>
<td>83.00 60.25 84.00 43.75 0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.60 0.35 0.60 0.20 0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR (mg/dl)</td>
<td>3.00 1.23 3.00 0.93 0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB (mg/dl)</td>
<td>13.75 13.50 15.53 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT (N/ml)</td>
<td>239.50 59.50 231.50 47.50 0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (N/ml)</td>
<td>5.63 1.50 5.95 2.15 0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FM (%)</td>
<td>33.00 9.60 36.10 13.90 0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diameter (mm)</td>
<td>3.45 0.77 3.45 0.58 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal velocity (cm/s)</td>
<td>4.95 7.28 6.20 8.87 0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD</td>
<td>8.16 8.42 5.33 8.17 0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NID</td>
<td>17.95 13.82 15.79 6.06 0.64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FMD: flow-mediated dilatation; (see Table 1 for the other abbreviations; NID: nitrate-induced vasodilatation.

---

**Metabolic profile**

The results of univariate analysis (Table 2) showed significant differences only in relation to LDL and total cholesterol. In fact in the placebo group, the median LDL and total cholesterol were respectively 137 mg/dl (IQR: 51.35 mg/dl) and 215 mg/dl (IQR: 64.85 mg/dl) while they were 103.50 mg/dl (IQR: 55.25 mg/dl) and 189 mg/dl (IQR: 56.25 mg/dl) after the eight weeks of pravastatin treatment (p < 0.01 in both cases). Pravastatin was not responsible for adverse metabolic events in that AST, ALT, and CK did not significantly affect between the two groups. It is interesting to observe that insulinema and HOMA both showed higher values in patients after treatment: they were 7.21 mU/l (IQR: 5.28 mU/l) and 1.51 (IQR: 0.78) respectively after eight weeks of pravastatin while they were 6.35 mU/l (IQR: 3.37 mU/l) and 1.28 (IQR: 0.90) in the placebo group. However, the differences were not statistically significant.

**Endothelial function**

As far as endothelial function was concerned, no significant differences were observed in flow-mediated dilatation (FMD) and nitrate-mediated vasodilatation (NID) (Table 2).

**Evaluation of circulating EPCs, senescence, and angiogenesis**

At day 7 in endothelial specific culture medium, PBMCs developed an elongated spindle-shaped morphol-
is now evidence that this favorable effect is present also in healthy postmenopausal women [8]. As expected, we observed that pravastatin significantly reduced total and LDL cholesterol compared to the placebo treatment.

The effects of statins administration on insulin sensitivity have been previously investigated, in particular in diabetic and dyslipidemic patients; within these subjects pravastatin improved glucose metabolism, including insulin resistance. In contrast other studies performed in healthy subjects showed that pravastatin treatment did not affect glucose and insulin levels. In our research, we observed that both insulinemia and HOMA did not change with regard to pravastatin treatment. Thus, it seems that pravastatin does not give beneficial effects on carbohydrate metabolism in healthy postmenopausal women. Similarly, we assessed that blood pressure was not modified after treatment with pravastatin. This finding is consistent with a previous study, showing that pravastatin treatment significantly reduced systolic blood pressure in hypertensive patients but not in normotensive [28]. Regarding the changes in inflammatory markers (CRP and white blood cells), our results showed no significant differences between the two treatments (pravastatin vs placebo). This can be attributed to the short treatment period with pravastatin (only eight weeks). Endothelial function, evaluated in vivo by flow-mediated dilatation (FMD) and nitrate-mediated vasodilatation (NID), did not change with respect to treatment. This result is consistent with those of Davis et al., who observed that therapy with pravastatin did not modify FMD in postmenopausal hypercholesterolemic women [29]. In contrast, other authors have demonstrated that the pravastatin administration improved FMD within ten days and this favourable effect occurred before any significant reduction in blood lipids in patients with unstable angina [30].

Increasing evidence indicates that the integrity and functional activity of the endothelial monolayer play an important role in global cardiovascular health. The traditional view of endothelial cell repair mediated exclusively by the adjacent endothelial cells has been changed by the discovery of EPCs, bone marrow derived cells, able repair the sites of endothelial injury and ischemia, through proliferation, differentiation and integration into the endothelial layer [31].

Aging may constitute a potential limitation both to EPC mobilization from bone marrow and to EPC ability to sustain repair of ischemic tissue. In fact Scheubel et al. have demonstrated that aging leads to a reduction in VEGF concentration, which might limit the mobilization and survival/differentiation of EPC. After menopause, EPC reduction attributable to aging, to change in the reproductive state characterized by estrogen withdrawal and to the worsened risk profile, may cause endothelial dysfunction and predispose to cardiovascular events [32].

In our study we showed that pravastatin is able to stimulate EPC colony formation and to delay the onset of EPC senescence in healthy postmenopausal women. These two mechanisms may contribute to increase EPC bioavailability in postmenopausal women. On the other hand, through in vitro angiogenesis assay, we could not observe an increase in tube-like formations by EPCs, obtained from postmenopausal women treated with pravastatin. Presumably more than one agent might directly influence the mobilization or half-life of EPCs and the mechanism through which pravastatin acts still remains to be determined. Previous studies aimed to evaluate the effect of other statins on EPCs. Dimmeler et al. hypothesized that statins were able to increase EPCs by the PI 3-kinase/Akt pathway [33]. Spadaccio et al. in a randomized double-bind, placebo-controlled two-way cross-over trial in 50 patients undergoing elective coronary surgery, observed that three weeks of treatment with atorvastatin increased levels of EPCs in comparison with a placebo group but on the other hand this statin did not affect the levels of VEGF [34]. Matsumura et al. in a study, using ischemic hindlimbs of rats, showed that treatment with low-dose of atorvastatin increased signif-
ically the regional blood flow and induced a parallel increase both in EPC colony formation and in proangiogenic factors such as VEGF, IL-8, Ang-1, Ang-2, ENOS and HO-1 [35].

A possible explanation of our data is that our study group included middle-age subjects without evidence of any vascular disease with conventional diagnostic tools; however we cannot rule out whether these subjects had any other subclinical alterations. It is questionable that reduced levels of VEGF typical of the postmenopausal period represent an important factor contributing to reduced responsiveness to pravastatin stimulus. Moreover it is possible that the duration of pravastatin treatment could be insufficient to stimulate EPC colony formation but not so effective to induce a modification in the functional capacity of EPC in tube-like structure formations and in FMD results.

In conclusion in healthy postmenopausal women eight weeks of treatment with pravastatin effectively reduced serum lipids, increased the count of EPC colonies but did not improve the endothelial function or insulin sensitivity. Thus pravastatin treatment (Pravaselect 40 mg) did not influence the latter cardiovascular risk factors in these subjects, at least after a short treatment period. Further studies are needed to better evaluate the potential beneficial effect of pravastatin as a tool to reduce cardiovascular risk in healthy postmenopausal women.

Acknowledgment

Our thanks to Dr. Elena Conti, MD, PhD, for contributing to the design of the study and to Dr. Eleonora Santucci, MD, for contributing to patient assessment.

References


Effect of pravastatin on endothelial function and endothelial progenitor cells in healthy postmenopausal women


Address reprint requests to:
G. PARADISI, M.D.
Department of Obstetrics and Gynecology
Catholic University of Sacred Heart
Largo A. Gemelli, 8
00168 Rome (Italy)
e-mail: giancarlo.paradisi@tin.it
Contraceptive consciousness and sexual behavior in three different female age groups in Greece: a retrospective study of the evolution during the last three decades


Tzaneio Hospital, General Hospital of Piraeus, Attiki (Greece)

Summary

The aim of the study is to describe the evolution of contraceptive and sexual behavior within our Greek society. Materials, Measures and Methods: We interviewed 508 females and made a statistical analysis of their answers. Conclusion: We tried to underline a strategy for the best promotion of the values in question. General, sexual and contraceptive education as well as the use and type of contraception are the weapons that will lead our endeavors to decreased involuntary pregnancy and towards responsible sexual behavior.

Key words: Contraception; Sexual behavior; Induced abortion; HPV infection; Evolution of sexuality; Sexual promiscuity; Sexual liberation; Contraceptive and sexual consciousness.

Introduction

There is a strong and extensive discussion on sexual behavior in Greece on the dawn of the third millennium, resulting in the contemporary average female to be tangled between sexual promiscuity, as a state of emotional instability and loose morals and sexual liberation. Early sexual intercourse among adolescents represents a major public health problem and a suitable indicator of the degree of sexual promiscuity within our society. Many factors are considered to be involved in early sexual activity, and among them, media is believed to play the most significant role. In film, television and music, sexual messages are becoming more explicit in dialogue, lyrics, and behavior. In addition, these messages contain unrealistic, inaccurate, and misleading information that young people accept as fact [1]. Furthermore, in spite of the persistence of the traditional importance placed on marriage and motherhood, the fertility rate in Greece is very low. Sex education is still not included in the school curriculum, and the lack of accurate information on contraception and the prevention of unwanted pregnancy, especially in adolescence, still have critical repercussions for women's life choices [2]. Additionally, as sexual behavior, especially the number of sexual partners as well as the type of current relationship, remain the dominant and individual risk factors for high HPV prevalence [3], the latter, together with induced abortions, unwanted pregnancies [4] and emergency contraception (EC), also reveals the size of the prevailing sexual degradation. Although sexual behavior is considerably influenced by each country's culture [4], these differences have been normalized lately under the current expanding globalization and thus, our results could easily be applied in several other countries as well. This study has been conducted in the spirit of a globalized society and its conclusions are saturated with the concept of a potential future global community, beyond the narrow barriers of a nation.

As sexual behavior consists at large of several other partial factors like the age at first coitus, frequency of sexual intercourse, number of lifetime sexual partners, use and type of contraception and the selection of proper sources of sexual education, our survey collected information on these topics to contract a realistic model of current sexual behavior. Moreover, since the study of sexuality consequences, either positive (childbirths, desired pregnancies, etc.) or negative (induced abortions, EC, HPV infection, etc.), determines its quality and nature, information on these topics was also gathered.

The purpose of this study was to investigate the evolution of the Greek sexual and contraceptive consciousness and estimate its contemporary status proposing strategies out of the current social dead end. The population we studied (15-45 years old women) covers a spectrum of the last 30 female reproductive years (1980-2010), being at the same time a dynamic and flexible pattern of multiple different mentalities through the flow of 30 years' time within our Greek society.

Materials and Methods

We interviewed 508 women, 15-45 years old, who came for some reason to our outpatient department, through anonymous questionnaires and studied their experience on certain issues concerning their contraceptive knowledge and sexual behavior (Table 1).

Sensitive questions, like those on abortion and HPV infection, were answered in a self-completed questionnaire. With their permission, the 508 female individuals were divided into three categories according to their age; females 15-25 years old...
Contraceptive consciousness and sexual behavior in three different female age groups in Greece: a retrospective study of the etc.

Table 1. — The questionnaire.

<table>
<thead>
<tr>
<th>Question</th>
<th>15-25 years</th>
<th>25-35 years</th>
<th>35-45 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>How old are you?</td>
<td>Greek</td>
<td>Foreign</td>
<td>Greek</td>
</tr>
<tr>
<td>Are you a Greek or a foreigner?</td>
<td>Orthodox</td>
<td>Christian?</td>
<td>Other?</td>
</tr>
<tr>
<td>What is your religion?</td>
<td>Elementary</td>
<td>Middle school</td>
<td>High school</td>
</tr>
<tr>
<td>What is your level of education?</td>
<td>15-20</td>
<td>20-25</td>
<td>25-30</td>
</tr>
<tr>
<td>When did you have your first coitus?</td>
<td>yes</td>
<td>no (z)</td>
<td>yes (EC)</td>
</tr>
<tr>
<td>Use of contraception</td>
<td>a, b, c, d, e, f, g, h, i, j, k, l, m</td>
<td>yes (EC)</td>
<td>no</td>
</tr>
<tr>
<td>Type of contraception</td>
<td>Use of emergency contraception (EC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If yes (EC), please write down how many times approximately you have used this method:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How many times have you been having sexual intercourse per week?</td>
<td>2/week (at least)</td>
<td>1/week</td>
<td>less frequently</td>
</tr>
<tr>
<td>What is the number of your lifetime sexual partners?</td>
<td>1 to 5</td>
<td>5 to 10</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Which is the main source of your sexual education?</td>
<td>Doctors</td>
<td>Family</td>
<td>Friends</td>
</tr>
<tr>
<td>How many births or miscarriages have you had before?</td>
<td>0 (s)</td>
<td>1-2 (q)</td>
<td>&gt; 3 (r)</td>
</tr>
<tr>
<td>Are you pregnant?</td>
<td>yes</td>
<td>no</td>
<td>yes (x)</td>
</tr>
<tr>
<td>How many induced abortions do you have in your medical history?</td>
<td>0 (s)</td>
<td>1-2 (l)</td>
<td>3-5 (u)</td>
</tr>
<tr>
<td>Have you ever been diagnosed to have been suffering from HPV infection before?</td>
<td>yes (x)</td>
<td>no (w)</td>
<td>yes (z)</td>
</tr>
</tbody>
</table>

The results of the questionnaire are cited, in Table 2, and the analysis of each potential risk factor follows.

Results

The results of the questionnaire are cited, in Table 2, and the analysis of each potential risk factor follows. Education: One hundred and ten women 15-25 years old (62.50% of group A) reported a university level education, while 107 individuals 25-35 years old (63.69% of group B) and 85 35-45 years old (51.83% of group C) reported the same. Taking into consideration the fact that there were several adolescents 15-18 years old in group A who will presumably enter the university in the future and then, using the chi-square test, we find: p value < 0.05 (between mainly group B and C (df = 1), while there is no statistical difference between groups A and B; but similarly, since the portion in group A will be augmented in the future (several girls will go to university), the same conclusion has to be approached at large for A and B groups) which means that there is a moderate, but countable positive relation between the two values (level of education, youth) and, more precisely, there is a negative correlation between the level of education, as a general factor that configurates contraceptive and sexual consciousness, and the women’s age during the last 30 years, depicting a Greek society that has been struggling for a long period of time towards the promotion of gender equality in several sectors such as education. However, did this social fight against the gender inequality eventually meet the standards of a highly inspired mentality in the field of contraception and sexual behavior? Use of contraception: One hundred and twenty-one young females (68.75%) of group A answered that they had mainly used traditional methods of contraception (withdrawal, condom, rhythm method, etc., that is, contraception a, b, e, f according to Table 2) during sexual

(176, Group A), 25-35 years old (168, Group B) and 35-45 years old (164, Group C). We obtained information on age, nationality, education, religion, medical history, number of children, contraception, number of induced abortions in the past, miscarriages, current pregnancy, HPV infection, sources of sexual education, use and type of contraception and number of lifetime sexual partners.

Age, nationality and religion were the information needed to confirm that the interviewees were 15-45 years-old, and Greeks and Orthodox Christians. In this way, we prevented interviewers from being trapped in the calculated logic of ourselves and not to be able to meet the standards of a highly inspired mentality in the field of contraception and sexual consciousness, and the women’s age during the last 30 years, depicting a Greek society that has been struggling for a long period of time towards the promotion of gender equality in several sectors such as education. However, did this social fight against the gender inequality eventually meet the standards of a highly inspired mentality in the field of contraception and sexual behavior? Use of contraception: One hundred and twenty-one young females (68.75%) of group A answered that they had mainly used traditional methods of contraception (withdrawal, condom, rhythm method, etc., that is, contraception a, b, e, f according to Table 2) during sexual
intercourse, while 84 (50.00%) of group B and 83 (52.61%) of group C reported the above. The chi-square test revealed a statistically significant increase of the traditional methods among young women 15-25 years (p < 0.001/df = 1, compared to use of the other contraceptive methods). On the other hand, a moderate (p < 0.05, when compared with the rest of the methods, df = 1) decrease in the percentage of “no use of any contraception” category was noticed in the group A women (15.91%) compared to those of group B (26.19%). Likewise, group C women that had not been using any type of contraception at all, were significantly fewer (9.76%) compared to their corresponding ones in the 25-45 years old women. The above analysis brings us face to face with a dilemma. Is the increased use of traditional contraceptive methods in young females, in combination with the decreased portion of “no use of any contraception” women in this group, attributed to a deeper sensitiv-

Table 2. — Pathologic findings - Group A Women 15-25 years old.

<table>
<thead>
<tr>
<th>Type of contraception</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>z</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of education</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Middle school</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>High school</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>University</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Age at first coitus</td>
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<td></td>
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<td></td>
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<td>15-20 y</td>
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<td>5</td>
<td>4</td>
<td>5</td>
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<td>5</td>
<td>q,s,x</td>
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<td>20-25 y</td>
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<td>5</td>
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<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
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<tr>
<td>Frequency of sexual intercourse</td>
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<td></td>
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<td></td>
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<tr>
<td>2 times/week (at least)</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>5 n,s,w</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
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<tr>
<td>5 n,s,w</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Sources of information on contraception topics</td>
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<td></td>
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<td></td>
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<tr>
<td>Doctors</td>
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<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Family</td>
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<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Friends</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
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<td>5</td>
<td>q,s,x</td>
</tr>
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<td>Media</td>
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<td>5</td>
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<td>4</td>
<td>5</td>
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<td>4</td>
<td>5</td>
<td>q,s,x</td>
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<tr>
<td>Others (rare)</td>
<td>4</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>Number of lifetime sexual partners</td>
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<td></td>
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<tr>
<td>1 to 5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
</tr>
<tr>
<td>&gt; 10</td>
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<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>q,s,x</td>
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</tbody>
</table>

- a = withdrawal; b = condom; c = pill; d = IUD; e = other method; f = a+b (withdrawal and condom); g = a+c (condom and pill); h = b+c (condom and IUD); i = b+d (condom and IUD); j = c+d (pill and IUD); k = a+b+c (withdrawal, condom and pill); l = b+c+d (condom, pill and IUD); m = a+b+c+d (withdrawal, condom, pill and IUD); n = 0 no childbirths and/or miscarriages and/or current pregnancy; p = 3 or more childbirths and/or miscarriages and/or current pregnancy; q = 0 abortions; t = 1-2 abortions; u = 3-5 abortions; v = 5 more than five abortions; w = no, absence of HPV infection; x = yes, presence of HPV infection; z = no contraception (neither emergency contraception).
normalizing degraded sexual behaviors and, thus, encouraging them among teenagers (1). Consequently, young women take them for granted as simply being natural facts, without feeling that such misleading messages are violently imposed on them by several corrupted sources through “media”. Moreover, “family” does not seem to have played an important role in the sexual education of female individuals for the last three decades as its portion as a source is very low (11.93% in group A, 2.38% in group B, 3.05% in group C). However, there was a significantly increased portion in 15-25-year-old women ($p < 0.01$), but this mainly concerned females with traditional contraception use. The question that arises leads us towards a controversy: do the above findings reveal a certain disintegration of our social structure on the matter of contraception and sexual information as time elapses, or do they present a hidden, decomposed reality on the matter in question that struggles to be organized in highly qualitative levels of social remodeling?

**Age at first intercourse:** One hundred and fifty-one (85.80%) women of group A answered that they had had their first sexual intercourse at 15-20 years old, while 103 of group B (61.31%) and 104 of group C (63.41%) females gave the same answer. There is an obvious statistical differentiation in this parameter between group A and the other groups ($p < 0.001$), while there is not any significant change between groups B and C. We have to mention that nine individuals of group C (5.49%) had had their first experience at 25-30 years old. Do these results agree with an epoch characterized as the era of sexual liberation, meaning the ostracism of several prejudices and taboos from the social background or, do these results reveal a weakened and confused society that regresses to conditions of very primitive behavior, to an epoch when human principles did not even exist?

**Number of lifetime sexual partners:** 68.75% of group A women had had less than five sexual partners in their lifetime, while 75.60% of group B and 46.95% of group C females had the same number. There is a significant statistical differentiation between the groups ($p < 0.05$), while there is not any significant change between group A and the other groups. Do these results agree with an epoch characterized as the era of sexual liberation, meaning the ostracism of several prejudices and taboos from the social background or, do these results reveal a weakened and confused society that regresses to conditions of very primitive behavior, to an epoch when human principles did not even exist?

**Number of lifetime sexual partners:** 68.75% of group A women had had less than five sexual partners in their lifetime, while 75.60% of group B and 46.95% of group C

### Table 2. — Pathologic findings - Group B. Women 25-35 years old.

<table>
<thead>
<tr>
<th>Level of education</th>
<th>Age at first coitus</th>
<th>Frequency of sexual intercourse</th>
<th>Sources of information on contraception topics</th>
<th>Number of lifetime sexual partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary school</td>
<td>15-20 y</td>
<td>2 times/week (at least)</td>
<td>Doctors</td>
<td>1 to 5</td>
</tr>
<tr>
<td>Middle school</td>
<td>20-25 y</td>
<td>1 week</td>
<td>Family</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>25-30 y</td>
<td>less frequently</td>
<td>Friends</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td></td>
<td>other (rare)</td>
<td>Media</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>other</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- The table presents the pathologic findings for group B, focusing on women aged 25-35 years old.
- The data include the type of contraception used, level of education, age at first coitus, frequency of sexual intercourse, and sources of information on contraception.
- The table also categorizes the number of lifetime sexual partners into three groups: 1 to 5, 5 to 10, and > 10.

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reported the same. Moreover, 53.05% of group C individuals, 24.40% of group B and 31.25% of group A had had more than five sexual partners in their lifetime. The above portions must be evaluated on the basis of different perspectives; although the differentiation between the portions in groups A and B is statistically insignificant, it could become stronger if we take into consideration the difference in the mean group age (younger women, as having had less sexually active years, were expected to have had a lower percentage of sexual partner and thus, the above result of statistical insignificance should be interpreted as a significant deviation of what was really expected to be found in our study, on the basis of the “age” parameter). Consequently, there is an important hidden differentiation that we have to mention in the percentages we cited, concerning the quality; the portions in group B and C females are mainly attributed to their older age which promotes the proliferation of sexual relations, while the corresponding one of group A is strongly related mostly to their attitude in the sexual sector. We can clearly reach the conclusion that the younger a woman is today, the looser her sexual choices concerning her partners are. Next, we have to wonder if this is a trend of our over-consuming epoch or, an expression of a current highly promoted ideal of freedom.

Frequency of sexual intercourse: One hundred and three (58.52%) group A individuals reported to have had sexual intercourse around two times per week, while 117 (69.64%) of group B reported the same (p < 0.05). On the other hand, 41.48% of women 15-25 years old reported having sexual intercourse less than two times per week and, 30.95% of group B and 48.78% of group C women
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Answered the same. We noted that, even if the number of lifetime sexual partners attributed a stronger desire of sexual variety was higher in the younger ages (increased number of lifetime sexual partners as proved in the previous paragraph), the frequency of sexual intercourse was statistically lower; a finding that confused us, urging us to think whether sex is normally “shrinking” today because of the current hustle and bustle or worse, that people tend to become alienated from each other resulting straightforwardly in values like sex becoming eliminated.

Upcoming results of a certain sexual and contraceptive behavior: This paragraph refers to the outcome of women’s entire sexual behavior and additionally, the practical side of their inner contraceptive consciousness, both reflected in phenomena like childbirth, abortion and sexually transmitted infectious diseases (STIs) such as HPV infection. We noted that the mathematical relation “(childbirths, miscarriages, current pregnancy): (abortions)” was equal to (42:18 =) 7:3 in group A, (102:54 =) 17:9 in group B women and (186:150 =) 31:25 in group C. In order to deeply comprehend the meaning of these portions, we have to take into our consideration two more facts: the age of women and percentage of women that had used emergency contraception (EC) before (in each group). We observed that 35.80% (63/176) of group A had used EC before (in each group). We observed that 35.80% (63/176) of group A had used EC before (in each group). The real portions of “induced paused pregnancies” (EC plus (+) abortions, as an aggregation of the induced paused potential (EC) and current (abortions) pregnancies) correct the previous math relation to: (42:18 =) 14:27 in group A, (102:94 =) 51:47 in group B and (186:178 =) 93:89 in group C (p < 0.01 between groups A and the other two, B and C). This finding in combination with the differentiation concerning women’s age group A women had fewer sexually active years, something that should normally suppress their portion of negative consequences emerged by their sexuality, such as unwanted pregnancies, induced abortions, use of EC, HPV infections, etc.) among groups, can lead us towards the conclusion that the younger a woman, who has experienced sex before in our epoch, the more negative the consequences of her sexual behavior are. This conclusion is enhanced by the fact that, according to several surveys, the percentage of unwanted pregnancies and childbirths are increasing among young female individuals [2]. Moreover, the HPV infection rate is higher in group A (19.89%) and group B (19.05%) females compared to the corresponding one in group C (14.02%) individuals (but not statistically significant). If we consider age to be a risk factor in the groups, we can clearly assume that women 15-25 years old are presenting a higher possibility of getting HPV infections in the future (attributed to their young age; proportionally we should expect group A females to have a significantly lower portion on HPV due to their less sexually active years of experience) and thus, their portion is supposed at large to be differentiated upwardly compared to group B and C females. We clearly reach again the previously cited conclusion that “the younger a woman is in our epoch, the more negative the consequences of her sexual behavior are”.

Discussion

Our results form a picture of the evolution of sexual and contraceptive attitudes in Greece during the last 30 years. The age of women we studied and the chi-square test applied in our questionnaire were co-evaluated so as several safe conclusions could be extracted. Women in group A were directly influenced by prevailing contemporary behavioral streams, while group B and mainly group C individuals were more typical of the changes through the flow of time, concerning female sexuality during the last three decades. Our study clearly proved that sexual and contraceptive behavior in our country have started to become victims of our current over-consuming society.

Young women appear today to be acquiring a higher general education, to be using more “traditional” than “modern” methods of contraception, to be advised on sexual and contraception topics mostly from unreliable sources (such as “friends”) in a constantly increasing rhythm, to be experiencing sex at even younger ages, to be reporting a higher number of lifetime sexual partners and lower frequency of sexual intercourse compared to their elder peers. On the other hand, there is an increasing portion of failed pregnancies (unwanted pregnancy, induced abortion, use of EC) and HPV infections among 15-25 year-old females. The fact that the new generation (group A) is highly educated [5], which is one of the great achievements of the feminist movement, has triggered several controversies in the developed world [6]. Although women have surpassed men at many levels of education (e.g., in the United States in 2005/2006, women earned 52% of Associate degrees, 58% of Bachelor degrees, 56% of Master degrees, and 50% of Doctorate degrees) [7], there is a growing doubt as to whether the higher educational attainment of female individuals is related either positively or negatively to sexual promiscuity [6], which is a negatively charged concept that refers to the degradation of several other partial concepts/constituents of female sexuality, like the age of first coitus, frequency of sexual intercourse, number of lifetime sexual partners and childbirths. In our study, the combination of three parameters, age at first coitus, number of lifetime sexual partners and frequency of sexual intercourse, revealed that young women today show a disproportionately high diversity in their sexual choices (younger age at first coitus and higher number of lifetime sexual partners than older women) compared to the quality/degree of their relationship bonds (lower frequency of sexual intercourse, as an indicator of the low quality/feeble bond of their sexual relationships) and thus, sexual promiscuity appears to have reached today the highest degree in the last three decades. This idea can also be extracted inversely from our study if we just apply the “upcoming results of a certain sexual and contraceptive behavior” conclusion: Since “the younger a woman is in our epoch, the more negative the consequences (induced abortions, HPV infection) of her sexual
behavior are” and since the degradation of sexual behavior is the main indicator of sexual promiscuity, the latter appears again to have today reached the highest degree in the last three decades. Moreover, the factors that configure at large to sexual and contraceptive consciousness, and mainly the general as well as sexual and contraceptive education, seem to be derived mostly from unreliable sources in contradiction to what was happening in this sector during the last 30 years. The asymmetry between the high level of young female individuals’ general education on the one hand and the low one of their sexual education on the other, reveals a superficial technologically orientated knowledge, a knowledge isolated from human values and from deeper understanding of human principles such as those that are given by a genuine and responsible sexual and contraceptive education within our society. Finally, the quality of contraceptive use in young people (the prevalence of traditional methods) shows that the new generation is inundated mainly with fear against an aggressive world, full of sexually transmitted diseases (HPV, HIV, etc.); a fear that can become an obstacle to any ideal of a prosperous social development.

On the other hand, there is a hidden and positive potentiality underneath the above-mentioned sexual promiscuity, which refers to a growing ideal of sexual liberation (lower age at first coitus, more lifetime sexual partners); an ideal that may be able to remodel our society into highly qualitative structures. Although recently it was noted that teenagers are sexually active in younger ages and demonstrate lower compliance to contraceptive methods [8], there is a moderate decrease in the “no use of any contraception” category in women 15-25 years old in our study that points a way towards a growing responsibility on the one hand and the low one of their sexual education on the other, reveals a superficial technologically orientated knowledge, a knowledge isolated from human values and from deeper understanding of human principles such as those that are given by a genuine and responsible sexual and contraceptive education within our society. Finally, the quality of contraceptive use in young people (the prevalence of traditional methods) shows that the new generation is inundated mainly with fear against an aggressive world, full of sexually transmitted diseases (HPV, HIV, etc.); a fear that can become an obstacle to any ideal of a prosperous social development.

As the sexual activity rate in adolescence is reportedly increasing worldwide, improving knowledge concerning sexual education in adolescents might contribute to improving reproductive health issues. This must be provided mainly by the school, family and doctors. Unfortunately, sex education is still not included in the school curriculum in Greece, and only sporadic information is given. We should improve the attitudes, beliefs and knowledge of Greek adolescents regarding sexual intercourse, contraception and sexually transmitted diseases through organizing better programmes on sexuality for young people [10]. Moreover, the institution of family must be promoted to a very important social cell where proper knowledge that results in healthy development of youths’ sexual and contraceptive consciousness, must be given mainly by the state through extensive scientific programmes, similar to those of schools that have already been mentioned. Finally, doctors (gynecologists) in Greece should undergo special training on female sexuality issues and become more sensitive and adequate on these matters without underestimating their importance to the social tissue; a practice that unfortunately is widespread in our country (e.g., some women are being treated in an adverse way by doctors and social workers in public hospitals [9]). In other words, we should never forget that the high quality of consultation contributes to the decrease of future and repeated unwanted pregnancies and induced abortions [10] and thus, to the suppression of sexual promiscuity. However, we have to mention that the least for all the above to be achieved remains always the high quality of the general education provided in our society (e.g., education of the young, mothers, of doctors, in families, through the media, etc.); an education which core is not inundated only with technological concepts (as today’s intensely happening) but mainly with substantial human values and furthermore, with a deeper understanding of the mechanisms and the principles that harmonically constitute the reality and define the well – and the mal – function of things and concepts. This general consciousness could become the fertile ground where a healthy sexual and contraceptive consciousness could grow and thrive.

One of the conclusions that is quite obvious from our study is that Greek society has not fully adopted the modern methods of contraception and, according to several other studies, it appears to have one of the lowest rates in modern contraceptive use in Europe [2]. Coitus interruptus and condom use are the most commonly used methods in our country, whereas the pill and other reliable contraceptive methods appear to have low use rates [10]. Consequently, it is very important that contraception-related topics be introduced as a part of sexual education, despite several adverse circumstances, like the generation gap between parents and children, the lack of teachers trained in sexual education and discussion and other barriers [10, 11]. In conclusion the evolution of sexual and the contraceptive consciousness in Greece is ambiguous today, being degraded on the one hand, while very promising for
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the future on the other. We must use this enormous potentiality, which is well hidden underneath our widespread social consciousness, to our general and universal benefit. General, sexual and contraceptive education as well as the use and type of contraception are the weapons that will lead our endeavors to success.

References


Address reprint requests to:
A. KOUMOUSIDIS, M.D.
Grigoriou Lambraiki 112-114
p.c. 18532 Pireas (Greece)
e-mail: kumusidi@yahoo.gr
Bilateral uterine and ovarian artery ligation in addition to B-Lynch suture may be an alternative to hysterectomy for uterine atonic hemorrhage

K. Gezginç, F. Yazıcı, T. Koyuncu, A.S. Mahmoud

Department of Obstetrics and Gynecology, Selçuk University Meram Medical School, Konya (Turkey)

Summary
Purpose of investigation: To evaluate the effectiveness of bilateral uterine arteries and ovarian artery ligation followed by B-Lynch compression suturing in controlling atomic postpartum hemorrhage. Methods: In this retrospective study, the data of eight patients that had uterine atony during cesarean section and treated by bilateral uterine and ovarian artery ligation followed by B-Lynch compression suturing during the period from February 2009 to September 2010 were collected and analyzed. Results: Eight cases were treated by the above protocol; the average age of the patients was 25.25 ± 5.09 years, and the mean gestational age was 35.75 ± 3.80 weeks. Seven of the patients were primiparous. They were hospitalized on average 5.25 ± 2.31 days. The mean operation time was 61.25 ± 24.60 minutes and mean estimated blood loss was 2787.5 ± 1573.38 ml. Internal iliac artery ligation was necessary in one patient only. Hysterectomy was not performed in any of the patients. Five patients had intraoperative or postoperative blood transfusion. Conclusion: The addition of uterine artery and ovarian artery ligation to the B-Lynch suture may be considered as a major hemostatic step before proceeding to hysterectomy in cases of uterine atony bleeding, and all gynecologic surgeons should be familiar with it.

Key words: Atonic postpartum hemorrhage; B-Lynch suture; Bilateral uterine and ovarian artery ligation.

Introduction
Early (primary) postpartum hemorrhage (PPPH) is the cause of high maternal morbidity and mortality. PPPH is defined by blood loss greater than 500 ml during the first 24 h after vaginal delivery or > 1,000 ml after cesarean section [1].

Uterine atony accounts for 75%-90% of primary PPH [2]. The traditional management of this condition begins with conservative methods such as bimanual uterine compression, medical therapy with uterotonic agents, uterine tamponade with balloons and occasionally, arterial embolization, the failure of which often mandates surgical intervention [2].

Surgical therapy is reserved for hospital settings, and is aimed at preventing uterine atony by compressive techniques or to achieve selective uterus devascularization, thus preventing hemorrhage (vascular ligation of uterine artery, ovarian artery, hypogastric artery; and selective transarterial embolization). According to current data, hysterectomy is only reserved for refractory PPH in multiparas [1].

Uterine compression sutures (B-Lynch) have proved to be valuable in the control of massive atomic postpartum hemorrhage as an alternative to hysterectomy [3, 4].

Bilateral UAL is the first step of a stepwise uterine devascularization approach that affords good control of postpartum hemorrhage [4, 5]. Performed with or without utero-ovarian ligament ligation, UAL does not appear to affect future fertility or obstetric outcomes [4-6]. Vascular occlusion is only temporary, as recanalization soon ensures normal uterine circulation [4, 5].

The aim of the present study was to evaluate the effectiveness of bilateral uterine arteries and artery ligation followed by B-Lynch compression suturing in controlling atomic postpartum hemorrhage.

Materials and Methods
In this retrospective study, we evaluated patients that had intraoperative uterine atony during cesarean section treated by bilateral uterine and ovarian artery ligation and B-Lynch procedure from February 2009 to September 2010. Bilateral uterine and ovary artery ligation and B-Lynch procedure were performed in eight women only after uterine atony which was defined by persistent uterine relaxation that did not respond to measures such as uterine massage, bimanual compression and the use of uterotonic, i.e., oxytoxin, ergometrine and misoprostol. All patients were delivered by cesarean section and were complicated by uterine atony during the operation. All cesarean deliveries were performed by the first author. A dose of 1 g of first-generation cephalosporin (Iespor, I. Ulagay, Istanbul) was administered intravenously 20 min prior to skin incision. The surgery was done under general anesthesia. The abdomen was opened by an appropriate-sized Pfannenstiel incision.

The inclusion criteria, intraoperative atonic postpartum hemorrhage, did not respond to conservative methods. Bimanual compression was first applied to assess the potential chance of success of the B-Lynch suturing technique. The vagina was swabbed to confirm adequate control of the bleeding. If vaginal bleeding was controlled, bilateral uterine and ovarian artery ligation and B-Lynch suture were performed. The B-Lynch compression suture was applied either starting from the right or the left corner of the lower segment of the incision, starting 3 cm
below the incision line, and brought out 3 cm over the upper side of the incision. This suture is taken over the uterine fundus and brought down symmetrically to the anterior incision at the posterior of the uterus, right over the sacrouterine ligaments. A horizontal bite is taken at the posterior segment of the uterus penetrating the whole uterine wall. The suture is then brought over the uterine fundus again to the anterior, and enters the uterine cavity again 3 cm above the anterior incision. Finally the suture is taken out of the uterine cavity passing 3 cm distally to the inferior segment of the incision. The ends are tied to compress the uterus [3]. The suture material used was No. 1 Vicryl (Pegesorb, Trabzon, Turkey).

The technique used for bilateral uterine artery ligation was the following: the peritoneum over the vesicouterine pouch was incised horizontally, the peritoneum over the uterine isthmus and cervix was bluntly dissected downwards, and this dissection was then extended laterally. To avoid including the ureter in the ligation of the ascending branch of the uterine artery, the peritoneum was carefully mobilized at the uterine angles to expose both. Uterine artery pulsations were palpated digitally at the level of the internal os. A 1.0 vicryl suture attached to a round-bodied needle was passed from posterior to anterior through the cervical tissues. The vaginal bleeding was controlled by vaginal examination. When bleeding stopped, the uterine incision was then closed in the normal way, in two layers with or without closure of the lower uterine segment of the peritoneum. Finally, the abdomen was closed using a regular technique.

Monitoring of maternal hematologic parameters 24 hours before cesarean delivery and two hours after the procedure is a requirement of the protocol. Blood transfusion was performed if the hemoglobin level was less than 7 g/dl and the hematocrit value was less than 21%. The following data were extracted from the hospital charts: age, parity, indication for cesarean delivery, weeks of gestation, newborn weight, cause of postpartum hemorrhage, method of anesthesia, operation time, amount of blood loss, and postoperative stay.

### Results

The B-Lynch procedure, uterine artery and ovary artery ligation were performed in a total of eight cases from February 2009 to September 2010. The results are summarized in Table 1. The average age of the patients was 25.25 ± 5.09 years (age ranged from 19 to 33 years) and mean gestational age was 35.75 ± 3.80 weeks. Seven of the patients were primiparous. They were hospitalized on average 5.25 ± 2.31 days. Mean operation time was 61.25 ± 24.60 minutes. All patients received a high dose of oxytocin (more than 20 U/l) and prostaglandin E2. All sutures were placed, and the uterine arteries were clamped. The mean estimated blood loss was 2787.5 ± 1573.38 ml (range 1000-4000 ml). Five patients had intraoperative or postoperative blood transfusions. Hysterectomy was not performed in any of the patients.

### Discussion

Postpartum hemorrhage (PPH) is one of the main causes of maternal death. The management of PPH includes mechanical, medical, and surgical procedures. Mechanical procedures include compression of the uterus and medical therapy includes uterotonics to induce uterine contraction, e.g., oxytocin, methylergometrine, or prostaglandin preparations that can be administered via intra-myometrial injection by the transabdominal, intracervical, or intracavitary route with the use of a Foley catheter [1, 7].

Uterine compression suture techniques such as the B-Lynch brace suture technique were first described in 1997 by B-Lynch et al. and since then many publications of successful application of the technique have appeared in various journals [3, 7]. Subsequently, there have been a total of 16 publications on the technique from 2000 to 2005, reporting an 80%-100% success rate of the B-Lynch procedure in controlling PPH with uterine preservation. Since 1997, more than 1,000 procedures have been performed worldwide [2]. They described a technique of oversewing the uterus with a continuous suture to apply ongoing compression. This procedure is much quicker and simpler than hysterectomy or internal iliac ligation, and the reported series suggest that it is very effective [8]. Other surgical methods used in combination with the B-Lynch suture with variable results have also been described, including uterine artery ligation, ovarian vessel ligation and oversewing of the placental bed [9].

Bilateral uterine artery ligation (UAL) is the first step of a stepwise uterine devascularization approach that affords good control of postpartum hemorrhage. The technique decreases blood perfusion through a temporary vascular occlusion. This effect may be combined with that of a B-Lynch compression suture, which probably also decreases blood flow. As recanalization occurs and normal uterine circulation resumes, no interference with future reproductive performance is expected [4, 5].

Sentilhes et al. [10] were the first to report on a woman who underwent stepwise uterine devascularization and B-Lynch compression suturing who became pregnant again, and showed no signs of the B-Lynch procedure on cesarean delivery. In our cases, in addition to B-Lynch suture we ligated the uterine and ovarian vessels that supply the uterus. Thus we applied an alternative treatment method in patients that might have a risk of hysterectomy inspite of a B-Lynch suture. There was no need for hysterectomy in any of our patients who were treated by this protocol. There have been isolated reports of adverse conse-
quences after B-Lynch application. Grotegut et al. reported one case of erosion of a B-Lynch suture through the uterine wall and partial ischemic necrosis of the uterus occurring 24 hours after the procedure. This complication was also reported in a 26-year-old primigravida [11, 12]. Long-term complications include the formation of bowel adhesions which was described in a patient who delivered by cesarean section [2]. In our patients, no complication was encountered on physical examination and ultrasonography in the postoperative control visits, and the patients had no complaints.

The compression B-Lynch suture is a successful, safe, inexpensive, and simple method for the treatment of PPH during cesarean section, which reduces the incidence of mutilating urgent peripartum hysterectomy due to uterine atony or coagulopathy, and preserves subsequent patient fertility [1].

In women who desire to have their fertility preserved, bilateral UAL and ovarian artery ligation followed by B-Lynch compression suturing represents an effective and safe combination for controlling atomic postpartum hemorrhage, and an alternative to hysterectomy.

Shahin et al. [4] found that their protocol did not impair fertility. When compared with other surgical interventions for uterine bleeding which are technically harder, have a slower learning curve and high complication rates, B-Lynch suturing is a rather easy and quick technique to learn and apply [3]. Some studies have reported that the B-Lynch suture is not always successful and hysterectomy may be needed in some cases. The addition of uterine devascularization to B-Lynch suturing is a simple, easy, and less time consuming technique, and we think that it is a good alternative to hysterectomy, especially in societies were hysterectomy forms a medicolegal problem. We certainly believe all gynecologic surgeons should be familiar with it.

In conclusion, we also believe that bilateral uterine and ovarian artery ligation plus B-Lynch suturing should be considered a major hemostatic step before hysterectomy in cases of uterine atony bleeding. We have suggested the addition of uterine and ovarian artery ligation to the B-Lynch suture to make it more effective and eliminate the need for hysterectomy [8]. Our protocol seems promising as none of our patients needed a hysterectomy and there have not been any complications.

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Address reprint requests to:
K. GEZGINÇ, M.D.
Selcuk University Meram Medical School
Department of Obstetrics and Gynecology
Akyokuş
42080 Konya (Turkey)
e-mail: kazimgezgine@hotmail.com
Combination of baclofen and antimuscarinics to reduce voiding difficulty in treating women with overactive bladders

H.Y. Chin¹, K.C. Lin², C.H. Chiang¹, C.J. Wang³

¹Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital at Keelung, Keelung
²Department of Management Information Systems, National Chung Hsing University, Taichung
³Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital at Linkou
Chang Gung University College of Medicine, Kwei-Shan, Tao-Yuan (Taiwan)

Summary

Purpose of study: To evaluate the efficacy of baclofen in combination with antimuscarinics to treat women with an overactive bladder (OAB) with abnormal voiding patterns. Methods: An action research and chart review was conducted in 245 OAB women. Women were prescribed tolterodine or oxybutynin with or without baclofen after urodynamics. The complaint of voiding difficulty was followed up one week later. Results: There was a significant difference in the occurrence of voiding difficulty after antimuscarinic administration in OAB women with abnormal voiding patterns compared with normal patterns (18% vs 4.9%, respectively; p = 0.013). The clinical difference of voiding difficulty after treating with antimuscarinics between both voiding patterns disappeared after adding baclofen (abnormal voiding pattern vs normal pattern; 11.1% vs 5.6%, respectively; p = 1.000). Conclusion: Combined use of baclofen and antimuscarinic agents could reduce voiding difficulty in treating women with overactive bladders with abnormal voiding patterns.

Key words: Overactive bladder; Antimuscarinics; Urodynamics; Voiding pattern; Baclofen.

Introduction

Overactive bladder (OAB) is a symptom complex. In 2002, the International Continence Society (ICS) announced a new definition of OAB, which is regarded as a syndrome whose diagnosis is made purely on the basis of symptoms presented without the need to perform any urodynamic investigation [1, 2]. This new definition allows greater ease in clinical practice. To date, antimuscarinic agents are the most common and currently the most effective drugs for treating OAB [3].

The most common side-effect of antimuscarinic agents is dry mouth which has long attracted much attention [4]. However, their clinical use has often been found to cause also voiding difficulty such as small caliber, decreased force of urinary stream, urinary hesitancy, or strained voiding, which can be very disturbing and distressing. It being unpredictable implies that it may have a profound impact on quality of life. Worse still, emergency catheterization may be required due to acute urinary retention. So far, there has been no protocol for treating OAB that can prevent voiding difficulty. Moreover, the idea that OAB symptoms can be caused by problems of the voiding phase has become more widely accepted. It meant that dysfunction voiding may be masked by urinary frequency and urgency, which may lead to a wrong diagnosis and inappropriate treatment [5-7]. Therefore, increasing attention has been given to the occurrence of incomplete emptying or voiding dysfunction in combination with OAB symptoms [8, 9].

Baclofen is a prototypic gamma-aminobutyric acid (GABA) subtype receptor agonist which could decrease external urethral sphincter resistance by depressing pudendal nerve reflex through spinal cord pre-synaptic hyperpolarization. Previous research has reported that baclofen can be employed to relieve certain kinds of voiding difficulty, which arises as a result of the external urethral sphincter or pelvic floor muscle being stressed [10]. This paper examines the effectiveness of combined use of baclofen and antimuscarinic agents, tolterodine and oxybutynin in prevention of voiding difficulty in women with OAB according to clinical symptoms without urodynamic examination. Exploration of other possible measures for avoiding voiding difficulty after antimuscarinic agents is one of the aims.

Materials and Methods

The study embraced action research as well as chart review. The database contained medical records of 245 women who had one or more typical OAB symptoms for more than one year as their chief clinical complaints between January 2004 and November 2006. The symptoms of OAB are defined as urinary urgency with or without urge incontinence, usually with urinary frequency (voiding eight times or more in a 24-hour period), and nocturia (awakening two times or more at night to void) [2, 11, 12].

In the study, patients enrolled had had no symptom of voiding difficulty and no abnormal urinary routine examinations. Other inclusion criteria were: no previous history of urinary tract abnormalities or lithiasis, no prior surgery of the pelvic floor and bladder, and no medical and neurogenic diseases. Patients on medication that could affect bladder function were not included. Combined symptoms of stress urinary incontinence and voiding difficulty also constituted the exclusion criteria. In

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addition, women had no chronic pelvic pain or painful bladder symptoms. During pelvic examination, no obvious cystocele, uterine prolapse or urogenital anomaly were found. Sonographic examination revealed no significant increase in size of the uterus or pelvic mass.

Patients were to undergo urodynamic examinations after being diagnosed with OAB syndrome. Thus, medicinal treatment was offered subsequently. Between January 2004 and October 2005, 100 women with OAB symptoms underwent a daily regimen of 2 mg tolterodine (Pfizer Italia S.R.L, Ascoli Piceno, Italy). Follow-up was scheduled one week later in clinics. Voiding difficulty associated with the regular protocol of tolterodine administration was observed. Therefore, a changeover to short-acting oxybutynine was employed. Daily 2.5 mg of oxybutynine (Panion & BF Biotec Inc., Taiwan) was given to 27 OAB patients for the period November 2005 to April 2006. Discomfort of voiding was still mentioned by the patients. Hence the successive medicine alternative was changed to a daily combination of 2 mg tolterodine along with 10 mg baclofen (Swiss Pharmaceutical Co. Ltd., Switzerland) for one week. Twenty-three patients were administered the modified protocol between May 2006 and August 2006. For cost effectiveness, the weekly regimen was changed to 2.5 mg oxybutynine daily plus 10 mg baclofen. A total of 13 patients underwent this protocol between the time frame of September 2006 and November 2006 (Figure 1).

Urodynamic studies were performed in all symptomatic patients, and consisted of measurement of post-micturition residual, urethral pressure profilometry, EMG and cystometry according to the criteria of the ICS [13]. Uroflowmetry was performed under natural circumstances. If the patient complained that the test was not performed through self-voiding as usual, the examination would be done again. In general, typical results recorded in the uroflowmetry showed a smooth single curve with the maximum flow rate exceeding 15 ml/sec under a voided volume above 200 ml. If the curve was not smooth, had multiple interrupted peaks or showed an abnormal low flow rate, the patients were considered to be suffering from an abnormal voiding pattern regardless of whether they had clinical symptoms or not.

A three-day urinary diary had to be completed to make sure that the included subjects voided more than eight times per day, awake two times or more at night to void, and had no fluid overload the whole day. No questionnaires were used to quantify the impact of symptoms, but all subjects had these symptoms as their chief complaints, which had affected their quality of life. Of note is that the patients did not report any voiding difficulty or discomfort when having OAB symptoms.

All statistical analyses were conducted using version 13.0 SPSS software program (SPSS, Inc., Chicago, IL, USA). Demographic characteristics of the patients are presented as the mean ± SD or percentage according to the variables. Furthermore, cross tabulation was employed to describe the relationship between voiding pattern in urodynamics, baclofen, antimuscarinic agents and voiding difficulty after treatment of OAB. Comparison of the categorical data was made by the chi-square test ($\chi^2$) and Fisher’s exact test with $p < 0.05$ considered significant.

**Results**

Of the 245 women with OAB symptoms in our original database, only 163 patients with complete follow-up data were enrolled in our study. Thirty patients did not meet the inclusion criteria. The remaining 52 patients did not return for follow-up or treatment after urodynamic examination. We conducted a telephone interview to explore the reasons why they refused to be followed up or receive treatment. Although we did not do any statistical analysis of their responses, we discovered two common reasons for the discontinuation of follow-up. They included discomfort felt during the invasive examination procedure and lack of knowledge concerning OAB, making them reluctant to seek medical treatment.

The demographic characteristics of the 163 subjects are listed in Table 1. All were Taiwanese. Their mean age was 48 years with no predominant age group found. Among them, 36.8% were postmenopausal, indicating that menopause is not a significant factor for the occurrence of OAB syndrome. However, the majority (71.8%) of the OAB patients had only normal spontaneous deliveries.

**Table 1. — Patient characteristics.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.1 ± 13.9 (22-79)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6 ± 3.8 (15.2-36.9)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>60 (36.8)</td>
</tr>
<tr>
<td>NSD only</td>
<td>117 (71.8)</td>
</tr>
<tr>
<td>CS only</td>
<td>17 (10.4)</td>
</tr>
<tr>
<td>NSD and CS</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>25 (15.3)</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation (range) or n (%).

BMI: body mass index; NSD: normal spontaneous delivery, CS: cesarean delivery.
Combination of baclofen and antimuscarinics to reduce voiding difficulty in treating women with overactive bladders

Table 2. — Voiding pattern in urodynamics with baclofen, antimuscarinic agents, and voiding difficulty after medication.

<table>
<thead>
<tr>
<th>Voiding pattern</th>
<th>Abnormal voiding pattern (n = 82)</th>
<th>Normal voiding pattern (n = 81)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen used</td>
<td>Yes (n = 15)</td>
<td>No (n = 67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 2)</td>
<td>(n = 13)</td>
<td></td>
</tr>
<tr>
<td>Tolterodine</td>
<td>Yes (n = 1)</td>
<td>No (n = 3)</td>
<td>0.503</td>
</tr>
<tr>
<td></td>
<td>(n = 1)</td>
<td>(n = 17)</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>0</td>
<td>1</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Concerning voiding difficulty after using pure antimuscarinics, 14 of 100 (14%) women with tolterodine treatment reported this side-effect, while for two of 27 women (7.4%) it occurred with oxybutynin. There was no clinical difference (14% vs 7.4%, respectively; p = 0.29). However, if analyzed thoroughly, taking the voiding pattern into consideration, there was a significant difference in the rate of voiding difficulty occurring after prescribin	olterodine only between different voiding patterns (abnormal voiding pattern vs normal pattern; 24% vs 4%, respectively; p = 0.008). In contrast to tolterodine, administration of oxybutynin only in treating OAB women did not demonstrate any relationship between voiding difficulty and different voiding patterns (7.1% vs 7.7%, respectively; p = 1.000). Worthy of mention is that the clinical difference of voiding difficulty after treating with antimuscarinics between both voiding patterns disappeared after adding baclofen (abnormal voiding pattern vs normal pattern; 11.1% vs 5.6%, respectively; p = 1.000) (Table 2).

Discussion

According to our original data, of 245 OAB women underwent urodynamic examination, 117 (47.8%) had involuntary detrusor contractions similar to the findings reported earlier [14, 15]. That is, not all clinical OAB symptoms can be attributed to involuntary detrusor contractions. Hence, the idea that OAB symptoms can be caused by problems of the voiding phase has become more widely accepted. In the past, voiding dysfunction among women was often ignored or attributed to anti-incontinence surgery and pelvic organ prolapse. With better understanding of pelvic floor dysfunction, dysfunctional voiding is known to be the result of spastic external sphincter urethra or levator ani muscle. It is common among women with wrong voiding habits, habitual refraining from voiding, or chronic pelvic pain [4, 16-18]. Often these women have complaints similar to the typical OAB symptoms [4, 5].

Antimuscarinics are the first-line pharmacotherapy for OAB, however, urine retention is one of the side-effects, although the incidence is rare (1.1 to 6%) [19, 20]. Owing to the experience of voiding dysfunction after the standard dosage of administration, is why we chose a low-dose in this study. Even so, we still observed 19 of 163 women (11.7%) with voiding difficulty, which implies administration of antimuscarinics in treating OAB has to be precautious. Moreover, low-dose antimuscarinics appeared to be sufficient for the Taiwanese women in this study. The discrepancy of dosage used in most published reports and our study might be due to the difference in body frame between Western and Eastern populations.

Overactive bladder can be diagnosed according to medical history, physical examination and clinical symptoms without the aid of urodynamic examination. In this study, OAB women with abnormal voiding patterns suffering from voiding difficulty after antimuscarinic treatment had a higher incidence compared to those with normal voiding patterns (18% vs 4.9%, respectively; p = 0.013). If no urodynamic examination is performed before giving patients prescriptions, these patients could be given an antimuscarinic agent directly (according to the routine protocol) and be caused a higher rate of voiding difficulty. In view of this, physicians should pay greater care to the use of antimuscarinic agents for treating OAB syndrome diagnosed purely on the basis of clinical symptoms.

Even though urodynamics plays an important role in confirming abnormalities in both the storage and voiding phases, its invasive nature and discomfort causes patients to shrink back at the sight. Of 245 OAB patients who underwent urodynamic examination during the study period, 52 (21.2%) opted not to continue with the follow-up or receive treatment. Telephone interviews of these patients...
cases revealed that most patients complained of discomfort felt during the examination. To avoid possible infection during examinations and discomfort caused by placing the catheter, non-invasive uroflowmetry examination followed by measuring residual urine after voiding by ultrasonography could be performed. This alternative can offer accurate assessment of the voiding function instead of a troublesome full course of urodynamics.

In this study, our subjects were OAB patients without clinical voiding difficulty. Even so, there were 50.3% (82 of 163 patients) with abnormal voiding patterns revealed by urodynamics in the final analysis. Therefore it is no wonder that using longer acting antimuscarinic agents such as tolterodine caused more voiding difficulty in OAB patients with an abnormal voiding pattern compared with a normal voiding pattern (24% vs 4%, respectively; \( p = 0.008 \)). The incidence of voiding difficulty after prescribing short acting oxybutynin with low dosage made no difference despite the voiding pattern. Baclofen is a muscle relaxant and has been used for a long time as an agent for voiding difficulty resulting from spasm of the external urethral sphincter. Results shown in Table 2 reveal that combined use of baclofen with antimuscarinic agents could reduce the incidence of voiding difficulty among patients with abnormal voiding patterns. In other words, baclofen has beneficial effects when used in combination with an antimuscarinic agent for treating OAB patients with abnormal voiding patterns in terms of reducing or eliminating side-effects of voiding difficulty.

The results of our study also showed that OAB might be caused by many more complicated factors and the pathological mechanism merits further investigation. Administration with antimuscarinics in treating OAB women with abnormal voiding patterns had a higher incidence of voiding difficulty than in OAB women with normal voiding patterns. In view of this, the clinical use of antimuscarinic agents for treatment of OAB syndrome should be applied with care and at least after performing uroflowmetry examination or measuring residual urine. A urodynamic study is not a requirement for diagnosis of OAB. The combined use of baclofen with antimuscarinic agents might be a practical way to treat OAB with prevention of voiding difficulty. This was a preliminary study involving only a small number of patients; we believe larger studies with more patients are required to investigate the feasibility and effectiveness of this regimen are crucial before definite conclusions can be drawn.

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References

Address reprint requests to:
C.J. WANG, M.D.
Department of Obstetrics and Gynecology
Division of Gynecologic Endoscopy
Chang Gung Memorial Hospital
Linkou Medical Center and Chang Gung University College of Medicine, 5 Fu-Hsin Street, Kwei-Shan Tao-Yuan (Taiwan)
e-mail: wang2260@gmail.com
Interruption of a study of cervical ripening with isosorbide mononitrate due to adverse effects


Department of Obstetrics, Federal University of São Paulo (UNIFESP), São Paulo, SP (Brazil)

Summary

Purpose of investigation: The objective of this study was to evaluate cervix length and the presence of cervical gland area (CGA) in ultrasounds performed before and after the administration of vaginal isosorbide mononitrate (IMN) for cervical ripening. Results: After enrolling 11 patients, the study had to be discontinued due to adverse effects. Three patients requested that they be withdrawn. Headaches were reported by all patients. Nausea, dizziness, dyspnea, and vomiting were also reported. The average cervical lengths at 0, 16, 24 and 36 hours were 27.6, 27.7, 25.9, and 23.0 mm, respectively. CGA disappeared in one of seven patients. Conclusions: The use of IMN appears to increase the MBS, slightly reducing cervical length without altering the appearance of CGA. Considering the importance of maternal wellbeing during labor, the routine use of IMN cannot be recommended for cervical ripening in the third trimester due to the frequency and intensity of side-effects.

Key words: Cervical ripening; Isosorbide; Adverse effects; Cervical length measurement; Induced labor.

Introduction

According to the World Health Organization, cesarean sections (C-sections) should ideally account for no more than 15% of all deliveries. However, rates of surgical deliveries are often much higher than this standard, especially in Brazil [1]. Studies analyzing patients submitted to a C-section have found that the failure to induce labor is one of the main indications for surgical birth, occurring particularly among patients with a Modified Bishop score (MBS) lower than six [2-7]. It is estimated that 20% of pregnant women need to have their labor induced due to maternal or fetal reasons [8].

Several cervical ripening treatments have been tested, but the existing literature does not always agree about the efficacy of such treatments, indications, side-effects, risks, and the criteria indicating their selection. The ideal method should be fast and effective in terms of inducing cervical ripening, with minimal maternal and fetal side-effects, and it should be compatible with use at home. Currently, the principal method of inducing labor in patients with unfavorable cervixes involves the use of prostaglandins and their derivates. This method has the characteristic of causing cervical ripening, increasing the chances of success in birth induction; however, the method also causes uterine contractions that may lead to hypersystole and hypertonia, requiring inpatient fetal vitality monitoring.

Isosorbide mononitrate (IMN) was originally used for the prevention and treatment of precordial pain [9], but over the past years, IMN has been administered as a pre-induction cervical preparation method. IMN is a precursor of nitric oxide (NO), which acts on the cervix by stimulating enzyme pathways that release prostaglandins and guanosine monophosphate, thus generating morphological alterations in the cervix similar to the alterations associated with spontaneous cervical ripening [10, 11]. The recent literature reports that this method has better results as compared with a placebo [12, 13], as well as having lower tachisystole levels [8, 9] when compared with prostaglandins. Nevertheless, the literature has not yet reached a consensus about optimal dosage, nor has it satisfactorily determined the incidence and intensity of side-effects. Twenty-four hours after the administration of 40 mg of vaginal IMN, Bullarbo et al. [12] found 22% of patients to have begun labor, while Osman et al. [8], after 24 hours and two 40 mg doses, found 55% of the patients either in labor or having a MBS less than or equal to six. Headache is the most commonly reported side effect, although there is great disagreement regarding its frequency, which may vary from 11 to 88% of cases according to different publications [8, 12-14]. The intensity of the headache is also contradictory: while some authors classify it as mild [8], other authors refer to it as moderate [12].

The evaluation of the method’s efficacy is based on the increase of MBS, a method that likely introduces significant intra-examiner variation due to the subjective nature of the criteria that compose the score (position, consistency, effacement, dilation, and fetal station) [15]. Cervical length, as evaluated by endovaginal ultrasound, is effective in predicting the success of full-term labor induction, and it may constitute a more objective method of evaluation. According to the literature, a cervical
length of less than 27 mm is associated with successful labor induction in 76% of patients [16-24].

The high rate of C-sections performed in Brazil, as well as the lack of consensus regarding both the best cervix preparation method and the criteria that should guide cervix preparation, motivated this study on the use of vaginally-administered IMN in cervical ripening. The study’s objectives were to compare the ultrasound (US) characteristics of the uterine cervix before and after the use of vaginal IMN in cervical ripening and to assess the efficacy and safety of the treatment.

Materials and Methods

The study, approved by the Ethics Research Committee of São Paulo Federal University (UNIFESP), used both observational and descriptive methods. Data were collected in 2009 at the University’s Obstetric Center. Patients with clinical indications for labor induction were invited to participate in the research when the following inclusion criteria were met: modified Bishop score of less than six; gestational age greater than 32 weeks; fetal vitality preserved for a live fetus (evaluated by normal cardiotocography, obstetric US and umbilical artery Doppler velocimetry); and an ability to understand the nature of the study. Patients were excluded when there were contraindications for vaginal delivery; two or more previous C-sections; breech presentation; chronic headache; if there were contraindications for the use of isosorbide mononitrate; or when the fetal or maternal condition required delivery in less than 72 hours. Patients who satisfied the inclusion and exclusion criteria were invited to voluntarily participate in the study and were carefully informed of the study objectives and procedures, as well as the medication’s mechanism of action, safety, and potential side-effects. After signature of the informed consents, the patients’ data were recorded in a spreadsheet.

Prior to the administration of the medication, an endovaginal US was performed to evaluate the longitudinal length of the uterine cervix and the presence or absence of endocervical glands. This procedure was performed using a SonoAce 8000 Live (Medison, Seoul, Korea) machine with a 7.5 MHz endovaginal transducer. A subsequent vaginal US evaluation was performed following the vaginal administration of 40 mg IMN. Sixteen hours after the first treatment, the cervical evaluation and US were repeated, and another dose of IMN was administered if the patient’s MBS was below six. Twenty-four hours after the first dose of the test medication, a final evaluation was performed, and a new dose of the medication was administered if the MBS was below six. After 36 hours, misoprostol was administered vaginally in cases in which the patient’s MBS was below six and no contraindications were present. In cases of MBS greater than six, patients were given oxytocin instead.

During each evaluation, patients were questioned about possible adverse events such as headaches, dizziness, palpitations, nausea, vomiting, abdominal pain, and vaginal bleeding. To evaluate the headaches’ intensity, we used a numeric pain scale (1 to 10).

Results

A pilot study was performed to determine the sample size that would be required for a full-scale study. However, due to the intensity of the side effects, the research had to be discontinued after enrolling only 11 patients. Due to the small number of cases, we report the results in a descriptive manner.

The average patient age was 25 years, varying from 19 to 33 years old. The average gestational age was 40 weeks and two days, varying from 32 weeks and six days to 41 weeks and five days. This study included eight nulliparae, two primiparae and one multipara. In ten cases (90.9%) the indication for cervical preparation was post-term gestation and in one case the indication was fetal death.

The initial analysis of each patient’s MBS through vaginal examination, longitudinal cervix length, and endocervical glands (CGA) was performed by the same examiner via endovaginal US. The average initial MBS was 2.6 (ranging from 1-4), with a standard deviation of 0.9. We observed that CGA was present in seven out of the 11 patients, and no correlation was observed between the presence of CGA and the patient’s MBS. The average functional cervical length was 27.6 mm. Sixteen hours after the first dose of IMN, average MBS increased from 2.6 to 3.9. Patient 3 presented an MBS increase from 4 to 6, indicating the need for intravenous oxytocin. Patient 8 was in labor. The remaining nine patients all had an MBS score lower than 6. Out of the 11 patients, nine (82%) experienced headaches, and three (27%) asked to withdraw from the study because of this side-effect (patients 2, 3, and 5). To evaluate pain intensity, patients were asked to rate their pain on an analogical scale (0-10). On average, they reported pain of 5.7 during the first reevaluation. The patients who withdrew from the study reported the following intensities in their headaches, respectively: 5, 6, and 10. In addition to headaches, the following side-effects were each cited once: nausea, vomiting, dizziness, and hyperthermia. Out of the remaining patients with cervixes resistant to induction, two asked to leave the study (patients 2 and 5), opting for vaginal administration of misoprostol instead. One woman with favorable cervix conditions also asked to withdraw from the study (patient 3) and opted to induce labor with oxytocin. One patient was in labor during the first reevaluation, and seven patients remained for the continuation of cervical evaluation. Following confirmation of fetal vitality, a new IMN dose was administered to these seven women. The US evaluation did not demonstrate alterations in average cervical length, which stayed at 27.6 mm. To measure the uterine cervix length, three measurements were taken during each evaluation and then averaged. This method was supported by the intraclass correlation coefficient (ICC). At 0, 16, and 24 hours after the initiation of treatment, the following measurements were obtained, respectively: 0.978, CI (95%) [0.941; 0.994]; 0.993, CI (95%) [0.980; 0.998]; and 0.993, CI (95%) [0.971; 0.999]. The CGA remained visible in six of the seven patients in whom CGA was revealed during the US at the beginning of the cervical preparation. Assessing patients’ blood pressure, we observed that mean arterial pressure (MAP) tended to decrease. In the initial evaluation, the average MAP was 87.64 mm Hg. The average MAP was 81.84 mm Hg two
Interruption of a study of cervical ripening with isosorbide mononitrate due to adverse effects

Table 1. — Characterization of physical and ultrasound exams at 0, 16, 24, and 36 hours.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bishop</th>
<th>CGA</th>
<th>Cervical length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
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<td>5</td>
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<tr>
<td>7</td>
<td>1</td>
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<td>-</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>4</td>
<td>AFD</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Average</td>
<td>2.6</td>
<td>3.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

L = labor; VD = vaginal delivery; CD = cesarean delivery; AFD = acute fetal distress; I = patient requested study withdrawal.

Three vaginal deliveries and five cesarean deliveries were performed. The average time from the beginning of cervical preparation with IMN to delivery was 50 hours and 30 minutes. Considering only vaginal deliveries, the average time was 60 hours and 33 minutes. The average weight of the babies was 3,405 g and the average Apgar score was 8.75 at the first minute and 9.5 at the fifth minute (Table 2).

Discussion

Recently published data regarding cervix preparation with IMN [8, 25] was the motivation for this study. Those preliminary results indicated that the drug is low risk and does not increase fetal distress, suggesting that IMN could be a good alternative for the outpatient setting. This research was initiated with a pilot study that, in agreement with the literature [8, 25], demonstrated that IMN is an efficacious method for preparing the cervix. However, in this sample the side-effects were intense and lasting, making continuation of the research unfeasible. The principal side-effects of the drug were headaches, which were mentioned by all patients and cited by three as their reason for withdrawing from the study. These complications forced us to interrupt the protocol and report these adverse events to the Research Ethics Committee.

Regarding cervical changes with IMN, our results demonstrated an MBS increase of 1.3 ± 1.9 after 16 hours and 2.2 ± 1.7 after 24 hours. These data accord with the findings of the study with the largest sample size in the current literature [8], which reported increases of 1.36 ± 1.26 and 1.35 ± 1.15, respectively. Through the exclusive use of the medication, two patients, or 18% of the study sample, reached MBS values greater than or equal to six. Five patients, or 45% of the sample, were in labor before all of the reevaluations could be performed (at 16, 24, or 36 hours). Four of the patients used oxytocin, which precluded a reliable MBS assessment. Therefore, out of a total of 11 patients, seven patients experienced successful cervical preparation or labor induction (63.6%). Based on

hours later, 77.33 mm Hg four hours later, and 78.87 mm Hg six hours later. The average maternal heart rate increased after drug administration. At the successive readings, women’s average heart rates were, respectively, 85, 92, 97, and 99 beats per minute. Twenty-four hours into the study, the average MBS was 4.8, indicating a 0.9 increase from the 16-hour reevaluation and a 1.2 increase since the initial physical exam. One patient (8) was in labor, and the unit shift staff recommended that she be given intravenous oxytocin. During the vitality evaluation, one of the study patients (patient 9) presented an altered cardiotocography, indicating the need for a C-section. In that case, the presence of meconium had been detected in the amniotic fluid. Among the remaining patients, average cervical length decreased from 27.6 to 26.2 mm, and there was no reduction in the prominence of the CGA. Two patients (patients 1 and 6), initially asymptomatic, reported headaches with a 5/10 intensity after 24 hours. During this same period, another patient described worsening headache symptoms with no improvement despite having received oral analgesics. However, this patient reported the same intensity on the pain scale (8/10). Drowsiness and dyspnea were reported by one of the patients. A new dose of IMN was administered to the five patients with MBS score lower than 6. After 36 hours, one patient (patient 6) had delivered her baby prior to the final cervical evaluation. Three patients were in labor (patients 1, 4 and 11) and receiving oxytocin, as recommended by the hospital shift staff. One of the patients (patient 10) maintained an MBS lower than 6. Her score did not increase from the second reevaluation, remaining at 3. Her cervix showed a reduction of 5 mm in average length, and the CGA remained present. The administration of vaginal misoprostol was indicated for the analysis.
evidence that reduced cervical length is associated with premature labor and that prior to labor induction reduced cervical length is associated with a higher success rate in inducing labor, a decrease in the cervical length was expected after IMN-induced cervical ripening. However, upon evaluating the study results, we observed no significant alteration in cervical measurements, which did not meet our initial expectations.

In 1988, Sekya et al. suggested that there is a correlation between CGA disappearance and the beginning of labor [26]. In agreement with this analysis, Asakura et al. demonstrated in 2009 that CGA absence should be considered as an independent factor in predicting labor in pregnant women suspected of premature labor [27, 28]. We hypothesized that IMN-induced cervical inflammatory alterations could lead to diminished CGA visibility. However, this hypothesis was not confirmed in our study. It is important to note that the small sample size of this study limited the evaluation.

Analysis of the interval between the beginning of treatment and the delivery was hindered by the high frequency of side-effects. These adverse effects caused patients to become dissatisfied and ask to be withdrawn from the study, which then resulted in a high rate of surgical labor induction. While Thomson et al. [14] found a headache incidence of 23%, Osman et al. [8] reported an incidence of 88%.

Variations in administration route and pregnancy status could explain the different incidences of this side-effect. The vaginal route of administration results in faster absorption than the oral route, therefore potentially leading to a greater frequency of side-effects. When analyzing headache incidence distribution compared to gestational age, we observed that headaches were more frequent in studies performed during the third trimester compared to those performed during the first three months (Table 3) [32-36].

Past the halfway point in the pregnancy, especially during the third trimester, a fourfold increase in vasopressinase occurs. This is an aminopeptidase produced by the placenta that degrades vasopressin, a molecule that stimulates vascular contraction and elevates blood pressure [37]. The use of IMN during the third trimester probably accentuates the vasodilatation state caused by reduced vasopressin levels. This phenomenon could explain the greater incidence of headaches in that period when compared to the first trimester of pregnancy. Similarly, such vasodilatation is probably also responsible for the decreased blood pressure and increased heart rates observed after the medication’s administration. In addition to recording headaches in 100% of the cases, this study found that 18% of patients suffered from nausea and 9% from dizziness, similar to findings reported in the literature (Table 3).

Regarding fetal vitality evaluations, one patient had an alteration in her cardiotocographic readings showing the presence of meconium during labor. Reviewing the side-effects described in the literature, we found no reports of fetal distress associated with IMN. Since the case in this study was an isolated event, it did not seem to be related to the use of medication. Labor represents a decisive, transitional period for the patient and the fetus. It is an emotional time characterized by intense maternal and familial involvement. In the context of human labor care, labor induction, even when necessary, must be a peaceful, efficient, and satisfactory procedure for the patient.

In our study, the use of IMN generated significant and intense side-effects. These side-effects, most notably

| Table 2. — Perinatal outcome of the cervical preparation with isosorbide mononitrate. |
|-----------------------------------|-----------------|-------------|---------|---------|
| Patient | Type of delivery | AT (h) | Weight (g) | Apgar |
| 1 | CD | 49 h45 m | 3550 | 9/10 |
| 2 | CD | 27 h | 3780* | 9/9 |
| 3 | VD | 32 h40 m | 2050* | OF |
| 4 | VD | 80 h20 m | 3295 | 8/9 |
| 5 | CD | 47 h30 | 2940* | 9/10 |
| 6 | VD | 37 h30 m | 3570 | 9/10 |
| 7 | CD | 51 h45 m | 3450 | 9/10 |
| 8 | CD | 48 h30 | 3195 | 8/9 |
| 9 | CD | 26 h14 m | 3130 | 9/9 |
| 10 | VD | 50 h30 m | 3225 | 9/10 |
| 11 | CD | 46 h20 m | 3830 | 9/9 |

Average — 50 h30 m** 3405*** 8/9 **5/9**

*Patients excluded from the study after 16 hours. These patients were submitted to induction with misoprostol.

**To calculate the average, the excluded patients were not considered.

CD = cesarean delivery; VD = vaginal delivery.

| Table 3. — Incidence of side-effects after administering isosorbide mononitrate. |
|-----------------------------------|-----------------|-------------|---------|---------|
| Symptom | N | T (h) | Trimester | Headache | Nausea | Dizziness |
| Thomssen et al. [14] | 66 | 3 | 1st | 23% | 5% | 5% |
| Chanrachakul et al. [32] | 35 | 24 | 3rd | 7.3% | 5.5% | 2.6% |
| Li et al. [33] | 21 | 3 | 1st | 33% | 17% | 7% |
| Ekerhovd et al. [34] | 30 | 4 | 3rd | 80% | 3.3% | 20% |
| David et al. [35] | 15 | 3 | 1st | 0 | 0 | 0 |
| Osman et al. [8] | 200 | 36 | 3rd | 88% | 19% | 8.7% |
| Ballbaro et al. [12] | 100 | 24 | 3rd | 88% | 19% | 0 |
| Rameez et al. [36] | 78 | 72 | 3rd | 60% | 0 | 0 |
| Habib et al. [9] | 51 | 36 | 3rd | 11.7% | 0 | 0 |
| Bollapragada et al. [25] | 130 | 48 | 3rd | 66% | 17% | 16% |

N = sample size; T = time of evaluation after administering the test drug.
strong headaches during labor, caused both patients and their families intense discomfort and fear that other factors could be influencing the labor process and increasing the risks to mothers and babies. We also perceived that the medical team exhibited anxiety and insecurity in the presence of side-effects. This whole context certainly influenced the high rate of cesarean delivery reported in the study: seven out of 11 pregnancies (63.6%). It is important to note that the side-effect was so serious that we felt the study should be discontinued. While we made the decision to discontinue the research on ethical grounds, communicating these negative data is also an essential step.

In Brazil, patients are used to a high rate of cesarean deliveries. This lack of incentive to undergo vaginal delivery leads patients to tolerate less pain, including headaches. It is very important that obstetricians know about these findings, which, small sample size notwithstanding, concisely demonstrate the presence of side-effects. It is also important to highlight that IMN is a method intended for preparing the cervix, not inducing labor. Accordingly, it should not be utilized in patients who need to deliver in a short period of time, which also limits the conditions under which the use of this medicament is indicated.

In conclusion, due to the intensity and frequency of side-effects, we cannot recommend the routine use of IMN to induce cervical ripening in vaginal delivery in patients who have already reached the third trimester of pregnancy.

References


Address reprint requests to:
A.R. HATANAKA, M.D.
Department of Obstetrics
Federal University of São Paulo (UNIFESP)
Rua Pedro de Toledo, 980 cj 85
Vila Clementino
São Paulo - SP (Brazil)
CEP 04039-034
e-mail: alahatanaka@alanhatanaka.com.br
Pregnancy outcome of Moroccan and Turkish women in Belgium


1Department of Obstetrics, Antwerp University Hospital, Edegem
2Study Center for Perinatal Epidemiology, Brussels
3Department of Obstetrics, Gent, University Hospital (Belgium)

Summary

Objective: To compare perinatal outcome in women from Turkish and Moroccan descent versus autochthonous women in Belgium. Methods: Retrospective cohort study, data from an existing database, coupled with sociodemographic data from birth certificates. Results: There were more teenage pregnancies in the Moroccan and Turkish group, Moroccan women delivered more frequently after age 40 but Turkish women less frequently. In Moroccan and Turkish women the level of education was lower; they had less hypertension, fewer pregnancies after artificial reproductive technology and preterm deliveries, more diabetes and more grand multiparity. Moroccan women demonstrated more HIV infection. Planned cesarean section was less frequent in the Moroccan and Turkish group, and there was no difference for secondary cesarean section. Belgian women had more induction of labor, instrumental vaginal delivery and epidural anesthesia. There were more babies with low birth weight in both the Moroccan and Turkish group. Moroccan woman had more babies with a birth weight above 4,500 g. Total perinatal death rate was higher for Moroccan women while there was no difference between Belgian and Turkish babies. Conclusion: Moroccan women demonstrated higher rates of HIV infection and perinatal mortality, while in both Turkish and Moroccan women diabetes was higher and hypertension less frequent. Belgian women underwent more interventions during pregnancy.

Key words: Obstetrics; Ethnicity; Cesarean section; Perinatal mortality; Induction of labor.

Introduction

Since the 1990s around 20% of the mothers giving birth in the Flanders region, the northern half of Belgium, are of non-Belgian origin, meaning that at the moment of giving birth the mother does not have the Belgian nationality. A major part of these women are of Moroccan or Turkish descent. Already in the 1970s the outcome of pregnancies in migrant women was studied in Europe [1]. Since the 1980s papers have been published on the perinatal outcome of immigrants, mainly Turkish and Moroccan, in Belgium [2-4]. At that time multiparity, teenage pregnancy and pregnancy at the age of 40 and above were more frequent in Moroccan and Turkish women, whereas induction of labor, epidural anesthesia, multiple pregnancies and cesarean section were more common in women of Belgian origin. Moroccan but not Turkish women also had a significantly lower rate of preterm birth. Diabetes was more frequent in Moroccan, and hypertension more frequent in Belgian women.

The aim of the current study was to assess the differences in perinatal outcome if any, between ethnic groups 20 years later and to examine determinants of differences observed.

Material and Methods

Study population and setting

The Study Centre for Perinatal Epidemiology in Flanders collects data on all deliveries in the region of Flanders, the northern part of Belgium, covering over 99% of all deliveries in the region. Only deliveries after 22 weeks of gestational age are recorded. The district councils collect social data at the time of birth registration. Both anonymized data sets are linked by a common code number. Data were collected for all deliveries in the period between January 1, 2002 and December 31, 2006. For the ethnicity we used the original nationality of the mother as a proxy.

The following data were available in the data base: maternal age, level of education, human immune deficiency virus (HIV) serum status, hypertension, diabetes, parity, singleton or multiple pregnancy, use of artificial reproductive technology, gestational age (as provided by the attending physician or midwife), position of the baby at birth, the use of the vacuum extractor or forceps, primary or secondary cesarean section, previous cesarean section, induction of labor, colonisation with group B Streptococci (GBS), the use of epidural analgesia, birth weight, fetal sex, transfer of the neonate to a neonatal intensive care unit, fetal death, early neonatal death, and perinatal death.

The level of education was subdivided as follows:
- no education or lower grade education (primary school)
- lower degree of middle school
- higher degree of middle school
- non university higher grade
- university

Hypertension was defined as systolic blood pressure more than 140 mmHg and/or diastolic more than 90 mmHg. During the study period no sub classification in chronic hypertension, gestational hypertension or preeclampsia was registered. The diagnosis diabetes was accepted as provided by the treating physician on the file; no further subdivision in gestational diabetes, type 1 or type 2 was possible. A primary cesarean section was defined as a planned cesarean section with intact membranes and no labor. Every other cesarean section was considered a secondary cesarean section.

Preterm birth was defined as birth before 37 weeks of com-
completed gestational age, and extremely preterm birth was birth before 28 completed weeks. Low birth weight was defined as birth weight less than 2,500 g, and extremely low birth weight as less than 1,500 g.

Fetal death was defined as every fetus born dead with a birth weight of at least 500 g. Neonatal death was defined according to WHO criteria as death during the first 28 completed days of life per 1,000 live births and was subdivided into early neonatal death, occurring during the first seven days of life and late neonatal death after the seventh day but before the 28th completed day of life.

Data analysis

The three groups were compared using chi-squared testing comparing the Moroccan and Turkish group with the Belgian group as a reference. Odd ratios (OR) and 95% confidence interval (CI) were calculated. For the chi-squared test significance was accepted at $p < 0.05$. Relevant differences found at univariate analysis were analyzed in a stepwise logistic regression including relevant items available in the data base. The statistical package used was SPSS 16.0.

Results

Tables 1 and 2 present the most relevant maternal and neonatal outcomes. There were more than twice as many teenage pregnancies in the Moroccan and Turkish group as compared with the Belgian group. Pregnancy after age 40 years was more frequent only in the Moroccan women, while Turkish women demonstrated significantly fewer deliveries in this age group. There were significant-

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<th>Table 1. — Sociodemographic factors.</th>
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OR: Odds Ratio; CI: Confidence interval; IVF: in vitro fertilisation.

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<th>Table 2. — Pregnancy-related factors.</th>
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<td>Early neonatal</td>
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OR: Odds Ratio; CI: Confidence interval; IVF: in vitro fertilisation; ICSI: intracytoplasmic sperm injection; Horm: pregnancy after hormonal stimulation.
ly more mothers in the Moroccan and Turkish group who had no education or only primary school. In the Moroccan group nine women (0.07%) screened positive for human immune deficiency virus versus 80 (0.03%) in the Belgian group (p = 0.03, OR 1.97; 95% CI 1.06-3.67). There was no difference between the Belgian and the Turkish groups (p = 5, 0.05%, p = 0.31, OR 1.55; 95% CI 0.66-3.64). Both the Turkish (n = 277, 3.1%, p < 0.01, OR 0.61; 95% CI 0.54-0.69) and the Moroccan (n = 311, 2.5%; p < 0.001, OR 0.50; 95% CI 0.44-0.56) group had significantly less hypertensive disease in pregnancy versus Belgian women (n = 11,520, 4.9%). The prevalence of diabetes was higher in Moroccan (n = 378, 3.0%, p < 0.01, OR 2.37; 95% CI 2.16-2.61) and Turkish (n = 153, 1.7%, p < 0.01; OR 1.38; 95% CI 1.19-1.62) as compared to Belgian women (n = 2779, 1.2%). The number of grand multiparous women, defined as having had four or more deliveries, was higher in both the Moroccan and Turkish group; in the Belgian reference population it was 998 (4.8%) versus 2,646 (21.2%, p < 0.001, OR 4.53; 95% CI 4.35-4.71) and in the Turkish group 1,019 (11.2%; p < 0.001, OR 2.40; 95% CI 2.25-2.55).

In the period studied there was no difference in incidence of twins between the three groups but there were more high multiples (≥3) in the Moroccan group, 24 (0.19% p < 0.001; OR 2.25; 95% CI 1.73-3.66) versus six (0.06% p = 0.88; OR 0.9495% CI, 0.43-2.07) in the Belgian and 162 (0.07%) in the Belgian group.

The breech position was significantly less frequent in Turkish (n = 406, 4.4%, p < 0.01, OR 0.79; 95% CI 0.71-0.87) and Moroccan (n = 516, 4.1%, p < 0.001, OR 0.71; 95% CI 0.67-0.79) women, while in Belgian women this occurred in 13,018 (5.5%) pregnancies. Instrumental vaginal delivery using a vacuum extractor or forceps was performed significantly more in Belgian women; 25,612 (10.9%), underwent vaginal instrumental delivery versus 1,197 (9.4%, p < 0.001, OR 0.98; 95% CI 0.97-0.99) in Moroccan and 813 (8.8%, p < 0.001, OR 0.97; 95% CI 0.96-0.98) in Turkish women. The differences were even more outspoken for cesarean section. For elective cesarean section there were 26,954 (11.4%) in the Belgian group versus 881 (9.5%, p < 0.001, OR 0.98; 95% CI 0.97-0.99) in Turkish and 1,107 (8.7%, p < 0.001, OR 0.96; 95% CI 0.95-0.97) in Moroccan women. There was no difference regarding emergency cesarean section; 17,751 (7.5%) in the Belgian group versus 950 (7.4%, p = 0.11, OR = 0.99; 95% CI 0.99-1.01) and 659 (7.1%, p = 0.01, OR 0.99; 95% CI 0.98-0.99) in the Turkish group. In the Belgian group 8,542 (19.1% of all cesarean sections) had undergone a previous cesarean section, while this was significantly more (n = 338; 21.9%, p = 0.005; OR 1.18; 95% CI 1.05-1.33) for the Turkish group and there was no significant difference between the Belgian and Moroccan women (n = 408; 19.8%; p = 0.41; OR = 0.96; 95% CI 0.88-1.05).

Labor was more frequently induced in Belgian (n = 67,450; 29.2%) versus 2,992 (23.9%; p < 0.001, OR 0.77; 95% CI 0.74-0.81) for Moroccan and 1,946 (21.4%, p < 0.001, OR 0.67; 95% CI 0.64-0.71) for Turkish women. Carriership for GBS was reported more frequently in Moroccan (n = 2,199, 17.6%, p < 0.001, OR 1.31; 95% CI 1.26-1.38) women as compared to Belgian women (n = 31,826, 13.8%). GBS were less frequent in the Turkish group (n = 1,027, 11.3%, p < 0.001, OR 0.80; 95% CI 0.75-0.86). During delivery epidural analgesia was more frequently applied in Belgian women (n = 153,030, 66.2%) versus 6,309 (50.5%, p < 0.001, OR 0.54; 95% CI 0.52-0.56) in the Moroccan women and 4,925 (54.2%, p < 0.001, OR 0.62; 95% CI 0.59-0.64) in Turkish women.

There were relatively more babies in the Moroccan group (n = 645, 5.1%, p < 0.001, OR 1.25; 95% CI 1.16-1.35) who had to be transferred to a neonatal intensive care unit, whereas in the Belgian group there were 9,629 (4.1%), and in the Turkish group these were comparable (n = 375, 4.1%; p = 0.873; OR 0.99; 95% CI 0.89-1.09).

For further analysis we performed multiple logistic regression analysis including ethnic groups (Belgian, Turkish and Moroccan). In the model for hypertension we included the ethnic group, maternal age (for logistic regression maternal age was subdivided in 3 categories: younger than 20 years, between 20 and 34.9 years, and 35 or more years), diabetes, hypertension, gestational age (for logistic regression gestational age was subdivided in less than 34 weeks and 35 or more weeks), parity, multiple pregnancy, diabetes and level of education. After forward stepwise regression all factors entered remained significant demonstrating that controlling for level of education, diabetes, parity, age and multiple pregnancy still resulted in an increased risk for hypertension in Belgian women. For diabetes we entered the ethnic group, maternal age, parity and the level of education, and here also all factors remained significant confirming that being Turkish or Moroccan increases the risk for diabetes when controlling for age, parity and level of education.

For preterm birth we entered the ethnic group, age, parity, hypertension, diabetes and level of education and here we noted that diabetes was not a significant predictor of preterm delivery (p = 0.34), but all other factors remained significant.

For elective cesarean section we entered ethnicity, parity, gestational age, having undergone a previous cesarean section, diabetes and hypertension and all factors remained significant confirming that a Belgian women had a higher risk of undergoing an elective primary cesarean section.

Concerning perinatal death we entered into the model the ethnic group, maternal age, birth weight (for logistic regression birth weight was subdivided in less than 2,500 g, 2,500-4,499 g and more than 4,500 g) and level of education. In this logistic regression being a Belgian woman still showed a significantly lower risk for perinatal mortality (p < 0.001).

The logistic regression for a birth weight above 4,500 g included the ethnic group, maternal age, parity, hypertension and diabetes, and here also all factors remained significant.
Discussion

Different studies [5-7] have demonstrated more teenage pregnancies, more pregnancies after age 40 and a higher rate of grand multiparity in migrant groups in Europe. Our study confirms that grand multiparity is more frequent in the Turkish and Moroccan group.

The higher frequency of human immune deficiency in Moroccan women is disturbing; it might be due to chance and low number in general. This finding is in conflict with the general belief of most practitioners that the more traditional muslim women have a lower risk of sexually transmitted diseases such as human immune deficiency virus. Women of Moroccan descent also seem to be more frequent carriers of GBS than women from Turkish descent. We have no further data to analyze any possible explanation for this.

David et al. [5] demonstrated that hypertension was significantly more common in German than in migrant, mainly Turkish, women whereas anemia was significantly more frequent in women of non German ethnicity. We have no data on anemia, but we can confirm that in Turkish women and also in Moroccan women hypertension in pregnancy is less of a problem than in Belgian women.

Worldwide ethnic differences in perinatal morbidity and mortality have been reported [8], the causes of which have not been completely elucidated: inequities in the provision of health care as a cause and the need for specific ethnic approaches as a solution has been suggested [9]. Looking only at the social disparities does not take into account eventual genetic (carriers of autosomal recessive disorders can be more prevalent in some ethnic groups resulting in a higher frequency of congenital anomalies), environmental and dietary factors [10-15]. Furthermore ‘ethnicity’ is dynamic, every ethnic group in our multicultural society is surrounded by other groups influencing each other. Dietary habits will evolve when people move from one country to another, environmental influences such as pollution can be different depending on where people live, accessibility of health services will become better when more graduated health professionals from each ethnic group will become available and genetic constitution shifts when more interethnic couples are formed. No studies are available on these interactions in Flanders and our data cannot provide these. In previous studies it has been demonstrated that perinatal morbidity and mortality was higher in Turkish and Moroccan women (4) both for the 1995 and the 1998 cohort, and that the educational level of the mother is the most important determinant of infantile mortality in Flanders.

Schulpen et al. [16] found that for deliveries between 1999 and 1993 in the Netherlands, perinatal death in the Mediterranean group including Turkish and north African, mainly Moroccan women, was significantly higher compared to the Dutch group. In a smaller audit study on 135 perinatal deaths in 1999 in Amsterdam, the Netherlands, both Turkish and Moroccan women had double the risk for perinatal death as compared to Dutch women [12], mainly caused by early preterm birth and substandard care in Moroccan but not in Turkish women. In the present study perinatal mortality is still higher for the Moroccan group, but there is no difference in the Turkish group.

This suggests that the integration of the Turkish population in the Belgian health system is better than for the Moroccan population, but we cannot substantiate this from our data.

A high birth weight into Mediterranean/Turkish women has been reported in the Netherlands [17, 18]. David et al. [5] demonstrated in a German cohort significantly more neonates weighing more than 4,000 g than in the Turkish group. Studying the role of constitutional (fetal gender, parity, maternal age and height) and environmental (education, cohabitation state, maternal pre-pregnancy body mass index, smoking, alcohol consumption, depression, and work stress) factors influencing ethnic differences in term birth weights, Goedhart et al. [14] demonstrated that constitutional rather than environmental determinants explain the difference in birth weight between Dutch, Turkish and Moroccan newborns, limiting possibilities for prevention. We performed a multiple regression to predict birth weight above 4,500 g including ethnicity, diabetes, gestational age, parity, which demonstrated that even when correcting for diabetes, Moroccan women still have a higher risk of having a baby with a birth weight above 4,500 g.

Preterm delivery before 34 weeks was more frequent in German women as compared to migrant Turkish women, especially in the multiparous lower class group [5]. In Amsterdam, the Netherlands, Goedhart et al. [18] found Moroccan women to have a significantly lower risk for spontaneous preterm birth as compared to the Dutch group. There was a lower, but not statistically significant, rate of preterm delivery in the Turkish versus Dutch women. Both Moroccan and Turkish women have a lower risk for preterm birth as compared to Belgian women and the ethnic group remains a significant factor in multivariate analysis including diabetes, level of education, parity, age and hypertension. Similar findings have been reported in Germany and in the Netherlands [5, 19].

We previously demonstrated [4] that artificial reproductive technology is used less frequently in Turkish and Moroccan women in 1994 and in 1999, and this did not change in the period 2002-2006. A lower rate of epidural anesthesia in ethnic minorities has been reported [4, 5, 19, 20]. This has been explained both by offering epidural anesthesia less frequently to migrants by the labor staff and/or by lower demand by the women, both possibly influenced by communication problems. We confirm that Turkish and Moroccan women used epidural analgesia less, even when correcting for level of education, parity, multiple pregnancy and cesarean section.

Elective cesarean section was more frequent in Belgian women as compared to Turkish and Moroccan women, and there was no difference for emergency section; a similar finding has been reported in Germany [5]. The rates
of cesarean section in different ethnic groups are highly variable, e.g. in an analysis of 553,491 live births in Norway, Vangen et al. [21] found an increased cesarean section rate in women from India, Africa and Latin America, but not for Turkish and Pakistani migrants. On the contrary, in Switzerland a significantly higher cesarean rate has been described in both Turkish and Moroccan women, even when adjusting for maternal (education, age, parity) and infant (sex, gestational age) characteristics [22].

One major drawback of our study is that we use nationality at birth of the mother as a proxy for ethnic group. This means that women who were born in Belgium with as primary nationality Belgian, were considered as Belgian, although they may well consider themselves as ethnically Turkish or Moroccan.

**Conclusion**

In Belgium ethnic inequality in obstetric outcome is a reality. Moroccan women still have a significantly worse pregnancy outcome, including more human immune deficiency virus infection, higher perinatal mortality, and more macrosomia. Both Turkish and Moroccan women carry GBS more frequently, have more diabetes and less hypertension and more babies below 2,500 g. Belgian women more often undergo interventions such as instrumental vaginal delivery, elective cesarean section, induction of labor and epidural anesthesia.

**References**


Address reprint requests to: Y. JACQUEMYN, M.D. Department of Obstetrics Antwerp University Hospital Wilrijkstraat 10 2650 Edegem (Belgium) e-mail: yves.jacqueyn@ua.be
Melatonin use in unilateral total salpingectomy in rats

E. Sapmaz1, A. Kale2, N. Akpolat3
1Obstetrics and Gynecology Department, Firat University School of Medicine, Elazığ
2Obstetrics and Gynecology Department, Numune Educational and Research Hospital, Adana
3Pathology Department, Firat University School of Medicine, Elazığ (Turkey)

Summary

Objective: To investigate the effects of melatonin use in unilateral total salpingectomy on ovarian histology in rats. Setting: Firat University, Medical School, Obstetrics and Gynecology Department, Elazığ. Material and Method: Thirty adult, female rats of Wistar albino species with regular cycles were randomly allocated to three groups in the estrus phase. G1 (n: 10): The group where the abdomen was opened and closed, and left oophorectomy was performed six months later. G2 (n: 10): The group where left total salpingectomy was performed and followed by left oophorectomy six months later. G3 (n: 10): The group where the abdomen was opened, left total salpingectomy was performed 15 min after 10 mg/kg/IP melatonin administration, and left oophorectomy was performed six months later. Samples of the left ovary were fixed in formaldehyde. The preparations were stained with hematoxylin-eosin, and primordial, primary, secondary and tertiary follicles were counted. All the numbers were added up to determine the ovarian follicle reserve. Atretic follicles were counted. Corpus luteum and corpus albicans were counted. Number of total corpuses was calculated. Regression of the presence of angiogenesis within the corpus luteum was examined. Presence of fibrosis on the ovarian stroma was examined. An ordinal scale was formed for the presence of regression of angiogenesis within the corpus luteum and presence of fibrosis (none: 0p, present: 1p, markedly present: 2p). Follicle cysts in the ovary were counted. Kruskal Wallis variance analysis was used in the statistical analysis of data; p < 0.05 was considered significant. Results: Primordial follicle count, ovarian follicle reserve and regression of angiogenesis in the corpus luteum were found to be significantly lower (p < 0.05, Mann-Whitney U test) in G2, when compared to G1 and G3 (p < 0.05, Mann-Whitney U test). None of the rats in G1 and G3 had ovarian cysts, whereas five rats in G2 were identified as having macroscopic follicle cysts. Other data were found to be similar in G1, G2, and G3 (Kruskal Wallis variance analysis). Conclusion: Left total salpingectomy reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic ovarian cysts and fibrosis development. It leads to the development of macroscopic follicle cysts in the ovary at a high rate (50%) in the sixth month. Melatonin use eliminates these harmful effects. Melatonin can be used to avoid the unfavorable effect of total salpingectomy on the ovary.

Key words: Melatonin; Ovarian histology; Rat; Total salpingectomy.

Introduction

IVF-ET results (implantation, pregnancy rates etc.) are adversely affected in hydrosalpinx cases [1-3]. The most common method used in the treatment of hydrosalpinx cases is salpingectomy. Both retrospective and prospective studies have found that implantation and pregnancy rates increase in hydrosalpinx cases that undergo salpingectomy [4-6].

The effects of the salpingectomy procedure on the ovary are still debatable. Dar et al. [7] did not find any negative effect on the ovaries of cases who had laparoscopic salpingectomy due to ectopic pregnancy. Chan et al. [8], on the other hand, reported that salpingectomy by laparotomy did not have any negative effect on ectopic pregnancy cases, but that laparoscopic salpingectomy adversely affected the ovary. Theoretically, salpingectomy can lead to the impairment (hypoxia and/or ischemia) of ovarian perfusion in humans. Branches of the uterine artery are located in the blood vessel network of the mesosalpinx, and are necessary to feed the ovary [9]. Moreover, uterine artery ligation (UL) in rats reduces ovarian blood flow, and the resulting hypoxia and/or ischemia impairs ovulation [10].

Ischemia and/or reperfusion injury lead to the formation of oxygen radicals (superoxide, hydroxyl, peroxyl, alkoxyl, and singlet oxygen radicals). These oxygen radicals have a destructive effect on lipids in all membranes. The most effective radical is hydroxyl [11]. Consequently, cell membrane, lisosome membranes, and membranes of such cell organelles as endoplasmic reticulum etc. are destroyed, cells break down, and necrosis results [12, 13]. This event is called lipid peroxidation.

However, lipid peroxidation stimulates collagen gene transcription in cell culture [14, 15]. Sugino noted that the oxygen radicals and antioxidant system in the ovary had a part in many events of reproductive physiology (follicle development, oocyte maturation, ovulation, C. luteum function, and follicular atresia development). Oxygen radicals in the ovary are produced by neutrophils and macrophages, and reside in C. luteum and follicles. Furthermore, it was shown that oxygen radicals (ROS) inhibited oocyte development and increased degenerated oocyte count as well as apoptosis [16].

Melatonin, a pineal secretory product, modulates ovarian function and reproduction in mammals [17].
Melatonin is present in human pre-ovulatory follicular fluid at concentrations 3-fold higher than in peripheral serum [18]. The ampullar ends of mammalian fallopian tubes, where fertilization occurs, are bathed by follicular fluid. Thus melatonin in follicular fluid may play a physiological role in fertilization and early embryo development after in vitro fertilization. That is because of the fact that melatonin is a reaction oxygen species (ROS) scavenger [20].

Takasaki et al. [21] used oral melatonin in infertile women with poor quality oocytes and found a high intra-follicular melatonin amount and a low lipid peroxide amount. Melatonin use reduced degenerated oocyte count, and increased fertilized oocyte count.

Melatonin is effective on hydroxyl radicals, singlet oxygen, peroxyl radical, and superoxide anion, among oxygen radicals. It protects nucleus DNA, membrane lipids, and cytosolic proteins against oxidative stress [22]. Moreover, it supports SOD, GSH-Px, glutathione reductase, and glyoxide-6-phosphate dehydrogenase of the antioxidant system [23]. It has an inhibitory effect on nitric oxide synthetase [24]. Besides, melatonin is easily absorbed, and rapidly passes through the morphophysiological barriers (blood-brain barrier, placenta, etc.), by whichever route it is administered. It protects the cells of the organ it penetrates against oxidative stress. Furthermore, it has a protective effect on mitochondria, which is a cell organelle [25].

A PubMed search (melatonin, salpingectomy, rat) did not show any experimental study on this topic. Thus, we attempted to examine the effects of melatonin use in unilateral total salpingectomy on ovarian histology in rats.

Materials and Methods
This study was conducted in the experimental animal laboratory of Fırat University Medical School. Thirty 14-week-old adult female rats of Wistar albino species, weighing 190-220 g and with regular cycles, were kept in a room with a 12-hour light (08-22) and 12-hour dark photoperiod, at 21-23°C fixed temperature, and fed with standard pellet feed and tap water. Permission of the Ethics Committee of Fırat University Medical School was given for the study. Feeding was interrupted 18 hours before the experiment, and only water was allowed. The rats that were found to be in the estrus phase by vaginal cytology follow-up were administered 400 mg/kg/IP chloral hydrate to induce anesthesia. The animals were laid on the operation table on their backs. The abdomen was opened with a midline incision. The rats were randomly allocated to three groups.

G1 (n:10): The group where the abdomen was opened and closed.
G2 (n:10): The group where the abdomen was opened, and left total salpingectomy was performed.
G3 (n:10): The group where the abdomen was opened, and left total salpingectomy was performed 15 min after 10 mg/kg/IP melatonin (melatonin 1 g flacon, N-acetyl-5-methoxytryptamine; Sigma Chemicals Co.) administration.

Layers of the abdomen and skin were closed with 3/0 silk suture. The rats were monitored throughout the study for blood pressure, heartbeat and body temperature measurements. They were kept in different cages in groups of five. On the post-operative 180th day, the animals were anesthetized in the same way. The abdomens were opened, and the ovaries were taken out. Ovarian tissue was fixed in 10% formaldehyde for histological examination, and placed in paraffin blocks, from which 4 µm cross sections were prepared. The cross sections were stained with hematoxylin-eosin. Primordial, primary, secondary and tertiary follicles were counted in the preparations examined under light microscopy. Total follicle reserve was calculated by the sum of all [26]. An atretic follicle count was made. Corpus luteum and corpus albicans were counted, and the total number of corpuses was calculated. Regression of angiogenesis in corpus luteum was examined. Presence of fibrosis on the ovarian stroma was examined. An ordinal scale was formed for regression of angiogenesis in the corpus luteum and presence of fibrosis (none = 0p, present = 1p, markedly present = 2p). Follicle cysts in the ovary were counted.

SPSS 9.0 computer software was used for the statistical analysis. Kruskal Wallis variance analysis was employed in the statistical analysis of continuous and ordinal data. Bonferroni correction Mann-Whitney U test was carried out for parameters for which the level of significance was set at $p < 0.05$.

Results
The experiment was successful in all rats. Comparison between G1 and G3 showed that all values were similar (Kruskal Wallis variance analysis).

Comparison of G2 with G1 and G3 revealed that primordial follicle count, ovarian follicle reserve, corpus luteum count, and regression of angiogenesis in the corpus luteum were significantly lower in G2 ($p < 0.05$, Mann-Whitney U test), and atretic follicle count, fibrosis, and microscopic follicle cysts were found to be significantly higher ($p < 0.05$, Mann-Whitney U test). There were no macroscopic follicle cysts in G1 and G3, whereas macroscopic follicle cysts were found in five rats (50%) in G2 ($p < 0.05$, X² test).

Ovarian follicle cysts that are formed due to total salpingectomy lead to a decrease in ovarian follicle reserve elements and corpus luteum count, while increasing fibrosis. In other words, cystic degeneration occurs in the ovary.

Table 1. — Parameters examined in all groups (values are presented as mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial follicle (no.)</td>
<td>9.5 ± 2.6</td>
<td>4.3 ± 2.7</td>
<td>9.7 ± 2.4</td>
<td>*</td>
</tr>
<tr>
<td>Primary follicle (no.)</td>
<td>8 ± 1.8</td>
<td>9 ± 2.6</td>
<td>8 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary follicle (no.)</td>
<td>1.8 ± 0.8</td>
<td>1.8 ± 0.9</td>
<td>1.8 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Tertiary follicle (no.)</td>
<td>2.2 ± 0.8</td>
<td>2.2 ± 0.8</td>
<td>1.9 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian follicle reserve (no.)</td>
<td>21 ± 2.6</td>
<td>17 ± 3.3</td>
<td>21 ± 3.4</td>
<td>*</td>
</tr>
<tr>
<td>Corpus luteum (no.)</td>
<td>6.4 ± 1.4</td>
<td>3.7 ± 1.2</td>
<td>6.6 ± 1.3</td>
<td>*</td>
</tr>
<tr>
<td>Corpus albicans (no.)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>6.4 ± 1.4</td>
<td>3.7 ± 1.2</td>
<td>6.6 ± 1.3</td>
<td>*</td>
</tr>
<tr>
<td>Atretic follicle (no.)</td>
<td>0.1 ± 0.3</td>
<td>1.8 ± 0.8</td>
<td>0.2 ± 0.4</td>
<td>*</td>
</tr>
<tr>
<td>Corpus luteum angiogenesis (point)</td>
<td>2 ± 0</td>
<td>0.9 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>*</td>
</tr>
<tr>
<td>Stromal fibrosis (point)</td>
<td>0 ± 0</td>
<td>1.9 ± 0.3</td>
<td>0 ± 0</td>
<td>*</td>
</tr>
<tr>
<td>Cystic follicle (microscopic)</td>
<td>0 ± 0</td>
<td>1.3 ± 1.4</td>
<td>0.2 ± 0.6</td>
<td>*</td>
</tr>
</tbody>
</table>

NS = $p > 0.05$, Kruskal Wallis variance analysis.
* = $p < 0.05$, Kruskal Wallis variance analysis.

Different superscripted letters over the means show that the difference among groups was significant ($p < 0.05$ Mann-Whitney U test). Means were placed in descending order in the numbering process.
Discussion

Left total salpingectomy performed on rats by the laparotomy method reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic follicle cysts and fibrosis development in the sixth month. Moreover, it leads to macroscopic cyst development in the ovary (G1 = G3 = 0%, G2 = 50%). These cysts have a negative effect on ovarian follicle reserve and the corpus luteum, and a positive effect on fibrosis and atretic follicle development. Melatonin use restores these harmful effects. Melatonin can be used to avoid the negative effects of total salpingectomy on the ovary.

According to a Pub-Med search (melatonin, rat, total salpingectomy), our study is the first of its kind, and thus original in this respect.

Primordial follicles in the left total salpingectomy group were significantly reduced. That is because oocytes are surrounded by cumulus cells in follicles. It has been reported that cumulus cells have a close connection with oocytes during maturation. Strong immunostaining of Cu/Zn-SOD was found in cumulus cells [27]. Interestingly, there is a report showing that cumulus cells protect oocytes against oxidative stress by increasing the glutathione content, an antioxidant, in the oocytes in gilts [28]. Primordial follicle does not have cumulus cells. Therefore, it is the most susceptible follicle to lipid peroxidation.

However, Suzuki et al. reported that Cu, Zn-SOD was detected in theca interna cells of preantral, non dominant and dominant follicles, and in granulosa cells of only dominant follicles in human [29]. In addition, Mn-SOD expression is detected in both granulosa cells and theca interna cells in human follicles [29]. Cu, Zn-SOD and Mn-SOD protect granulosa cells and theca interna cells by scavenging superoxide radicals for normal steroidogenesis and follicular development. Primordial follicles do not have theca interna and granulosa cells. Therefore, they are the most susceptible follicles to lipid peroxidation. Granulosa and theca cells emerge in the primary follicle, and cumulus cells in the secondary follicle. Thus, primordial follicles were found significantly lower, and primary and tertiary follicles were found similar in G2. Our results are consistent.

An increase was established in macroscopic and microscopic follicle cysts in our left total salpingectomy cases in the sixth month. The ovarian cysts identified macroscopically in G2 were quite large, and were filled with a yellow fluid. Microscopic examination showed small follicle cysts. Ovarian structures (ovarian follicle reserve, corpus luteum) were atrophied, and fibrosis and atretic follicle count were significantly higher. As the number of macroscopic cysts increased, a decrease was found in ovarian follicle reserve and corpus luteum count, with an increase in fibrosis and atretic follicle count. This may be proof that the follicle cysts that develop in the ovary after salpingectomy adversely affect ovarian reserve. This event, called cystic degeneration [30], is consistent with our results.

Anderson et al. [31] found that there were luteolytic agents in the uterus (which affect mitochondria and lysosomes in the luteal cells, and ensure regression of the corpus luteum), and that regression in the corpus luteum was delayed when these substances could not be transported to the ovary due to the impairment of blood flow. Mean corpus luteum value in G2 was lower than that in G1 and G3. There seems to be a contradiction in the corpus luteum results of our study. Corpus luteum that is expected to increase in G2 was found lower, relative to G1 and G3. This may be explained by the fact that the corpus luteum structure in the ovaries of rats with macroscopic follicle cysts in G2 was found lower, since in the presence of an ovarian cyst, cystic degeneration is seen in the ovary. Our results are consistent with those of Tenney et al. [30]. The highest corpus luteum value was found in G3, which had fewer ovarian cysts. Our results are also consistent with those of Anderson et al. [31].

It was found that changes associated with angiogenesis in the corpus luteum did not regress in G2. In a normal rat ovary, capillary structures that are formed in the corpus luteum regress. Vascular endothelial growth factor (VEGF) plays the largest part in the formation of these structures in the corpus luteum. One of the major stimulants of VEGF is hypoxia [32, 33]. As we impair the blood flow in the utero-ovarian anastomosis during left total salpingectomy, hypoxia results [9, 10], which in turn possibly leads to an increase in angiogenesis in the corpus luteum, and a decrease in the regression of angiogenesis, through VEGF; this is how regression in G2 was lower than in G1 and G3. This may be attributed to hypoxia in the acute phase. The effect of hypoxia may relatively decrease because of anastomoses that develop in the long-term, as the effect of ovarian artery ligation (OL) on ovulation increases in time, whereas the effect of uterine artery ligation (UL) decreases in time [10]. This event may lead to damage like the one in ischemia-reperfusion. Melatonin curtails neutrophil infiltration and tissue destructive effects of neutrophils during ischemia-reperfusion, and particularly in reperfusion [34]. The fact that the damage in G3 was less than the damage in G2 can be attributed to this effect of melatonin. Our results are consistent.

Increase in VEGF secretion under hypoxic conditions has been shown in both human ovaries and fallopian tubes. VEGF increases vascular permeability, and regulates lumen secretion in the fallopian tube. The increase in vascular permeability in the ovary causes fluid formation in ovarian cysts [32, 33, 35]. VEGF secreted in the hypoxic environment may be responsible for the follicle cysts found in G2 and G3. Especially, the higher number of follicle cysts in G2 indicates that hypoxia is more effective in this group. Melatonin reduces this hypoxic effect through various mechanisms [22-25, 34]. The lower cystic development in the melatonin group can be explained by melatonin reducing the hypoxic effect. Our results are consistent.

Hypoxia-induced factor-1 (HIF-1) is activated in both the ovary and other organs in case of acute or chronic
Melatonin use in unilateral total salpingectomy in rats

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hypoxia [36-38]. HIF-1 alpha and hypoxic environment bring about regression and apoptosis in follicles, and result in an increase in atretic follicles and a decrease in follicular reserve [36]. The increase in atretic follicles and fibrosis, observed in G2, may be associated with the apoptotic effect of chronic hypoxia [38]. Our results are consistent. Melatonin has a favorable effect on microvascular perfusion, as it has a supportive effect on endothelium [39]. Restoration of microvascular perfusion will reduce the effect of hypoxia (HIF-1 alpha, VEGF). This may be one of the reasons why there is less damage in the melatonin group. Our results are consistent.

HIF-1 alpha also increases VEGF secretion. VEGF helps angiogenesis, increase in vascular permeability, normal functioning of folliculogenesis in the ovaries, development of follicle cysts in the ovary, and in the long-term, development of fibrosis via fibroblast growth factor 2 from the third week on [32, 33, 35, 40]. Moreover, VEGF directly stimulates collagen synthesis [41]. The increase in fibrosis and follicle cysts in G2 may be explained by VGEF.

Ovarian fibrosis and atretic follicle count were found low in G1 and G3, but significantly high in G2. Ovarian stroma contains collagen, contractile and interstitial cells [42]. In case of blood or lymphatic circulation impairment, collagen neoformation is stimulated [26]. Uterine and tubal lymphatics are very close to each other on the broad ligament [43]. Lymphatic circulation may be damaged during left total salpingectomy, which may cause an increase in collagen formation. Our findings are consistent with G2, but contradict G3. This suggests that other factors may be influencing fibrosis in the ovary. Actually, VEGF, the secretion of which increases under hypoxic conditions, boosts collagen synthesis both directly and through fibroblast growth factor 2 [40, 41]. The increase in fibrosis may be a mechanism compensating the decrease in ovarian follicle reserve [26]. Mean ovarian follicle reserves in G1 and G3 alike were higher than that in G2. Our results are consistent.

These data may explain why fibrosis was lower in the melatonin group. Our results are consistent. Melatonin may reduce fibroblast proliferation and collagen synthesis. Increased collagen levels in both intact skin and wounds have been observed following pinealectomy, whereas exogenous application of melatonin has caused the opposite effect [44]. Fibrosis caused by lipid peroxidation and its products decreases after the administration of antioxidants (melatonin, Vit E) in animal models [45-47]. That is why the melatonin group has no fibrosis. The protective action of melatonin may be related with its antioxidant activity.

Conclusion

Left total salpingectomy reduces primordial follicles, ovarian follicle reserve and regression of angiogenesis in the corpus luteum, while increasing atretic follicles, microscopic ovarian cysts and fibrosis development. It causes a high rate (50%) of macroscopic follicle cyst development in the ovary in the sixth month. Melatonin use corrects these harmful effects. Melatonin can be used to avoid the adverse effect of total salpingectomy on the ovary.

References


Address reprint requests to: A. KALE, M.D.
Yeni baraj mah 68070
sok no: 11/4
Seyhan/Adana (Turkey)
e-mail: ahmetkale5@yahoo.com
Clinicopathological changes of uterine leiomyomas after GnRH agonist therapy

C. Grigoriadis¹, E. Papaconstantinou², A. Mellou¹, D. Hassiakos¹, A. Liapis¹, A. Kondi-Pafiti²

¹12nd Department of Obstetrics-Gynecology
²Pathology Laboratory, Aretaieon Hospital, University of Athens, Medical School, Athens (Greece)

Summary

Objective: Gonadotrophin-releasing hormone agonist (GnRHa) has been commonly used for the medical treatment of prostate cancer, precocious puberty, endometriosis, adenomyosis and uterine leiomyomas. GnRHa therapy in cases of symptomatic uterine leiomyomas aims for the reduction of their size and remission of symptoms such as menometrorrhagia, causing a state of hypo-estrogenemia. This is considered to be a helpful preoperative strategy in cases of large myomas, or anemia because of abnormal vaginal bleeding. The aim of this retrospective study was to examine the clinicopathological changes in uterine leiomyomas exposed to preoperative GnRHa therapy for two up to six months. Materials and Methods: The study group consisted of 10 premenopausal patients who were treated with GnRHs prior to surgery. Results: In all cases the size of leiomyomas was reduced after GnRHa therapy. A microscopic review of the surgical specimens showed increased cellularity and ischemic type of necrosis. Conclusion: Morphological changes of uterine leiomyomas are often associated with preoperative GnRH agonist therapy. The differential diagnosis from uterine leiomyosarcomas includes absence of mitotic activity.

Key words: GnRH agonist; Uterine leiomyomas; Morphological changes.

Introduction

With the advent of isolation and synthesis of the gonadotrophin-releasing hormone (GnRH) by Schally et al. (1971) in the early 1970s, interest in the clinical application of GnRH agonist (GnRHa) has grown [1]. GnRH analogues are a class of drugs that downregulate hypophysial receptors, resulting in a direct refractoriness of the gland to new GnRH stimulus. That leads to a reduction of gonadotropins and ovarian steroid serum levels, with important changes in cell growth, cell cycle progression, apoptosis and expression of growth factors that determine regressive alterations in myometrial cells [2, 3].

Uterine leiomyomas are considered to be hormone-sensitive neoplasms, since estrogens have been shown to promote their growth as the concentration of receptors for estradiol is higher in leiomyomas compared to normal myometrium [4]. Chronic administration of GnRHa causes a ‘medical oophorectomy’ with a state of hypo-estrogenemia through suppression of ovarian follicular activity. This is followed by reduction of the volume of the estrogen-sensitive uterine leiomyomas and by remission of symptoms such as menometrorrhagia.

From a surgical point of view the operation is easier after GnRHa treatment because of the reduction of both uterine volume and blood flow. The surgical field is better and there is less bleeding [5, 6]. This is very important in order to avoid hysterectomy in cases of young premenopausal women where myomectomy is the only surgical approach for fertility preservation reasons. GnRHa preoperative therapy is also helpful in cases of anemia due to symptomatic uterine leiomyomas, in order to decrease abnormal vaginal bleeding prior to surgery and the need for transfusion during the operation. Although the endometrial morphology following GnRHa therapy has been described [7], the possible morphologic changes within leiomyomas have not been thoroughly studied [8-10]. The aim of this study was to analyze the clinical, surgical and histopathologic changes of uterine leiomyomas removed after GnRHa treatment.

Materials and Methods

From September 2009 to February 2011, ten patients who had received GnRHa therapy for two to six months prior to surgery, because of uterine leiomyomas, were seen in our Department. All women were premenopausal. They presented with either an asymptomatic pelvic mass diagnosed in a typical ultrasound (US) examination or with symptoms such as menometrorrhagia, menstrual disorders, infertility, and urine retention. The leiomyomas were resected by operative hysteroscopy, laparoscopic/abdominal myomectomy or hysterectomy. None of the patients had any history of recent pregnancy, other uterine surgery, or hormone replacement/contraceptive therapy.

This was a retrospective study. All necessary parameters (patient’s age, symptoms, number and size of uterine leiomyomas prior and after GnRHa therapy, type of surgery, surgical findings, blood loss during surgery and need for transfusion, type-dosage-duration of GnRHa treatment and time between the last GnRHa dose and the operation) were collected from the hospital history records of the study group patients as well as from their surgical and histopathological reports.

In all cases the specimens were fixed in 4% neutral buffered formaldehyde, examined and sectioned, and blocks were selected and processed. Hematoxylin-eosin stained slides were examined. Mitotic activity, cellularity, fibrosis degree, edema between the smooth muscle cells, vascular changes and cytologic atypia or necrosis – with cytoplasmic and nuclear changes – were studied during the histopathological examination.

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Results

Ten patients (27-48 years old, mean age 38.1 years), with prior surgical removal of their uterine leiomyomas and GnRHa treatment, were seen in our Department during this study period. The most common symptoms and signs were menometrorrhagia/menstrual disorders (50%), infertility (10%) and urine retention (10%). In the rest of the cases, typical gynecological and US routine examination revealed an asymptomatic pelvic mass.

Eight patients had been treated with triptorelin. Five out of these eight patients had received depot intramuscular injections of triptorelin (3.75 mg) administered every four weeks for periods varying from two months (two doses) to six months. Two patients received two doses, one patient three doses and in the other two cases the patients underwent six cycles. Three out of the eight patients who were treated with triptorelin received depot intramuscular injections of triptorelin pamoate (11.25 mg) administered every 12 weeks, from three months (one dose in one case) up to six months (two doses in another one patient). Two patients had been treated with another GnRH analogue, leuprorelin acetate, again using depot intramuscular injections (3.75 mg) at four weekly intervals for periods of two and three months, respectively.

All patients underwent surgery in a period of two up to five months after cessation of treatment with GnRH analogues. In one case hysteroscopic resection of a submucosal leiomyoma was performed. Seven patients underwent myomectomy and in two cases hysterectomy was necessary because of multiple large leiomyomas. In five cases a single leiomyoma was resected and examined, in three cases two leiomyomas, and in the last two cases multiple leiomyomas were removed within the surgical specimen of the uterus. In all cases leiomyomas maximum diameter at the time of surgery was reduced from 1.4 up to 3.2 cm in comparison with the US findings before GnRHa therapy. In only one case was there the need for transfusion during surgery.

The pathologic examination of the leiomyomas removed after GnRHa treatment showed cellular areas with moderate cellular and nuclear atypia of smooth muscle cells. Areas of ischemic type necrosis, hyalinization or hydropic degeneration of stroma were observed as well. No remarkable mitotic activity (0-1 mitoses/10 high-power fields HPF), or geographic type necrosis were observed (Figures 1, 2, 3). The morphology was consistent with the effects observed after GnRHa therapy.

Discussion

GnRH agonists are commonly used as an adjunct to surgery since the need for transfusion during surgery may be reduced and leiomyoma shrinkage may lead to an easier and safer operation, avoiding hysterectomy [11]. Their use is also indicated in cases of perimenopausal women with leiomyomas as a temporary treatment to reduce menometrorrhagia and associated anemia prior to the onset of menopause. On the other hand, long-term treatment with GnRHa can cause osteopenia, osteoporosis and vaginal dryness due to prolonged hypoestrogenism. That is why patients under GnRHa treatment have to receive calcium in order to avoid osteopenia. None of our patients received GnRHa therapy for more than six months and all were under calcium during the treatment period. Response to intramuscular GnRHa therapy occurs within three weeks of the initiation of the treatment [12], and the maximal rate of reduction of uterine and leiomyoma size occurs during the first month of GnRH agonist treatment [10]. However it is also well known that most leiomyomas return to pretreatment size in cases of premenopausal women after cessation of treatment if it is not followed by surgical resection [13].

Many studies demonstrate that the reduction of uterine volume is caused by the reduction of both leiomyoma and nonleiomyoma components of the uterus [14]. It is believed that hypoestrogenism caused by GnRHa leads to a significant reduction in uterine blood flow in pathologic lesions or in surrounding myometrial tissues as Doppler US examinations have shown [10, 15, 16]. These findings as well as the severe decrease in micro-vessel density in leiomyomas after GnRHa treatment could give an explanation to the mechanism of action of GnRHa therapy in the reduction of uterine size. In all our cases the diameter of leiomyomas was reduced after GnRHa therapy.
Also only in one case was transfusion necessary. This is probably because of reduced blood loss during myomectomy after GnRHa therapy.

In agreement with other studies [8, 9] we did not reveal any significant differences in mitotic activity, edema, fibrosis or vascular changes. The histopathologic findings of our study demonstrate hypercellularity and ischemic type of necrosis in leiomyomas that had been exposed to GnRHa therapy.

These results lead in many cases to diagnostic problems. However the absence of remarkable mitotic activity and cellular atypia is enough to exclude leiomyosarcomas from the differential diagnosis of these smooth muscle tumors.

In conclusion, pathologic changes in leiomyomas following GnRHa treatment, such as increased cellularity, ischemic type of necrosis, hyalinization or hydropic degeneration are often probably associated with this preoperative therapy. On the other hand this treatment leads to an easier surgery with less bleeding in order to avoid hysterectomy especially in young premenopausal women. Also in perimenopausal women it could be an ideal strategy to control menometrorrhagia and avoid the risks of an unnecessary surgery prior to menopause.

These are the main reasons for which the conclusion of many studies is that GnRHa therapy could result in a more cost-effective use of hospitalization and support services [9, 17].

References


Address reprint requests to:

C. GRIGORIADIS, M.D.
Kavafi 44
Dionysos Athens 14576 (Greece)
e-mail: xarisgrigoriadis@yahoo.gr
Clinical risk score to recognize macrosomia at the time of delivery

J. Patumanond¹, C. Tawichasri¹, S. Khunpradit²

¹Clinical Epidemiology Unit, Faculty of Medicine, Chiang Mai University, Chiang Mai
²Department of Obstetrics and Gynecology, Lamphun Hospital, Lamphun (Thailand)

Introduction

Macrosomia, defined as birth weight 4,000 g or more at the time of delivery, is one of the most recognized obstetric problems in many parts of the world. The prevalence varies roughly from 5% to 20% [1]. A postulated upward trend in some developing countries may be explained partly by adoption of westernized lifestyles, which brought to elevated direct risks of macrosomia [1, 2].

Macrosomia increases morbidity and mortality of both the mothers and newborns. In mothers, the rate of cesarean delivery increases, and in those with vaginal deliveries, pelvic floor injury, perineum laceration and postpartum hemorrhage may occur. In newborns, protracted labor, shoulder dystocia, birth trauma, brachial plexus injury, Bell’s palsy, hypoglycemia, polycythemia and jaundice are well documented [3-5].

Although macrosomia is highly associated with gestational diabetes mellitus (GDM), non-diabetic macrosomia is still an obstetric dilemma, as there is no clear consensus regarding its antepartum prediction and management [6]. A high proportion of macrosomic infants are born to non-GDM mothers. While screening and treatment may prevent macrosomia in GDM mothers, a high proportion of mothers carrying macrosomic infants are either not screened or screened as negative for GDM. Among all macrosomic newborns, 10% were undetected, leaving 90% unsuspected antenatally [7]. Many efforts to detect macrosomia earlier in the course of pregnancy for preventive intervention have been attempted by disclosing macrosomia risk factors and its prediction. Nevertheless, there was no evidence that macrosomic cases were centralized to larger, better equipped, maternity units, nor that planned cesarean delivery was scheduled for such cases [5].

Estimating birth weight with ultrasound has been reported in many studies [8], both using standard infant biometry and more sophisticated measurements [9]. Successful prediction by its combination with clinical characteristics was also reported [10-12].

In remote areas of many developing countries, ultrasound is not available. Ignorance of and incomplete antenatal care makes it more difficult to detect a relatively large-sized baby early in the course of pregnancy and it is not uncommon that women appear in labor rooms just before the time of delivery [13]. Precautious detection of macrosomia at the time of delivery in such women may still be important to help obstetricians in remote areas to centralize delivery to better equipped maternity units, or to translate such risk to pregnant women.

Patients and Methods

Study design and setting

A case-control study was designed from retrospective data at a university-affiliated general hospital in Lamphun, Thailand. Macrosomic cases were 67 women who delivered babies weighing at least 4,000 g. Controls were 779 women with babies weighing between 2,500 g. and < 4,000 g. The best predictors were selected by multivariable logistic regression and transformed into clinical risk scores. Result: The best combination of predictors included parity, gestational age at delivery, weight at delivery and symphysis-fundal height. The scores predicted macrosomia correctly with an AuROC of 94.1% (95% CI; 92.3, 95.6). The likelihood ratio of positive for macrosomia was 0 in the low risk category and 10.68 (95% CI; 7.76, 14.68) in the high risk. Conclusion: A simple clinical risk score may help obstetricians suspect macrosomia at the time of delivery in areas where antenatal care services are inadequate.

Key words: Macrosomia; Large for gestational age; Birth weight; Risk factors; High risk pregnancy; Clinical prediction rule.
Table 1. — Clinical characteristics of cases vs controls, evidence of difference (p value), area under receiver operating curve (AuROC) and 95% confidence interval (CI).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 67)</th>
<th>Controls (n = 779)</th>
<th>p value</th>
<th>AuROC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>28.9 ± 5.6</td>
<td>26.4 ± 5.3</td>
<td>&lt; 0.001</td>
<td>0.63 (0.60, 0.67)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.0 ± 0.9</td>
<td>1.6 ± 0.8</td>
<td>&lt; 0.001</td>
<td>0.65 (0.61, 0.68)</td>
</tr>
<tr>
<td>Parity</td>
<td>0.8 ± 0.7</td>
<td>0.4 ± 0.6</td>
<td>&lt; 0.001</td>
<td>0.66 (0.63, 0.69)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.2 ± 6.5</td>
<td>155.4 ± 6.0</td>
<td>0.020</td>
<td>0.57 (0.54, 0.60)</td>
</tr>
<tr>
<td>Prepregnancy weight (kg)</td>
<td>59.9 ± 13.6</td>
<td>50.6 ± 8.5</td>
<td>&lt; 0.001</td>
<td>0.74 (0.72, 0.78)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 4.5</td>
<td>20.9 ± 3.2</td>
<td>&lt; 0.001</td>
<td>0.74 (0.71, 0.77)</td>
</tr>
<tr>
<td>Weight at delivery (kg)</td>
<td>76.0 ± 13.1</td>
<td>65.2 ± 9.8</td>
<td>&lt; 0.001</td>
<td>0.77 (0.74, 0.80)</td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>16.2 ± 4.2</td>
<td>14.7 ± 4.8</td>
<td>0.013</td>
<td>0.59 (0.56, 0.63)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.4 ± 1.1</td>
<td>38.8 ± 1.2</td>
<td>&lt; 0.001</td>
<td>0.63 (0.59, 0.66)</td>
</tr>
<tr>
<td>Symphysis–fundal height (cm)</td>
<td>38.2 ± 2.2</td>
<td>33.7 ± 2.1</td>
<td>&lt; 0.001</td>
<td>0.94 (0.92, 0.95)</td>
</tr>
<tr>
<td>Gestational DM (n,%)</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>NA</td>
</tr>
<tr>
<td>Not screened</td>
<td>20 (29.9)</td>
<td>687 (88.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Screened negative</td>
<td>41 (61.1)</td>
<td>84 (10.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Screened positive</td>
<td>6 (9.0)</td>
<td>8 (1.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mode of delivery (n, %)</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>NA</td>
</tr>
<tr>
<td>Normal vaginal</td>
<td>22 (32.8)</td>
<td>494 (63.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Operative vaginal</td>
<td>13 (19.4)</td>
<td>112 (14.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cesarean</td>
<td>32 (47.8)</td>
<td>173 (22.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male infant (n, %)</td>
<td>46 (68.7)</td>
<td>427 (54.8)</td>
<td>0.030</td>
<td>0.57 (0.54, 0.60)</td>
</tr>
</tbody>
</table>

Data collection

All clinical characteristics were extracted from obstetric case notes. These included maternal age, height, prepregnancy weight, weight before delivery, pregnancy weight gain, gestational age at delivery, symphysis–fundal height, mode of delivery, birth weight and newborn gender.

Data analysis

Cases and controls were compared for evidence of differences (p value) in clinical characteristics with t-tests, rank sum tests or exact probability tests as appropriate. Prediction by each characteristic was calculated by univariable logistic regression and presented as an area under receiver operating characteristic (AuROC) curve and its 95% confidence interval (CI). Distribution of cases vs controls into low, moderate and high probability categories, likelihood ratio of positive (LHR+) and 95% confidence interval (CI), logistic regression beta coefficient (β) and assigned item scores.

Table 2. — Best multivariable clinical predictors, odds ratio (OR), 95% confidence interval (CI), logistic regression beta coefficient (β) and assigned item scores.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
<th>β</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>0.100</td>
<td>reference</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2.34</td>
<td>1.16, 4.72</td>
<td>0.017</td>
<td>0.85</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>6.23</td>
<td>2.22, 17.48</td>
<td>0.001</td>
<td>1.83</td>
<td>2.5</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 38</td>
<td>1.00</td>
<td>reference</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>38-39</td>
<td>2.07</td>
<td>0.53, 8.16</td>
<td>0.296</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 39</td>
<td>5.36</td>
<td>1.34, 21.45</td>
<td>0.018</td>
<td>1.68</td>
<td>2.5</td>
</tr>
<tr>
<td>Weight at delivery (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 63</td>
<td>1.00</td>
<td>reference</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>63-80</td>
<td>8.04</td>
<td>1.76, 36.86</td>
<td>0.007</td>
<td>2.08</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>13.23</td>
<td>2.62, 66.78</td>
<td>0.002</td>
<td>2.58</td>
<td>3.5</td>
</tr>
<tr>
<td>Symphysis–fundal height (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>1.00</td>
<td>reference</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>35-37</td>
<td>21.29</td>
<td>4.90, 92.48</td>
<td>&lt; 0.001</td>
<td>3.06</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 37</td>
<td>142.27</td>
<td>31.58, 640.90</td>
<td>&lt; 0.001</td>
<td>4.96</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3. — Distribution of cases vs controls into low, moderate and high probability categories, likelihood ratio of positive (LHR+) and 95% confidence interval (CI).

<table>
<thead>
<tr>
<th>Probability categories</th>
<th>Score</th>
<th>Cases (n = 67)</th>
<th>Controls (n = 779)</th>
<th>LHR+</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt; 5</td>
<td>0 (0)</td>
<td>422 (54.2)</td>
<td>0</td>
<td>-</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Moderate</td>
<td>5-10</td>
<td>22 (32.8)</td>
<td>308 (39.5)</td>
<td>0.83</td>
<td>0.58, 1.18</td>
<td>0.299</td>
</tr>
<tr>
<td>High</td>
<td>&gt; 10</td>
<td>45 (67.2)</td>
<td>49 (6.3)</td>
<td>10.68</td>
<td>7.76, 14.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td>11.4 ± 2.3</td>
<td>4.8 ± 3.2</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Results

There were a total of 67 cases of macrosomia and 779 controls. In comparison to controls, study cases were older (28.9 ± 5.6 vs 26.4 ± 5.3 years, p < 0.001), higher in gravidity (2.0 ± 0.9 vs 1.6 ± 0.8, p < 0.001) and parity.
Clinical risk score to recognize macrosomia at the time of delivery

(0.8 ± 0.7 vs 0.4 ± 0.6, \( p < 0.001 \)), were taller (157.2 ± 6.5 vs 155.4 ± 6.0 cm, \( p = 0.020 \)), had more prepregnancy weight (59.9 ± 13.6 vs 50.6 ± 8.5 kg, \( p < 0.001 \)), body mass index (24.1 ± 4.5 vs 20.9 ± 3.2 kg/m\(^2\), \( p < 0.001 \)), weight at delivery (76.0 ± 13.1 vs 65.2 ± 9.8 kg, \( p < 0.001 \)), and total pregnancy weight gain (16.2 ± 4.2 vs 14.7 ± 4.8 kg, \( p = 0.013 \)), and had larger symphysis-fundal height (38.2 ± 2.2 vs 33.7 ± 2.1 cm, \( p < 0.001 \)). Cases had a higher proportion of GDM screening (70.1% vs 11.8%) and positive results (9.0% vs 1.0%, \( p < 0.001 \)). More cesarean deliveries or operative vaginal deliveries (67.2% vs 36.6%, \( p < 0.001 \)) and male infants (68.7% vs 54.8%, \( p = 0.030 \)) were also observed. Among all clinical predictors, the prediction ability as measured by the AuROC curve was highest for symphysis-fundal height (Table 1).

The best multivariable clinical predictors for macrosomia were parity, gestational age at delivery, weight at delivery and symphysis-fundal height. These clinical predictors were each categorized into three levels; the optimal cut-off points for each characteristic was determined by the values at which the level yielded the smallest \( p \) values, and also the largest likelihood ratio obtained in logistic regression. An item score was assigned to each level of the four clinical characteristics by simple transformation of its logistic regression coefficient (Table 2). A summary risk score was obtained by adding up the item scores.

The discriminative ability of the derived risk score, which ranged from 0 to 15.5, could directly be observed by the different percentage distribution between cases and controls (Figure 1). The risk score predicted macrosomia with an AuROC curve of 94.1% (95% CI; 92.3, 95.6) (Figure 2) and with the \( p \) value for the Hosmer-Lemeshow goodness-of-fit test of 0.552. Internal validation by the bootstrapping method reduced the AuROC curve to 90.2%. When translating into absolute risks, the score predicted risk of macrosomia increased when the risk score moved upward, with close calibration to the actual or observed risks (Figure 3).

The risk scores were categorized into three risk groups, low (below 5) when the slope of the risk curve was lowest, moderate (5 to 10), and high (above 10) when the slope was highest, to facilitate clinical interpretation. The likelihood ratio of positive for macrosomia was 0 in the low risk category, 0.83 (95% CI; 0.58, 1.18) in the moderate and 10.68 (95% CI; 7.76, 14.68) in the high category (Table 3).
Discussion

Prediction of birth weight and macrosomia has been a challenge of practice in obstetrics. Undetected macrosomia results in perineal trauma, birth asphyxia and neonatal trauma related to surgical vaginal deliveries. On the other hand, false detection results in unnecessary cesarean delivery, followed by the risk of operative morbidity, mortality and an increase in costs of care [14]. Focus has been drawn mostly to high-risk pregnancy, especially among GDM. In day-to-day practice, a large proportion of macrosomic babies are born to non-gestational diabetic mothers and the majority of them were not detected before delivery [6, 7]. Focusing only on, and intervention given to, diabetic mothers are therefore not the global solution. We believe that prediction of macrosomia – not only in diabetic mothers – should be reconsidered.

Ultrasound and related techniques have been emphasized mainly when considering birth weight prediction [15, 16]. Their performance may be enhanced with more parameters [17], or in combination with clinical characteristics [10-13, 16, 18]. Although predictive accuracy varied from study to study, with some over or under estimation [6, 19], prediction of birth weight and macrosomia is still universally accepted valuable, particularly in a preventive context. The earlier the prediction capacity, the more valuable it should be [11]. This may be true in areas where ultrasound is easily accessible, where adequate antenatal care services are also achieved. In remote areas of many developing countries, where ultrasound may not always be accessible, and/or in areas where antenatal care may be ignorant or inadequate, it is likely that macrosomia may not be recognized at all until the time of delivery. In such situation, ultrasound prediction may be inapplicable. Obstetricians may be faced with the risk of macrosomia at the time of labor. We believe that prediction of macrosomia at the time of labor using only clinical characteristics (without ultrasound facility) may still be valuable.

Clinical characteristics known to increase the risk of macrosomic or large-for-gestational-age babies were previous delivery of macrosomia [20], increasing maternal age [2, 4, 19-21], gravidity and/or parity [3, 4, 21], maternal height [2, 20], prepregnancy weight [2, 20-22], pregnancy weight gain [20, 23, 24], weight at delivery [3, 20], gestational age at delivery [2, 3, 20, 22] and symphysis-fundal height [15]. Many of these clinical characteristics are recorded and readily accessible in routine practice. On the other hand, a male infant, also reported as one of the other hand, a male infant, also reported as one of the factors [2, 4, 20] is not known to all pregnant women, and its value on prediction of macrosomia is therefore limited. Likewise, screening for GDM [1,20] is not universal in many parts of the world, and its prediction value is also limited, such as observed in our setting, where up to nearly 30% of cases and 90% of controls were not screened for GDM. Mode of delivery as has been used in some prediction models represented a consequence of macrosomia, not its predictor, and was therefore not considered applicable in our study.

In a univariable analysis, most of the above-mentioned clinical characteristics were significantly different between cases and controls. However, their prediction value varied. In multivariable analysis, when considering all clinical characteristics simultaneously, the best predictors were symphysis-fundal height, weight at delivery, parity and gestational age at delivery. This is not surprising, as many of the clinical characteristics are highly correlated, such as prepregnancy weight and weight at delivery, or pregnancy weight gain and symphysis-fundal height [20]. The predictive values of the four clinical characteristics chosen by the model have previously been reported [1, 10, 11, 13, 15, 16, 18, 19, 25]. Their practical values are also enhanced by their routine accessibility and simplicity in application.

In our setting, women who scored below 5 were free from macrosomia (none out of 422 women). In those who scored more than 10, the likelihood of macrosomia increased by approximately ten times. Forty-five out of 94 (47.9%) women in this category were correctly identified by the derived clinical risk score. In this category, it may be worth while to centralize pregnant women in better equipped maternity units, in case macrosomia may result in unexpected consequences. Although cesarean delivery is not routinely recommended to non-diabetic mothers with expected macrosomia, such risks and delivery options may be worth mentioning, to let pregnant women decide on their own risks.

Retrospective data collection obtained under routine clinical practice may be limited by its precision. The validity of the results should be evaluated with a planned prospective data collection with calibrated instruments.

Like any other risk score prediction approaches, our derived score is likely to be space and domain specific, due to a different patient mix across health care facilities. Clinical characteristics used as clinical predictors in our setting may not be directly applicable to other settings. Model adjustment, either selection of different clinical predictors, and/or different scoring weight, should always be considered for application to a new setting.

Conclusions

A simple clinical risk score may help obstetricians suspect macrosomia at the time of delivery in remote areas where antenatal care services are less than adequate. Women in a high-risk category may be informed about their risk or centralized to deliver in better equipped maternity units.

Acknowledgments

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References

Clinical risk score to recognize macrosomia at the time of delivery


Address reprint requests to:
J. PATUMANOND, M.D., DSc
Head, Clinical Epidemiology Unit
Faculty of Medicine
Chiang Mai University
Chiang Mai 50200 (Thailand)
e-mail: jpatumanond@gmail.com
Outcomes of pregnancies in women with parity ten or more: a case control study

B. Demir¹, A.I. Guzel¹, S. Demir¹, F. Demir², Y. Celik³

¹Department of Gynecology and Obstetrics, Ergani State Hospital, Diyarbakir
²Diyarbakir Children’s Hospital, Diyarbakir
³Department of Biostatistics and Medical Informatics, Dicle University, Medicine Faculty, Diyarbakir (Turkey)

Summary
Purpose: To determine the outcomes of pregnancies in women with parity ten and more. Materials and Methods: We designed this study in a government hospital in rural Turkey. Pregnant women with parity of ten or more (n = 126) were evaluated and compared with pregnant women with parity lower than ten (n = 90). The risk factors recorded were maternal age, parity, gestational age (weeks), delivery mode, fetal birth weight and Apgar scores. Statistical analyses were carried out using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Results: During the study period, a total of 12,551 deliveries were delivered at the current clinic. One hundred and twenty-six mothers were delivered with parity ten or more with a ratio of 1.01%. There was a statistically significant difference between the study and control group by means of maternal age, parity, fetal birth weight and 1- and 5-min Apgar scores (p < 0.05). There was no difference in delivery mode between the groups. Conclusion: According to this study, pregnant women with parity ten or more showed no adverse clinical characteristics when compared with pregnant women with parity lower than ten.

Key words: Pregnancy; Parity; Grand multiparity; Clinical characteristics.

Introduction
Women that had ≥ 10 pregnancies are referred to as grand grand multiparas by Silva [1]. Grand multiparity is used to define women with parity of four [2] or five parity [3]. Juntunen et al. [4] reported a high incidence of grand multiparas because of religious beliefs of the Laestadius movement within the Lutheran church. These women do not accept any contraception method due to their religious belief. These pregnancies have been reported to be at increased risk for adverse obstetric outcomes. Schechter et al., [5] found in their study that patients with grand and grand grand pregnancies were at increased risk of labor dystocia and perinatal mortality. In a retrospective study, Kale et al., [6] reported that being at advanced maternal age with very high parity is not always related with adverse outcomes.

In the current study, we aimed to evaluate the outcomes of pregnancies with parity ten or more delivered in our clinic.

Materials and Methods
This retrospective and case control study was conducted at Ergani State Hospital in the south-east region of Turkey, from January 2002 to November 2010. One hundred and twenty-six pregnant women with parity ten or more were enrolled in this study. The control group included 90 pregnant women with parity lower than ten.

During the study period, a total of 12,551 deliveries were delivered at the current clinic. One hundred and twenty-six mothers were delivered with parity ten or more with a ratio of 1.01%.

The clinical characteristics of the groups are depicted in Table 1. The mean age of the patients and control group was 40.90 ± 3.76 years and 28.82 ± 5.12 years old, respectively. There was a statistically significant between the age of the groups (p < 0.05).

Mean parity of the patients and the control group was 10.95 ± 1.46 (range, 10-16) and 2.24 ± 2.01 (range, 0-9). The difference between the parity of the groups was statistically significant (p < 0.05).

Mean gestational weeks and fetal birth weight of the patients was 38.26 ± 2.35 weeks and 3387.80 ± 613.90 g, respectively. The mean 1-min Apgar scores of the patients and control group were 7.19 ± 2.01 and 6.42 ± 1.47, respectively. The mean 5-min Apgar scores of the patients and control group were 9.14 ± 0.86 and 8.40 ± 0.86, respectively. The difference between the Apgar scores was statistically different between the groups (p < 0.05).

In the patient group, 60 (47.61%) delivered spontaneously by the vaginal route, while 66 (52.38%) delivered by cesarean section. In the control group 39 (43.34%) of the patients delivered spontaneously by the vaginal route, and 51 (56.66%) delivered by cesarean section.

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Table 1. — Demographic and clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnant women with parity ≥ 10 (n = 126)</th>
<th>Pregnant women with parity &lt; 10 (n = 90)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.90 ± 3.76</td>
<td>28.82 ± 5.12</td>
<td>(&lt; 0.001^*)</td>
</tr>
<tr>
<td>Parity</td>
<td>10.95 ± 1.46</td>
<td>2.24 ± 2.01</td>
<td>(&lt; 0.001^{**})</td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>38.26 ± 2.35</td>
<td>38.22 ± 1.22</td>
<td>0.896*</td>
</tr>
<tr>
<td>Fetal birth weight (g)</td>
<td>3387.80 ± 613.90</td>
<td>3578.10 ± 550.77</td>
<td>0.020*</td>
</tr>
<tr>
<td>Apgar score - 1 min</td>
<td>7.19 ± 2.01</td>
<td>6.42 ± 1.47</td>
<td>0.02*</td>
</tr>
<tr>
<td>Apgar score - 5 min</td>
<td>9.14 ± 2.44</td>
<td>8.40 ± 0.86</td>
<td>0.07*</td>
</tr>
<tr>
<td>Delivery type</td>
<td></td>
<td></td>
<td>0.677**</td>
</tr>
<tr>
<td>SVD</td>
<td>60 (47.61)</td>
<td>39 (43.34)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>66 (52.38)</td>
<td>51 (56.66)</td>
<td></td>
</tr>
</tbody>
</table>

SVD: Spontaneous vaginal delivery; \( n \): Data presented as n (%); Bold \( p \) values are statistically significant. * \( p \) values were obtained by Student’s t-test; ** \( p \) values were obtained by chi-square test.

Discussion

In the present study, we evaluated the demographic and clinical characteristics of pregnant women with parity ten or more delivered at our clinic and also compared them with a control group of pregnant women with parity lower than ten. The age and parity were higher in the patient group. Fetal birth weight was higher in the control group. According to our study, pregnant group with parity ten or more was associated with lower fetal birth weight. The Apgar scores of the patient group was higher than the control group. Also there was no statistically significant difference between the groups for the delivery mode.

In a retrospective study, Humphrey [7] reported the characteristics of pregnant women with high parity. According to this study, pregnancies in women with high parity were not associated with poor maternal and obstetric outcomes. In these patients, spontaneous vaginal birth was higher than the pregnant women with lower parity.

Seidman et al. [8] reported their experience in pregnant women with parity seven or more. They found that these pregnancies were at decreased risk of small-for-gestational age infants and found no increased obstetric complications or neonatal morbidity and mortality. We also found no adverse maternal and fetal outcomes in our study group.

Kale et al. [6] studied 23 women with very high parity. They found no association between adverse maternal outcomes (hypertensive disorders, preterm labor, cesarean section) and fetal outcomes (lower Apgar scores, lower birth weight and perinatal mortality) in their study.

Similarly to this study, we also found no adverse outcomes in pregnant women with parity ten or more.

Brunner et al. [9] conducted a case control study to evaluate the obstetric risk factors in grand multiparous pregnant women. They reported that women were at increased risk of placental complications such as placenta previa and ablatio placenta. They also reported that with proper and good obstetric care no adverse maternal and fetal outcomes are seen in grand multiparous women. Similar to this study, we also found that being with parity ten or more did not increase the risk of adverse maternal and fetal outcomes.

In conclusion, according to the present study the maternal and fetal outcomes of pregnant women with parity ten or more were similar to the control group. Therefore, pregnant women with parity ten or more should not be at increased risk of adverse maternal and fetal outcomes.

References


Address reprint requests to: B. DEMIR, M.D.

Diyarpark 1 Sitesi, E blok, No: 15 Kayapinar, Diyarbakir (Turkey)
e-mail: bulentdemirmd@hotmail.com
Emergency peripartum hysterectomy cases in Agri: a 6-year review

M. Kara

Department of Obstetrics and Gynecology, Bozok University Medical Faculty, Yozgat (Turkey)

Summary

Purpose of Investigation: The aim of this study was to detect the incidence, indications, maternal and perinatal outcomes, and complications of emergency peripartum hysterectomy (EPH) as a life-saving operation. Methods: Fifty-four cases of emergency peripartum hysterectomy between June 2003 and June 2009 at the Obstetrics and Gynecology Department of the Agri Maternity and Children’s Hospital in Turkey, were analyzed retrospectively. Results: The incidence of EPH was found to be 1.87 per 1,000 deliveries. The most common indication for the procedure was uterine rupture (37.03%). There were three maternal deaths (5.56%). Wound dehiscence and wound infection were seen in eight (14.81%) and nine patients (16.67%), respectively. Discussion: EPH continues to be an important factor for maternal morbidity and mortality. In our study, we aimed to investigate the risk factors for peripartum hysterectomy. The most common three risk factors were uterine rupture, atony, and placenta accreta.

Key words: Peripartum hysterectomy; Incidence; Indication; Complication.

Introduction

Emergency peripartum hysterectomy (EPH) is a surgical procedure performed at the time of delivery or in the immediate postpartum period. Incidence is different in various parts of the world due to socioeconomic status, level of antenatal care, and family planning. Yucel et al. found an incidence of 0.29 per 1,000 births [1], Forna et al. reported the incidence in their study as 0.80 per 1,000 births [2]. Aryee et al. [3] found that the incidence of EPH was 4.34/1,000 deliveries. The indications of EPH have changed as time has passed. Previous studies have reported that the first two indications of EPH are uterine atony and rupture [4, 5], but in recent years placenta accreta and abnormal placentation have become the most common indications due to higher number of cesarean sections [6, 7]. EPH is associated with high maternal and fetal mortality and morbidity. In spite of the recent advances in modern obstetric practice, it remains a life-threatening complication of pregnancy [2].

This retrospective study was conducted to evaluate the incidence, indications and outcomes of emergency peripartum hysterectomy.

Material and Methods

The clinical records of emergency peripartum hysterectomy cases that were managed at the Obstetrics and Gynecology Department of the Agri Maternity and Children Hospital, Agri, Turkey, from June 2003 to June 2009 were analyzed retrospectively. EPH was defined as a hysterectomy for a lifesaving indication performed for hemorrhage within 24 hours of delivery. Subtotal and total hysterectomy operations were performed for the patients. Information was collected on patient characteristics, including age, socioeconomic status, parity, weeks of gestation, prior cesarean sections and maternal and fetal mortality/morbidity.

Low socioeconomic status was defined as yearly income of US$ ≤ 1,000. SPSS 9.05 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The mean and standard deviation were calculated for continuous variables; p < 0.05 was considered significant.

Results

During the study period 28,776 deliveries occurred in our clinic and cesarean section was performed in 4,052 of them. The rate of cesarean section was 14.08%. EPH was performed in a series of 54 cases. The incidence was 1.87 per 1,000 deliveries. The main indications for emergency hysterectomy were uterine rupture in 20 cases (37.03%), uterine atony in 18 cases (33.34%), and placenta accreta in seven patients (12.97%), respectively. The indications for EPH are shown in Table 1. The mean maternal age was 26.31 ± 7.12, gravidity was 4.06 ± 2.14, and parity was 2.92 ± 2.05. Demographic and clinical characteristics of the patients are shown in Table 2. Interestingly, low socioeconomic status was a factor in 38 women (70.37%).

Table 3 details the comparison of demographic and clinical characteristics according to the operation type. Subtotal abdominal hysterectomy was performed in 31 cases (57.40%), and total abdominal hysterectomy was performed in 23 cases (42.60%). There were no statistically significant differences between these two groups in terms of maternal age, gravidity, parity, gestational age, previous cesarean section, low socioeconomic status, fetal and maternal mortality.

Subtotal and total hysterectomy were compared regarding operation time, blood loss, blood transfusion, hospital stay, wound dehiscence, and wound infection (Table 4). Although operation time in the subtotal hysterectomy
Emergency peripartum hysterectomy cases in Agri: a 6-year review

Discussion

Most studies report the rate of occurrence for peripartum hysterectomies as between 0.26 to 5.40 per 1,000 deliveries [8, 9]. This variation is due in part to the different definitions regarding the time period for peripartum hysterectomy used in different studies, either within 24 hours of a delivery or during the same hospitalization period [9]. The high incidence of EPH could be related to the increasing number of cesarean sections, which in turn gives rise to an increased number of abnormal placenta and placenta previa. Sakse et al. reported that uterine scarring, especially with increased number of previous cesarean deliveries, increases the risk of peripartum hysterectomy, even in the absence of placenta previa [10]. The incidence was 1.87 per 1,000 deliveries and the rate of cesarean section was 14.08% in our study. The number of previous cesarean sections was 17 (31.48%).

Emergency peripartum hysterectomy is an important cause of maternal mortality, morbidity and perinatal mortality. We found that uterine rupture, atony, placenta accreta, obstetric hemorrhage due to other causes, abruptio placentae, and uterine inversion were associated with peripartum hysterectomy, respectively. Our findings were consistent with a previous study by Aryee et al. [3]. The most common indication for EPH was uterine rupture with a rate of 37.03% in our study, Yalinkaya et al. found a uterine rupture rate of 30.71% in 2010 [11]. The most important predisposing factor for uterine rupture is previous cesarean section [12, 13]. Uterine atony, defined as the lack of efficient uterine contractility after placental separation, is the most common cause of EPH and complicates 1/20 deliveries. Atony was seen at the rate of 33.34% as a risk factor for EPH in our study.

Previous cesarean section is also a risk factor for abnormal placental adherence. Placenta accreta is a condition where conservative measures such as curettage or suturing are of very limited success [14]. Our placenta accreta rate for EPH was 12.97% and this low proportion could be explained by the low previous cesarean section rate.

Hysterectomy is accepted as a definitive treatment for EPH. Prompt control of uterine hemorrhage is vital to decrease morbidity and prevent death. The operative technique for hysterectomy depends on the timing and indication for the procedure. Total hysterectomy should be considered when active bleeding occurs from the lower segment or cervix. Kastner et al. reported that the subtotal hysterectomy rate was 80% [15]. Our subtotal hysterectomy rate was 57.40%. We found that operation time, length of hospital stay, blood loss, blood transfusion, and wound dehiscence rates were lower in the subtotal hysterectomy group than the total hysterectomy group. On the other hand, wound infection rate was slightly higher in the subtotal hysterectomy group than the total hysterectomy group. Both subtotal and total hysterectomy are associated with high maternal and fetal mortality [16]. When the groups were compared in relation to fetal and maternal mortality there were no statistically significant differences in our study. Our total maternal mortality rate of 5.56% was similar to previous studies [1, 17].

Table 1. — Indications for emergency peripartum hysterectomy.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Number (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine rupture</td>
<td>20</td>
<td>37.03</td>
</tr>
<tr>
<td>Uterine atony</td>
<td>18</td>
<td>33.34</td>
</tr>
<tr>
<td>Placenta accreta</td>
<td>7</td>
<td>12.97</td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>4</td>
<td>7.40</td>
</tr>
<tr>
<td>Obstetric hemorrhage due to other causes</td>
<td>4</td>
<td>7.40</td>
</tr>
<tr>
<td>Uterine inversion</td>
<td>1</td>
<td>1.86</td>
</tr>
</tbody>
</table>

n = number of patients; % = percentage.

Table 2. — Demographic and clinical characteristics of the patients (n = 54).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age, year</td>
<td>26.31 ± 7.12</td>
</tr>
<tr>
<td>Gravidity (SD)</td>
<td>4.06 ± 2.14</td>
</tr>
<tr>
<td>Purity (SD)</td>
<td>2.92 ± 2.05</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>37.26 ± 3.91</td>
</tr>
<tr>
<td>Previous cesarean section (n, %)</td>
<td>17 (31.48)</td>
</tr>
<tr>
<td>Low socioeconomic status (n, %)</td>
<td>38 (70.37)</td>
</tr>
<tr>
<td>Fetal mortality (n, %)</td>
<td>18 (33.34)</td>
</tr>
<tr>
<td>Maternal mortality (n, %)</td>
<td>5 (5.56)</td>
</tr>
<tr>
<td>Number of referred patients (n, %)</td>
<td>16 (29.62)</td>
</tr>
</tbody>
</table>

n = number of patients; % = percentage.

Table 3. — Comparison of the demographic and clinical characteristics according to operation type.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subtotal hysterectomy (n = 31)</th>
<th>Total hysterectomy (n = 23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age, year (SD)</td>
<td>27.32 ± 7.24</td>
<td>25.81 ± 6.62</td>
<td>0.39</td>
</tr>
<tr>
<td>Gravidity (SD)</td>
<td>4.22 ± 2.26</td>
<td>3.76 ± 1.88</td>
<td>0.76</td>
</tr>
<tr>
<td>Purity (SD)</td>
<td>3.18 ± 2.14</td>
<td>2.71 ± 2.01</td>
<td>0.61</td>
</tr>
<tr>
<td>Gestational age, week (SD)</td>
<td>37.64 ± 4.09</td>
<td>36.75 ± 4.50</td>
<td>0.18</td>
</tr>
<tr>
<td>Previous cesarean section (n, %)</td>
<td>10 (32.5)</td>
<td>7 (30.43)</td>
<td>0.33</td>
</tr>
<tr>
<td>Low socioeconomic status (n, %)</td>
<td>21 (67.74)</td>
<td>17 (73.91)</td>
<td>0.84</td>
</tr>
<tr>
<td>Fetal mortality (n, %)</td>
<td>10 (32.5)</td>
<td>8 (34.78)</td>
<td>0.45</td>
</tr>
<tr>
<td>Maternal mortality (n, %)</td>
<td>2 (6.45)</td>
<td>1 (4.34)</td>
<td>0.76</td>
</tr>
<tr>
<td>Number of referred patients (n, %)</td>
<td>8 (25.80)</td>
<td>8 (34.78)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

n = number of patients, % = percentage, SD = standard deviation.

Table 4. — Comparison of the complications according to operation type.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Subtotal hysterectomy (n = 31)</th>
<th>Total hysterectomy (n = 23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time, minute (SD)</td>
<td>99.46 ± 36.84</td>
<td>112.37 ± 42.28</td>
<td>0.960</td>
</tr>
<tr>
<td>Blood loss, ml (SD)</td>
<td>1243.28 ± 179.25</td>
<td>1437.63 ± 246.08</td>
<td>0.089</td>
</tr>
<tr>
<td>Blood transfusion, unit (SD)</td>
<td>3.82 ± 2.84</td>
<td>4.61 ± 3.52</td>
<td>0.110</td>
</tr>
<tr>
<td>Hospital stay, day (SD)</td>
<td>6.41 ± 3.72</td>
<td>7.56 ± 5.03</td>
<td>0.288</td>
</tr>
<tr>
<td>Wound dehiscence (n, %)</td>
<td>5 (16.12)</td>
<td>3 (13.04)</td>
<td>0.364</td>
</tr>
<tr>
<td>Wound infection (n, %)</td>
<td>4 (12.90)</td>
<td>5 (21.73)</td>
<td>0.061</td>
</tr>
</tbody>
</table>

n = number of patients, % = percentage, ml = milliliter, SD = standard deviation.

Group was shorter than for the total hysterectomy group (99.46 ± 36.84 vs 112.37 ± 42.28) the difference was not statistically significant. There were no statistically significant differences between these two groups for any of these variables.
As a result, lack of antenatal care, normal vaginal deliveries which are performed by midwives, and especially low socioeconomic status might have been responsible for the increase of EPH in our clinic. However, the low previous cesarean section rate may have contributed a positive effect to decrease EPH in our region. Ultimately, EPH rates are not decreasing because of these risk factors and it could be possible to prevent hysterectomy by closely monitoring the women at particularly high risk and with early recognition of their risk factors.

References

Address reprint requests to:
M. KARA, M.D.
Department of Obstetrics and Gynecology
Bozok University Medical Faculty
66200 Yozgat (Turkey)
e-mail: mustafa.kara@bozok.edu.tr
Effects of daily intake of zidovudine-stavudine on rat pregnancy outcome: biological essay

E. Restum Antonio¹, T.M. Pereira Fontes¹, R.S. Simões², A. Moreira de Carvalho³, S. Espiridião³, M. Uchiyama Nakamura¹, L. Kulay Jr.¹

¹Department of Obstetrics, Sao Paulo Federal University, School of Medicine (UNIFESP-EPM), São Paulo (SP)
²Department of Obstetrics, Sao Paulo University, Faculty of Medicine (FMUSP), São Paulo (SP)
³Department of Internal Medicine, School of Medicine, Jose do Rosario Vellano University, Alfenas (MG)
⁴Department. of Obstetrics and Pediatrics, ABC Medical Foundation (FUABC), São Paulo (SP) (Brazil)

Summary

Purpose: To evaluate the effects at term of a highly active antiretroviral drug association when administered for the whole period of rat pregnancy. Methods: Forty pregnant rats weighing about 200 g were randomly divided into four groups: a control group (Ctr = drug vehicle control, n = 10) and three experimental groups, which were treated with an oral solution of zidovudine-stavudine (Exp1x = 10/1 mg/kg b.w., n = 10; Exp3x = 30/3 mg/kg b.w., n = 10; Exp9x = 90/9 mg/kg b.w., n = 10) from “day 0” up to the 20th day of pregnancy. Maternal body weights were recorded at the start of the experiment and on the 7th, 14th and 20th day thereafter. At term (20th day) the rats were anesthetized and submitted to hysterotomy. Implantations, reabsorptions, living fetuses, placentae and intrauterine deaths were looked for and recorded. The collected fetuses and placentae were weighed and the concepts were examined by a stereoscopic microscope looking for external malformations. Results: No significant alterations due to the antiretroviral drug treatment could be detected regarding the number of implantations, fetuses, placentae, absorptions and malformations nor regarding maternal and fetal mortality. Conclusions: Administration of the association zidovudine/stavudine for the whole period of rat pregnancy did not interfere with the maternal, fetal and placental weight gain as well as abnormalities detectable by the employed methodology.

Key words: Rat; Pregnancy; Zidovudine; Stavudine; Adverse effects.

Introduction

Due to the worldwide situation of the HIV infection, taking into consideration epidemiological and clinical aspects, there is urgent necessity to know the obstetrical challenges related to such condition in HIV-infected women [1, 2].

In September 2009 the United Nation Organization published a report estimating that HIV had infected 33.4 million people. About two-thirds of such population lived in the sub-Saharan region of Africa [3, 4].

In 1994, the Aids Clinical Trial Group (ACTG) 076, under subvention of the National Institute of Allergy and Infectious Diseases of the United States of America, pointed out that zidovudine (AZT) had reduced the perinatal transmission of HIV to newborns of infected women in two-thirds [5, 6]. From this outcome, zidovudine came to be the ideal monotherapy drug for prevention of perinatal HIV transmission. Its use in different countries resulted in lower levels of vertical transmission. Later on another experiment (ACTG 175) showed that there was a desirable effect of zidovudine on pregnant women at a level of CD4 < 200 cells/mm³ (very immunodepressive) as well as on women under use of AZT before the pregnancy period [7]. Between 1994 and 1997, other reverse transcriptase inhibitors were discovered. Among them is stavudine (d4T) which is analagous to nucleoside. Later on other classes of antiretrovirals were discovered. From that point on an associated therapeutic treatment was proposed that proved to be superior in relation to administrating one drug alone. The association resulted in a significant reduction of viral load in plasma. Sometimes the reduction of the viral load carried to undetectable levels of the virus [8, 9].

The associated therapy for women infected by HIV in fertile age has been used more and more. Due to this it is urgent to know the possible effects of such drugs on pregnancy as well as on the fetus. Because stavudine is very cheap, in spite of not being an eligible drug alone for AIDS treatment, it is largely used in some regions where such disease is prevalent and the population is very poor. Certainly one can not extrapolate from animal effects of drugs to human beings. On the other side, probably studying the effect of drugs on animal pregnancy is the best model to comprehend the effect of such drugs on humans. This experimental way of studying the effect of drugs on pregnancy was adopted by the Department of Experimental Obstetrics at the Federal University of Sao Paulo, Brazil. Drugs have been studied solely or in association with others. The aim of such studies is to verify any pharmacokinetic change of the antiretroviral drugs that can provoke adverse effects. It is well known that there are not a lot of trials of drug effects on rat pregnancy. This is one of the reasons that led us to such experiment. The choice for stavudine is related to it being an inexpensive drug and easy to use, in accordance with the WHO [10]. On the other hand, zidovudine has been systematically recommended for use in pregnancy since the ACTG 076 [11].

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Materials and Methods

Wistar female rats (Rattus norvegicus albinus) of the EPM-1 variant, with approximately 250 g of body weight, provided by the Center for the Development of Experimental Models (CEDEME) of the Federal University of Sao Paulo (UNIFESP- EPM) were used throughout. Experiments were approved (Report No. 1516/06) by the local Animal Care Committee, following guidelines which comply with those of the Canadian Council on Animal Care [11].

The animals were kept in plastic cages under controlled room temperature set at 22°C and artificial light by fluorescent lamps with a photoperiod of 12 h (lights on at 7 a.m.) with free access to pelleted Purina rat diet and tap water.

After a 7-day period of adaptation, the animals were mated in the proportion of one male to three females for 2 h. The immediate 24-h period after mating was taken as day 0 of pregnancy if spermatozoids were detected in vaginal smears [12]. Forty pregnant rats were then distributed at random into four animal groups, as follows: Control (Ctrl) = drug vehicle (propyleneglycol); experimental animals were treated daily with the association zidovudine/stavudine by gavage for the whole period of pregnancy: Exp1x = 10/1 mg/kg, Exp3x = 30/3 mg/kg, and Exp9x = 90/9 mg/kg b.w. respectively.

Results and Discussion

The zidovudine/stavudine association did not interfere with the maternal body weight directly. In fact, there was maternal body gain for the whole period of pregnancy. The control and experimental groups showed a normal curve represented by Figure 1. The matrix weight gain increased gradually up to the 14th day. The weight increase continued until the 20th day of pregnancy which means that the association of drugs did not interfere with such variable. Even high-dose zidovudine did not provoke changes in maternal weight gain when used solely (60 and 100 mg/kg daily). Such result is in accordance with Mamede et al. [13, 14] and Figueiró Filho et al. [15]. Similarly to stavudine used in 1-3 mg/kg daily and solely, it did not interfere with the matrix weight gain for the whole period of pregnancy [16].

The zidovudine/stavudine association did not interfere with the number of implantations and reabsorptions (Figure 1). This outcome is similar to the research of Mamede et al. [13] who used zidovudine alone. On the other side, the research of Barreto Mamede [16] that used stavudine solely showed increased reabsorptions when the administered dose was nine times the control one. This can be understood taking into consideration that zidovudine inhibits phosphorylation of stavudine. It mainly competes with stavudine in a phosphorylated way which provokes intracellular diminishing of stavudine triphosphate (active form) [17, 18]. In vitro studies of zidovudine/stavudine showed antagonistic results as well [19].

In relation to antiretroviral drugs it is known that reverse transcriptase inhibitors cross the syncytiotrophoblastic membrane quite well [20]. Through facilitated diffusion, drugs can get to intervillous spaces, since they fulfill some conditions: molecular weight less than 800, soluble in lipids, and not conjugable. Stavudine has a molecular weight of 224.2 Da [21]. The association AZT + dT4, due to the related antagonism, diminishes the mean effective
biodisponibility of stavudine which is 86.4% [22]. For this reason, it has a lower chance of promoting reabsorptions.

Our results showed that the association of zidovudine/stavudine had no effect on the weight, number of fetuses and/or placenta taking into consideration the three experimental groups (GExp1x, GExp3x e GExp9x) in relation to the control (Figure 1).

Zidovudine crosses the placenta barrier by simple diffusion at plasmatic levels similar to those observed in the maternal [23]. Crossing through the placenta is by passive diffusion. It is known that zidovudine is not able to change the placenta and fetus weight nor the fetus and placenta number, except in Figueiró et al.’s [15] research. In accordance with such authors diminishing of the number of fetuses was observed when zidovudine was used in a higher dose, i.e., ten times the therapeutic dose [13, 24, 25]. Research on the effect of zidovudine used solely on Wistar pregnant rats showed that it does not interfere with mean fetal and placenta weight and the number of fetuses and placenta even in high doses (9 times higher than the therapeutic dose). Only the group which had an intake of ten times the therapeutic dose showed diminishing of fetal weight and the number of fetuses [13, 15]. In our research stavudine alone taken at 1, 3 and 9 mg/kg daily had no effect on the fetus and placenta weight. In short, it had no adverse effect on albino rat pregnancy when taken in 1 and 3 mg/kg daily. But at 9 mg/kg daily there was increase in implantations and reabsorptions [16].

At the same time such association of drugs neither caused any malformation nor maternal and fetal deaths (Table 1). Other researchers administered zidovudine or stavudine alone for the whole period of albino rat pregnancy but observed no malformation or fetal deaths [13, 16].

Expansion of the use of associated antiretroviral drugs in human pregnancy diminished the occurrence of perinatal HIV infection leading to levels lower than 2%. On the other hand, this has brought into discussion the possibility of adverse effects of such associations on pregnancy immediately, and the impact on women and their children in the future [26].

Taking into consideration the modern antiretroviral triple therapy for HIV infection, it is often difficult to visualize the sort of interactions among the used drugs and which drugs are responsible for this or that effect. This challenge may be overcome by future biological assays using animal models when associated antiretroviral drugs can be used for a better comprehension of their effect on maternal and fetal bodies.


Address reprint requests to:
E.R. ANTONIO, M.D.
Av. Borges de Medeiros 2415 apto 201
Lagoa- Rio de Janeiro- RJ
Rio de Janeiro (RJ) (Brazil)
CEP 22470-002.
e-mail: elianarestum@yahoo.com.br
Importance of acupuncture on premenstrual syndrome

A. Anıl¹, T. Peker¹, T. Göktas², S. Kilic³, D. Erbaş²

¹Department of Anatomy, ²Department of Physiology, Gazi University Faculty of Medicine, Beşevler, Ankara
³Department of Reproductive Endocrinology and IVF, Dr. Zekai Tahir Burak Women’s Health Education and Research Hospital, Cebevi, Ankara (Turkey)

Summary

Aim: Premenstrual syndrome (PMS) is a complex group of symptoms. The clear reasons for PMS have not been understood completely. PMS includes emotional symptoms but mostly physical symptoms. Methods: The study was carried out on 11 patients (23-40 age range) diagnosed as having PMS. DSM IV was taken into account as the criteria for diagnosis. Ren2, Ren6, Ren12, LI4, LI11, P6, Liv3, Sp6, St36 and Du20 points were used on patients for the effects of acupuncture. The treatment of acupuncture was applied for three menses. Furthermore, NOx, MDA and GSH values in blood were studied. Results: The complaints of patients were observed to decrease or disappear completely. The most obvious changes were observed in myalgia, mastalgia and dysmenorrhea complaints (p < 0.000). Moreover, before starting the treatment of acupuncture, former blood values of NOx, MDA and GSH were compared with blood values of NOx, MDA and GSH after three cycles. An increase was observed for NOx levels after acupuncture treatment (p < 0.05). While there was no change in the oxidant stress indicator, MDA, an increase in antioxidant indicator GSH levels was observed (p ≤ 0.05). Conclusion: Acupuncture to treat premenstrual syndrome can be considered as an effective treatment modality.

Key words: Acupuncture; Premenstrual syndrome (PMS); NOx; MDA; GSH.

Introduction

The etiology of premenstrual syndrome (PMS) is not completely known today. Moreover, in epidemiological studies, in over 60% of women in the reproductive period, many symptoms in the premenstrual phase of the cycle are observed [1]. The symptoms, which are affective and somatic, are over a hundred [2]. While a group of women with some changes in lifestyle and conventional treatments reduce these symptoms, a large number have these problems in every menstruation cycle.

Acupuncture and acupressure have widely been used for the treatment of symptoms all over the world in recent years [3]. According to traditional Chinese Medicine theory, PMS is mainly due to functional disorders of the liver, spleen and kidneys leading to disturbances in blood and qi flow inside the body. Acupuncture has the effect of opening up the meridians and maintaining balance in the body, which generally eases the emotions and pain associated with menstruation [4].

Nitric oxide (NO) is a key regulator of local vascular tone and blood flow [5-7]. Local levels of NO vary according to various pathophysiological events and metabolic alterations. In the case of acupuncture, Ma et al demonstrated that NO synthase (NOS) expression is higher in meridian skin regions, including acupoints [8]. Li et al. reported an increase in NO content after warm needling [9]. These findings show that acupuncture stimulation could be a modulator of in vivo NO levels [7, 9].

In this study, we aimed to test the effect of acupuncture on the symptoms and blood NOx, reduced gestation (GSH), malondialdehyde (MDA) levels of PMS diseased women.

Material and Methods

In this study, the effects of acupuncture on PMS were studied on women who had a university degree, aged 23-40, and working in Zekai Tahir Burak Women’s Health Education and Research Hospital. Ethic committee approval and patient consents were taken. For the diagnosis of PMS, Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criterion was applied [10-13].

Eleven women who were diagnosed with PMS were treated with acupuncture. For the treatment of PMS, Ren2 (Qugu), Ren6 (Qihai), Ren12 (Zhonwan), LI4 (Hegu), LI11 (Quchi), P6 (Neiguan), Liv3 (Taichong), Sp6 (Sanyinjiao), St36 (Zusanli) and Du20 (Baihui) acu-points were used (Figure 1) [1, 3, 14, 15]. After determining the acupuncture points to be used for the treatment of PMS, the therapy was started. Treatments were applied every other day by starting one week before menstruation and ending at the onset of menses. Every session lasted for 20 min. The success of needling to the points was verified through the radiating deqi sensation starting from the needling point. Each treatment started with the DU20 point.

Any improvement from the treatment was registered on the complaint forms. In addition, by filling in three different scales – Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), and physical indications, the patients’ physical and psychological complaints from PMS were diagnosed. In the Beck depression test, including psychological changes, the participants were asked 21 questions in total. While grading, points were evaluated as: between 0-9 minimal, points between 10-16 slight, points between 17-29 middle and points between 30-63 severe depressive indicators. In the Beck anxiety test that consists of behavioral signs (0 = none, 1 = slight, 2 = middle, 3 = severe), 0-7 points were considered minimal, 8-15 points were slight, 16-25 were middle and 26-33 points were considered severe signs of anxiety. In the test involving physical symptoms, the degree of physical problems were graded with the same scores: (0 = none, 1 = slight, 2 = middle, 3 = severe).

In addition to these, blood samples were evaluated before the acupuncture treatment and one day after the last acupuncture session. Changes of the serum NOx, MDA and GSH were tested.
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The paired Student’s-t test was used to compare the psychological, behavioral signs (BAI) and physical changes of PMS women before and after acupuncture (BDI).

Results

The BDI, BAI and the test for physical signs were conducted before and after the acupuncture sessions, and $p < 0.001$ was considered as a statistically significant difference (Table 1).

The BDI has 21 items; 18 out of 21 have a significant decrease of problems. As a result of the BDI, a feeling of fatigue, anger, sadness and depression were significantly changed (respectively $p < 0.004$, $p < 0.006$, $p < 0.011$ and $p < 0.016$). Moreover, in the BAI, consisting of ten items, significant improvements were observed in seven of them.

Especially for the feelings: hot sensation, fatigue and vibration in the legs, and in the feeling of “bad things will happen”, significant improvements were observed (respectively $p < 0.004$, $p < 0.011$). In accordance with this, the problems of patients who had slight depressive symptoms and slight anxiety problems gradually decreased towards the end of the third cycle and came to a minimal level after the last session of acupuncture. The most apparent changes among the physical differences of PMS patients before and after acupuncture were seen for myalgia, mastalgia and dysmenorrhea ($p < 0.000$) (Table 2). However, almost no difference was observed in nausea problems before or after acupuncture.

Moreover, NOx, MDA and GSH blood levels before the acupuncture treatment and in the end of the third cycle were compared. For the NOx in relation to treatment with acupuncture, increased levels were observed ($p < 0.05$). For MDA, which is an oxidant indicator, the blood levels did not change; on the other hand, for GSH levels, an antioxidant indicator, increased levels were observed (Table 3).

Discussion

In the pathophysiology of PMS, many factors could be involved and play an important role: low progesterone level, high estrogen level, decrease in $\beta$ endorphins, lack of serotonin, increased activity of aldosterone, hypoglycemia, inhibition of the steroid hormone, thyroid malfunction, lack of magnesium, and lack of pyridoxine [16, 17].

According to NIMH criteria for the diagnosis of PMS, at least a 30% increase in symptoms must be observed, complaints must be noted for at least two to three months and the acuteness must be scored [18]. In our study, test results – applied to each person individually – were recorded separately. The mid-level complaints were reported to decrease to around a 50% lower degree.

As the cause of PMS is not exactly known, treatment modalities are changeable [19]. There are many types of treatment and the basic principle is to stimulate ovulation and regulate the hormonal changes in blood. Oral contraceptives are the most commonly used drugs for this purpose [20]. In addition to these, for the dominant symptoms, symptomatic treatments are conducted. For instance, to resolve edema from the body, diuretics like spironolactone are given and salt-fluid consumption is limited. For head, waist, legs and groin ache, painkillers such as naproxen, ibuprofen, and mefenamic acid are

Table 1. — Psychological, behavioral and physical symptomatic changes in the pre- and post-acupuncture period.

<table>
<thead>
<tr>
<th>Test</th>
<th>Test scores before acupuncture</th>
<th>Test scores after acupuncture</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological changes</td>
<td>16.82 ± 9.41</td>
<td>7.36 ± 4.86</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>(Beck depression test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavioral symptoms</td>
<td>9.9 ± 6.93</td>
<td>5 ± 3.52</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>Physical symptoms</td>
<td>14.45 ± 4.69</td>
<td>7.82 ± 4.38</td>
<td>$p &lt; 0.05$</td>
</tr>
</tbody>
</table>

Figure 1. — Acupuncture points for the treatment of premenstrual syndrome.

Ren 2 (Qugu), Ren 6 (Qihai), Ren 12 (Zhonwan) points on ren meridian;
LI 4 (Hegu), LI 11 (Quchi) points on large intestine meridian;
P 6 (Neiguan) point on pericard meridian;
Liv 3 (Taiqian) point on liver meridian;
Sp 6 (Sanyinjiao) point on spleen meridian;
St 36 (Zusanli) point on stomach meridian;
Du 20 (Baihui) point on du meridian.
Importance of acupuncture on premenstrual syndrome

Table 2. — Substantial differences related to the psychological, behavioral and physical tests in the period of pre- and post-acupuncture.

<table>
<thead>
<tr>
<th>Test</th>
<th>Before acupuncture</th>
<th>After acupuncture</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeling sad and depressed</td>
<td>1.00 ± 0.89</td>
<td>0.55 ± 0.69</td>
<td>p &lt; 0.016</td>
</tr>
<tr>
<td>Hopeless and pessimistic view for the future</td>
<td>1.00 ± 1.09</td>
<td>0.36 ± 0.50</td>
<td>p &lt; 0.026</td>
</tr>
<tr>
<td>Feeling unsuccessful</td>
<td>0.45 ± 0.69</td>
<td>0.09 ± 0.30</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>Not enjoying too many things</td>
<td>1.00 ± 0.63</td>
<td>0.55 ± 0.52</td>
<td>p &lt; 0.053</td>
</tr>
<tr>
<td>Feeling guilty</td>
<td>0.90 ± 0.83</td>
<td>0.54 ± 0.52</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>Not to be happy</td>
<td>0.90 ± 0.53</td>
<td>0.52 ± 0.50</td>
<td>p &lt; 0.006</td>
</tr>
<tr>
<td>Criticizing herself due to own mistakes</td>
<td>0.81 ± 0.60</td>
<td>0.45 ± 0.52</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>The feeling of crying</td>
<td>1.09 ± 0.83</td>
<td>0.36 ± 0.50</td>
<td>p &lt; 0.024</td>
</tr>
<tr>
<td>Aggression</td>
<td>0.81 ± 0.75</td>
<td>0.18 ± 0.40</td>
<td>p &lt; 0.011</td>
</tr>
<tr>
<td>Not talking and meeting others</td>
<td>1.00 ± 0.89</td>
<td>0.54 ± 0.52</td>
<td>p &lt; 0.053</td>
</tr>
<tr>
<td>Seeing old and ugly when looking at a mirror</td>
<td>1.00 ± 0.77</td>
<td>0.27 ± 0.14</td>
<td>p &lt; 0.024</td>
</tr>
<tr>
<td>Not being able to work as effectively as before</td>
<td>1.27 ± 0.90</td>
<td>0.72 ± 0.64</td>
<td>p &lt; 0.025</td>
</tr>
<tr>
<td>Feeling tired quickly</td>
<td>1.18 ± 0.87</td>
<td>0.45 ± 0.52</td>
<td>p &lt; 0.004</td>
</tr>
<tr>
<td>Anxiety of being punished</td>
<td>0.90 ± 1.22</td>
<td>0.27 ± 0.46</td>
<td>p &lt; 0.026</td>
</tr>
<tr>
<td>Numbness and tingling</td>
<td>0.72 ± 0.64</td>
<td>0.27 ± 0.46</td>
<td>p &lt; 0.016</td>
</tr>
<tr>
<td>Hot abscess/ Temperature</td>
<td>1.18 ± 1.16</td>
<td>0.45 ± 0.68</td>
<td>p &lt; 0.004</td>
</tr>
<tr>
<td>Weakness and tremor in the legs</td>
<td>1.00 ± 1.18</td>
<td>0.36 ± 0.67</td>
<td>p &lt; 0.011</td>
</tr>
<tr>
<td>Fear of very bad events in the future</td>
<td>1.18 ± 1.25</td>
<td>0.54 ± 0.68</td>
<td>p &lt; 0.011</td>
</tr>
<tr>
<td>Palpitation of heart</td>
<td>0.72 ± 0.90</td>
<td>0.18 ± 0.40</td>
<td>p &lt; 0.025</td>
</tr>
<tr>
<td>Fear of losing balance</td>
<td>0.72 ± 0.78</td>
<td>0.36 ± 0.50</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>Flushing</td>
<td>1.18 ± 1.40</td>
<td>0.54 ± 0.68</td>
<td>p &lt; 0.046</td>
</tr>
<tr>
<td>Headache in the pre-menstruation</td>
<td>1.18 ± 0.98</td>
<td>1.09 ± 0.70</td>
<td>p &lt; 0.004</td>
</tr>
<tr>
<td>Migraine in the pre-menstruation</td>
<td>0.72 ± 1.10</td>
<td>0.36 ± 0.67</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>Pain, repletion and swelling in the breasts</td>
<td>2.45 ± 0.68</td>
<td>1.27 ± 0.78</td>
<td>p &lt; 0.000</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2.27 ± 0.78</td>
<td>1.27 ± 0.64</td>
<td>p &lt; 0.000</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>1.36 ± 1.20</td>
<td>0.72 ± 0.78</td>
<td>p &lt; 0.011</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>2.45 ± 0.68</td>
<td>1.09 ± 0.83</td>
<td>p &lt; 0.000</td>
</tr>
<tr>
<td>Chin and face ache (TME ache)</td>
<td>0.81 ± 0.87</td>
<td>0.45 ± 0.68</td>
<td>p &lt; 0.038</td>
</tr>
<tr>
<td>Forgetfulness</td>
<td>1.90 ± 0.94</td>
<td>1.18 ± 0.60</td>
<td>p &lt; 0.012</td>
</tr>
</tbody>
</table>

Table 3. — Changes of the rates of NOx, MDA and GSH in the pre- and post-acupuncture.

<table>
<thead>
<tr>
<th>Test</th>
<th>Before acupuncture</th>
<th>After acupuncture</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx (nmol/ml) (n = 11)</td>
<td>38.42 ± 5.15</td>
<td>33.41 ± 6.22</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MDA (µmol/g tissue) (n = 11)</td>
<td>2.31 ± 0.30</td>
<td>2.40 ± 0.36</td>
<td>ns</td>
</tr>
<tr>
<td>GSH (µmol/g tissue) (n = 11)</td>
<td>2.03 ± 0.56</td>
<td>1.65 ± 0.23</td>
<td>p ≤ 0.05</td>
</tr>
</tbody>
</table>

As an estrogen antagonist, danazol is also preferred as a treatment modality [22]. Moreover, while foods consisting of refined sugar, red meat, alcohol and caffeine products (coffee, coke, chocolate) and unsaturated fat consumption are limited, green vegetables, fruit and pulse consumption are recommended [21, 23].

Acupuncture, as a treatment method, is being used increasingly for gynecological problems [3]. Habek et al., with the aim of determining the activity of acupuncture in PMS patients, practiced real acupuncture and placebo acupuncture for two groups of PMS patients. While the symptoms related to PMS were improved in 77.8% of the real acupuncture group, the symptoms were improved in only 5.8% of the placebo acupuncture group [1]. In the present study, differently from Habek et al., behavioral differences, and behavioral and physical signs of PMS women were compared before and after acupuncture. For each of the three parameters, a significant decrease was observed after acupuncture.

Acupuncture treatment, which has an important place in traditional Chinese medicine, has been used to control pain and treat psychosomatic diseases for many years. Chronic pain affects endorphinergic functions and probably consumes the neurotransmitters in endorphinergic neurons. Affective disorders and endorphine function in schizophrenia and pain sensitiveness are significantly affected [24, 25]. Stimulation of acupuncture points leads to the release of β-endorphins and ACTH from the hypophysis. It is believed that cutaneous afferents which rise from the analgesic or from the sedation of acupuncture spots occur by means of natural or electrical stimulation, and they are released from the interneurons that contain enkephalin in substantia gelatinosa which is placed in the posterior horn of the spinal cord [25, 26]. As pain is a partially negative emotional state which attempts to neurophysiologically and neuroendocrinologically increase emotional awakening by arranging the balance have pain killing profits [1]. The effect of acupuncture on the endorphinergic and enkephalineric system has been known for a long time.
In our study, it was observed that patient complaints about mastalgia, myalgia and dysmenorrhea in the pre-acupuncture period, were diminished remarkably in the post-acupuncture period, a most notable decrease for physical complaints.

According to traditional Chinese medicine, as the etiologic factor, insufficiency in the kidney meridian, liver qi stagnation and liver-blood insufficiency are responsible for the development of unwanted gynecological conditions [27]. Sp6, which is the most important point in the treatment of PMS, is the intersection point of the spleen, liver, and kidney meridians [14]. Sp6 regulates dysmenorrhea and PMS [28-31]. Furthermore, Ren2, Ren6 and Liv3 regulate menstruation and cure dysmenorrhea [32]. It has been observed that by using the points mentioned above, mild or severe dysmenorrheal complaints of women with PMS have been mitigated to a great extent.

Ren 12 is the most important acupuncture point which eradicates mental and physical failure [33]. It has been observed that patients with PMS, who in their menstruation period claim to be using much more effort than they normally do, can work during the menstruation period as easily as normally. Moreover, the feeling of fatigue easily in the pre-acupuncture period has been observed to have decreased or disappeared in the post-acupuncture period.

LI4 and LI11 decrease pain and fever [34]. In our study it was observed that by using these points, mild or severe myalgia and headache decreased after acupuncture treatment. When examined in terms of osteoarthritis, mild or severe complaints of women with PMS in the pre-acupuncture period were lessened after acupuncture treatment. When premenstrual migraine complaints were examined in the pre- and post-acupuncture periods, statistically some improvements have been observed.

P6 is one of the main points when curing nausea and vomiting. Moreover, it also opens the channels. ST36 is the point which strengthens the whole body and tonifies blood and qi [34]. These points are also very effective when curing mastalgia. Therefore, it has been observed that mastalgia complaints of women with PMS have lessened or disappeared.

The LI11 point is for edema and LI4 is for edema and spasm. LI4 also regulates the stomach. Du20 is the sedative regulator point. It is also the general physiologic and coordinator point.

Acupuncture enhances the level of NO concentrations substantially. NO is the key biological signal molecule for neurotransmission and causes vasodilatation in the blood vessels. Acupuncture increases NO levels in treated regions, thereby increasing local circulation. The importance of local circulation, and NO itself, in pain suggests that these effects might contribute to the pain relief obtained with acupuncture [7]. When the effects of acupuncture to the NO levels are studied, high NOS activity has been observed in the meridian acupuncture points of the rats [8]. It is concluded that the NOS system regulates the level of NO in the acupuncture points. Another explanation for the occurrence of NO is that it provides sympathetic tonus. Acupuncture is known to enable sympathetic activity changes [35]. Recent studies indicate that acupuncture provides anesthesia in the parts far from the point where acupuncture is applied, and it also has some cardiac and antiemetic effects [36]. The central neurological system and opioid peptides are believed to contribute to such characteristic effects [37].

Siu et al. did not observe any change in the antioxidant enzyme activity in rats on whose brains – without ischemic occurrence – electroacupuncture was applied. However, they observed electroacupuncture effects in ischemic reperfused brain tissues and increased antioxidant enzyme activity, while lipid preoxidation decreased [38]. We also noted that acupuncture increased GSH levels.

It has been observed that physical and emotional complaints of PMS patients decreased and the quality of life of these patients increased after acupuncture treatment. Biochemically this condition displays a parallelism with increased GSH and NOx levels. Acupuncture diminished PMS symptoms (lessening depression and fewer physical complaints), and improved biochemical parameters.

References

Importance of acupuncture on premenstrual syndrome


Address reprint requests to:
A. ANIL, M.D.
Gazi University Faculty of Medicine
Department of Anatomy
Building of Deanship, 3rd floor
06500 Beşevler, Ankara (Turkey)
e-mail: afitap@gazi.edu.tr
Sleep disturbances in pregnant patients and the relation to obstetric outcome

M.G. Ugur¹, K. Boynukalin², Z. Atak³, I. Ustuner⁴, R. Atakan⁵, C. Baykal⁶

¹Department of Obstetrics and Gynecology Gaziantep University School of Medicine, Gaziantep; ²Anatolia Women's Health Center, Ankara; ³Ministry of Health Kecioren Hospital, Ankara; ⁴Rize University School of Medicine, Rize; ⁵Anatürk University, School of Medicine, Erzurum; ⁶Florence Nightingale Kadıköy Hospital, Istanbul (Turkey)

Summary

Purpose: To compare obstetric outcomes between patients with positive and negative Berlin Questionnaire results. Methods: An observational study comparing outcome between these two groups was carried out in seven hospitals, representing seven different regions of Turkey. In each center, pregnant women who were admitted for normal pregnancy follow-up or labor, were consecutively recruited in the study. Each participant completed a sleep apnea questionnaire from the Berlin Questionnaire. This questionnaire tests snoring and daytime sleepiness. Fetal outcome and pregnancy outcome are recorded from patient files. Results: A total of 465 consecutive patients who completed the Berlin Questionnaire were analyzed. Patients with a positive questionnaire had a higher BMI, pre-pregnancy medical disorder rate and cesarean rate (68% vs 51%) than the negative group. Preeclampsia and gestational diabetes were more prevalent in the positive questionnaire group than the negative questionnaire group [19 (28%) vs 18 (5%) for preeclampsia, 8 (12) vs 13 (3%) for gestational diabetes, respectively]. At logistic regression analysis, gravidity, gestational age at birth and a positive questionnaire were independent predictors for preeclampsia. BMI and history of maternal medical disorders were independent predictors of gestational diabetes mellitus. Conclusion: Obstructive sleep apnea may be related to preeclampsia.

Key words: Berlin Questionnaire; Sleep; Disordered breathing; Pregnancy.

Introduction

Sleep disorders are a frequent but usually unrecognized problem that seem to be related to important health topics in women of childbearing ages. Sleep disturbances have been reported to be associated with hypertension, coronary artery disease, diabetes and depression [1, 2]. Most of the trials reporting these relationships have been established in middle-aged or older populations, but younger populations are being reported to have some association in recent studies [3-5].

Pregnancy is known to be associated with several changes in the body like hormonal, anatomic and mechanical changes. These changes in pregnant women alter sleep patterns and quality of sleep [6]. Sleep disorders may be divided in two major topics as obstructive sleep apnea (OSA) and upper airway resistance syndrome. These two disorders cause upper airway obstruction and hypoxemia during sleep and they may cause more severe results in pregnant women because of the fetus’s need for oxygenation. OSA is known to affect 2% of the general female adult population [7]. The true incidence is thought be higher because of the wide population of undiagnosed women. The incidence in pregnant patients and the association with adverse pregnancy outcomes are still unknown. There is no doubt about the adverse effects of hypoxemia of mothers on the fetus. Some well known pregnancy complications like intrauterine growth retardation (IUGR), preeclampsia and gestational hypertension can easily be related to maternal hypoxemia due to their pathophysiology [8, 9]. Some sleep-related problems like insomnia, snoring and restless leg syndrome are commonly reported by pregnant women [6, 10-11]. Some previous reports on sleep and pregnancy have provided important associations but there is great need for more detailed and larger studies to understand this new and underdiagnosed pregnancy problem [8, 12-18]. Life styles, socioeconomic status, physical activity degree and regional differences may affect sleep habits and pregnancy outcomes. We aimed to evaluate sleep disorder complaints and the relation to pregnancy outcome with a nationwide self-test questionnaire.

Materials and Methods

This study was conducted in seven different busy obstetrics clinics in different universities and centers. These centers were selected from different sociocultural, geographic and economic regions to be representative for all Turkey.

We conducted a prospective study comparing Berlin Questionnaire scores and pregnancy outcome between September 2010 and June 2011 in six cities (Ankara, Istanbul, Sanliurfa, Erzurum, Rize and Gaziantep). The study group consisted of pregnant women who were admitted to clinics for pregnancy follow-up or labor. All patients were interviewed by an obstetrician or midwife to obtain routine medical history and the team member used a standard verbal consent for study participation. Available and volunteer participants completed the Berlin Questionnaire (Appendix A) by themselves or with the help of a team member. The Berlin Questionnaire consists of three different categories.
Appendix A.

Center:
Name-Surname:
Gestational Age:
Complications in Pregnancy:

Route of delivery: Vaginal Birth C/S

Weight gain during pregnancy:

**CATEGORY 1**
1. Do you snore?
   a) Yes
   b) No
   c) I don’t know.

2. How loud do you snore?
   a) A little louder than sound of breathing.
   b) Like speaking voice.
   c) Much louder than speaking voice.
   d) Very severe- One can hear from next room.

3. Do other people become disturbed because that you snore?
   a) Yes  b) No c) I don’t know.

4. How often do you snore?
   a) Almost every night.
   b) 3-4 nights a week.
   c) 1-2 nights a week.
   d) 1-2 nights a month.
   e) Almost never

5. Did anybody tell you that you stop breathing during your sleep?
   a) Almost every night.
   b) 3-4 nights a week.
   c) 1-2 nights a week.
   d) 1-2 nights a month.
   e) Almost never

**CATEGORY 2**
1. How often do you feel tired and weak when you wake up from your sleep?
   a) Almost every night.
   b) 3-4 nights a week.
   c) 1-2 nights a week.
   d) 1-2 nights a month.
   e) Almost never

2. How often do you feel tired and weak during daytime?
   a) Almost every night.
   b) 3-4 nights a week.
   c) 1-2 nights a week.
   d) 1-2 nights a month.
   e) Almost never

3. Have you ever found yourself sleeping or falling asleep during driving?
   a) Yes, b) No

4. How often do you fall asleep or feel very sleepy during driving?
   a) Almost every night.
   b) 3-4 nights a week.
   c) 1-2 nights a week.
   d) 1-2 nights a month.
   e) Almost never

**CATEGORY 3**
1. Do you have a history of hypertension or obesity (BMI > 30 kg/m²)?
   a) Yes,
   b) No

designed to illicit information regarding snoring (category 1), daytime somnolence (category 2) and the presence of obesity and/or hypertension (category 3). In categories 1 and 2, patients answering “almost everyday” or “3-4 times per week” were considered to have significant symptoms. The presence of obesity (body mass index -BMI- > 30 kg/m²) and/or hypertension in category 3 was considered significant [1]. Hypertension was accepted as positive only in patients who had a “preeclampsia” diagnosis. A patient was considered to have a likelihood of sleep disordered breathing if significant symptoms existed in two out of three categories [19]. Demographic data of the patients were collected from their hospital files and medical history charts. Apgar scores and fetal data were obtained from pediatric files.

Statistical analysis

The primary outcome variable was the proportion of subjects with a high likelihood score on the Berlin Questionnaire (positive results). Categoric data were tested for significance with the χ² or Fisher’s exact test. Continuous data were tested for significance with a 2-tailed Student’s t-test or Mann-Whitney U test if not normally distributed. The Kolmogrov Smirnov test was used to test for normality of distribution. Multivariate analysis was performed by stepwise logistic regression. Results of the multivariate analysis are expressed as odds ratios with their 95% confidence intervals. A p value of 0.05 was required to reject null hypothesis. Statistical analysis was performed using SPSS software, version 14 (Chicago, IL).

Results

A total of 485 consecutive patients from seven centers were recruited for the study. Twenty patients were excluded from analysis because the Berlin Questionnaire was not filled in completely. A total of 465 patients constituted the study group. Patients’ demographic characteristics are given in Table 1. The mean BMI for all patients was in the overweight range. Patients who had a positive questionnaire result were older and had higher BMI, gravidity, and parity than the negative questionnaire group. Pre-pregnancy medical disorders were more prevalent for the positive questionnaire group than the negative group. Chronic
pregnancy and the relation between these disorders and obstetric outcomes did not differ between groups. Although number of fetuses with an Apgar score lower than 7 was higher in the positive questionnaire group it was the same at 5 min. Preeclampsia and IUGR, a 5% of patients with preeclampsia. The regression model, however, predicted 5% of patients with gestational diabetes mellitus. Many authors report short sleep duration and sleep-disordered breathing symptoms in pregnancy as frequent problems, and that these may be the cause of obstetric complications like preterm birth, preeclampsia and IUGR as a result of inflammation and oxidative stress [36-38]. There are some limited data from retrospective and case control studies pointing to these mechanisms as the cause of preeclampsia [8, 39, 40]. The main problem for this important topic is the testing method for sleep disorders; the Berlin Questionnaire is not the gold standard for this diagnosis, and that these may be the cause of obstetric complications.

### Table 1: Demographic characteristics of the pregnant women by the Berlin Questionnaire for assessment of sleep disorders. Data are given as mean ± standard deviation, median (min-max), n (%) where appropriate.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 465)</th>
<th>Berlin Questionnaire (n = 396)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.1 ± 6.2</td>
<td>27.6 ± 6.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Gravida</td>
<td>2 (1-14)</td>
<td>2 (1-11)</td>
<td>0.024</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0-13)</td>
<td>1 (0-9)</td>
<td>0.017</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.4 ± 5.3</td>
<td>162.3 ± 5.4</td>
<td>0.362</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.5 ± 12.7</td>
<td>74.0 ± 11.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.6 ± 4.1</td>
<td>28.1 ± 3.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Weight gain in pregnancy</td>
<td>14.2 ± 5.1</td>
<td>14.0 ± 5.0</td>
<td>0.027</td>
</tr>
<tr>
<td>Maternal medical disorders</td>
<td>44 (9)</td>
<td>29 (7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>11 (2)</td>
<td>3 (1)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>5 (1)</td>
<td>5 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (1)</td>
<td>2 (1)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Asthma</td>
<td>12 (3)</td>
<td>9 (2)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>4 (1)</td>
<td>4 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (1)</td>
<td>6 (2)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

### Table 2: Obstetric outcomes of pregnant women by the Berlin Questionnaire for assessment of sleep disorders. Data are given as mean ± standard deviation, median (min-max) where appropriate.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 465)</th>
<th>Berlin Questionnaire (n = 396)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>38.6 ± 1.3</td>
<td>38.5 ± 1.1</td>
<td>0.530</td>
</tr>
<tr>
<td>Meconium staining</td>
<td>21 (5)</td>
<td>7 (10)</td>
<td>0.161</td>
</tr>
<tr>
<td>Apgar score at 1 min &lt; 7</td>
<td>13 (3.3)</td>
<td>9 (13)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apgar score at 5 min &lt; 7</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>0.475</td>
</tr>
<tr>
<td>Cesarean</td>
<td>203 (51)</td>
<td>47 (68)</td>
<td>0.01</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3280.3 ± 458.9</td>
<td>3155.2 ± 650.8</td>
<td>0.055</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>18 (5)</td>
<td>19 (28)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GDM</td>
<td>13 (3)</td>
<td>8 (12)</td>
<td>0.002</td>
</tr>
<tr>
<td>IUGR</td>
<td>6 (2)</td>
<td>2 (3)</td>
<td>0.415</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>8 (2)</td>
<td>1 (1)</td>
<td>0.751</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>4 (1)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td>0.366</td>
</tr>
</tbody>
</table>

*Intrauterine growth retardation; *Gestational diabetes mellitus.

### Table 3: Significant independent predictors of preeclampsia with multiple logistic regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravida</td>
<td>1.4</td>
<td>1.1-1.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Gestational Age</td>
<td>0.6</td>
<td>0.4-0.9</td>
<td>0.011</td>
</tr>
<tr>
<td>+ Berlin Questionnaire</td>
<td>12.4</td>
<td>4.9-31.9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 4: Significant independent predictors of gestational diabetes mellitus with multiple logistic regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.1</td>
<td>1.02-1.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Maternal medical disordes</td>
<td>0.6</td>
<td>2.4-18.3</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Body mass index (kg/m²)

hypothesis is that enhanced inflammatory and oxidative stress response caused by these sleep disorders promote endothelial damage and metabolic derangements, which ultimately lead to conditions such as hypertension and non-insulin dependent diabetes mellitus [32-35]. There are some limited data from retrospective and case control studies pointing to these mechanisms as the cause of preeclampsia [8, 39, 40]. The main problem for this important topic is the testing method for sleep disorders; the Berlin Questionnaire is not the gold standard for this diagnosis.
and an overnight polysomnographic study is required for an exact diagnosis [41]. This test may be cumbersome and also expensive for the patient and many physicians prefer to use the questionnaire instead of this test.

We believe sleep disorders, especially OSA, are an important problem in pregnant women and related to some pregnancy complications based on our results. Larger and polysomnographic prospective studies are needed to show the relation between these two entities.

References

Address reprint requests to:
M.G. UGUR, M.D.
Batikent Mahallesi, 74 Nolu Cadde
No: 4 A Blok Daire: 3
27560, Gaziantep (Turkey)
e-mail: metegurolugur@hotmail.com
Transumbilical single-port laparoscopic hysterectomy using traditional laparoscopic instruments: a report of 20 cases

M. Li, Y. Han, Y.C. Feng
Division of Minimally invasive Gynecology, Central Hospital of Fengxian District, Shanghai-City (China)

Summary

Purpose of investigation: We studied 20 cases of transumbilical single-port laparoscopic hysterectomy (TSPLH) to evaluate the feasibility and safety of the TSPLH. Methods: Perioperative items and complications were observed: mean operation time, blood loss, gas pass time, out of bed activity time, postoperative analgesic rate, pain perception by visual analogue score (VAS), port site infection, hospital stay, postoperative fever rate and patient satisfaction score were measured. Results: All procedures were performed successfully, and no case was transferred to 4-port laparoscopic hysterectomy (LH). No postoperative complication occurred during the period of two month follow-up. Conclusion: TSPLH is a feasible and safe method for hysterectomy, although it may be little more time consuming. Nonetheless, it is welcomed by patients who are more concerned about cosmetic outcomes. Future studies are needed to improve the instruments to shorten the surgery time and confirm its advantages.

Key words: Transumbilical single-port laparoscopic hysterectomy; Complications; Feasibility and security.

Introduction

Since the first laparoscopic surgery through a single incision was reported in 1997 [1], single-port laparoscopic surgery (SPLS) has been used successfully to perform nephrectomy, prostatectomy, hemicolectomy, cholecystectomy, thoracoscopic decortication, and appendectomy, etc. [2-4]. In gynecology, SPLS has been used to perform oophorectomy, salpingectomy, bilateral tubal ligation, ovarian cystectomy, surgical treatment of ectopic pregnancy, and both total and partial hysterectomy [5-7]. Transumbilical single port laparoscopic hysterectomy (TSPLH) is still a new field to explore. The first case of TSPLH was reported by Langebrekke in 2009 [8], but due to technical and instrumental limitations, TSPLH has developed slowly. We report our initial experience of 20 cases of TSPLH performed with some traditional standard laparoscopic instruments, and evaluate the safety and feasibility of the operation.

Methods

From February to July 2011, 20 patients (12 uterine myomas and 8 adenomyomas) were admitted to our department and underwent TSPLH. The average age was 46.8 years. The size of the uterus ranged from 8-12 gestational weeks. All procedures were performed by the same surgeon under general anesthesia and accomplished by one articulating grasper and other standard laparoscopic instruments (Table 1). No prophylactic antibiotic was administered.

Data regarding patient operation time, blood loss, conversion rate, gas pass time, activity time, and postoperative analgesic rate were prospectively collected. Pain perception by visual analogue score (VAS) [9], port site infection, port hernia, postoperative hospital stay, postoperative fever rate, patient satisfaction (0-100) scores [10] were also measured during the perioperative period.

Result

TSPLH was successfully performed in all 20 cases, and no case was transferred to 4-port LH. No intraoperative complication occurred. Ancillary trocars were not necessary in any of the cases. All patients recovered from the operation and no postoperative complication occurred during a median follow-up period of 60 days. Results of the observed items are as follows. Average operation time was 126.4 ± 26.7 minutes, one of the longest procedure times due to patients having a history of cesarean section accompanied by uterine isthmus subserous myoma, which increased the difficulty of surgery. Mean blood loss was 179.3 ± 103.7 ml; no case needed blood transfusion interoperatively. Time of postoperation pass gas was 19.3 ± 8.7 hours, out of bed activity time was 9.5 ± 4.2 hours, postoperative analgesic rate was 2.1%, and almost no one needed intravenous analgesic. The average VAS score was 356, port site infection rate was mean 0.12%, no port hernia occurred, time of average hospital stay was 5.1 ± 1.42 days, and patient satisfaction score was 91.2 ± 6.9 (0-100).

We compared the last ten subjects of the TSPLH with the initial ten cases, however, the mean operation time obviously decreased (Table 2).
Some gynecologists began to explore vaginal access, has been successfully performed in some procedures. Moreover, there is no visible scar on the body [13]. Today, of laparoscopic technology and instrument upgrades, and the procedure was not as difficult as before with the development of laparoscopic surgical techniques. Surgeons found single-incision laparoscopic cholecystectomy to be beneficial for patients. In recent years, total laparoscopic cholecystectomy has been widely performed in many medical centers, and the procedure has been performed in some hospitals [14, 15]. Some gynecologists began to explore laparoscopic surgery using traditional laparoscopic instruments: a report of 20 cases.

Table 1. — Instruments of TSPLH.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triport single port system</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Articulating grasper</td>
<td>Yida (China)</td>
</tr>
<tr>
<td>Bipolar forcep</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Unipolar hook</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Suction apparatus</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Laparoscopic scissors</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Laparoscope (10 mm)</td>
<td>Olympus (USA)</td>
</tr>
<tr>
<td>Ultrasonic scalpel</td>
<td>Olympus (USA)</td>
</tr>
</tbody>
</table>

Table 2. — Operation results of the first ten cases compared to the last ten cases.

<table>
<thead>
<tr>
<th></th>
<th>First 10 cases (n = 26)</th>
<th>Last 10 cases (n = 10)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time (min)</td>
<td>136.4 ± 24.9</td>
<td>113.7 ± 19.1</td>
<td>9.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Hospital day (days)</td>
<td>5.3 ± 1.5</td>
<td>5.4 ± 1.37</td>
<td>1.8</td>
<td>0.67</td>
</tr>
<tr>
<td>Patient satisfaction score (0-100)</td>
<td>91.34 ± 6.83</td>
<td>94.7 ± 611</td>
<td>0.7</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Discussion

The hysterectomy procedure has evolved tremendously over the last century. The benefits of minimally invasive surgery – including less pain, faster recovery, and improved cosmesis – are well known [11]. Standard laparoscopic hysterectomy is performed with two 10-mm major manipulating trocars and two 5-mm ancillary trocars. There has been increasing attention to decreasing incisional morbidity and improving cosmetic outcomes in laparoscopic surgery by using fewer and smaller ports. In recent reports, traditional laparoscopy has been replaced by single-port operative laparoscopy from a great array of procedures. Innovative techniques of natural orifice transluminal endoscopic surgery (NOTES) and single-incision laparoscopic surgery (SILS) have been applied in gynecologic disease as a step towards even less-invasive procedures. SPLS represents the latest advance in minimally invasive surgery. Using flexible endoscopes and articulating instruments, the surgeon can complete complex procedures through a single 2-cm incision in the umbilicus. In the early years, only a few works were reported in journals and most surgeons did not think such a “difficult” operation, other than providing scar reduction, would be beneficial for patients. In recent years, total laparoscopic thyroidectomy and cholecystectomy have been widely performed in many medical centers, and the concept of a mini-invasive esthetic operation has been accepted by many mini-invasive surgeons because it has been welcomed by many patients [12]. More general surgeons found single-incision laparoscopic cholecystectomy was not as difficult as before with the development of laparoscopic technology and instrument upgrades, and moreover, there is no visible scar on the body [13]. Today, natural orifice transluminal endoscopic cholecystectomy, such as endoscopic cholecystectomy through gastric and vaginal access, has been successfully performed in some hospitals [14, 15]. Some gynecologists began to explore the technique of TSPLH. In the early period of TSPLH use, surgeons were mainly confronted with the difficulty of how to abate interactions between working apparatus when they were operating through three trocars in the same incision.

We present a novel technique to perform a single-port, total laparoscopic hysterectomy in patients with benign uterine diseases. Through our study, we found that three methods could be adopted to solve the problem: (1) using articulating instruments [16] such as a flexible-tip laparoscopic [17] articulating grasper which can provide adequate working room; (2) using a triport trocar or self-made apparatus, such as sterile gloves; (3) suturing the cuff through the vagina can make the operation possible and easier using the standard instrument. All of these reduce operative difficulty, but increase operation fees. In our study, we used one articulating grasper combined with other traditional instruments to complete the whole procedure. Suturing through a single port can be a challenge. When possible, closure of the vaginal cuff following a total laparoscopic or laparoscopic-assisted vaginal hysterectomy should be performed from below. When endoscopic suturing is required, standard suturing using both intracorporeal and extracorporeal methods is possible. Since we have no bidirectional self-retaining sutures [8], we suture the cuff through the vagina. During the surgery, instrument interaction is a crucial problem which needs to be resolved and it could be overcome by a surgeon’s adroit skill. Twenty cases of TSPLH were successfully performed without transference to 4-port LH; TSPLH is feasible in practice. No postoperative complication such as incision hernia or wound infection occurred. It seemed that TSPLH showed the same safety as traditional LH. In the last ten subjects of the TSPLH group, however, the mean operation time obviously decreased from the initial cases. More time may be needed to complete the operation in the early period (Figure 3). We are convinced that as experience increases and instruments develop, operation time will become shorter as traditional LH. TSPLH achieved a high patient satisfaction score indicating it was attractive, especially to young women. The authors’ experience has shown that single-port laparoscopic hysterectomy with the TriPort system is safe and feasible. Prospective randomized studies comparing single-access and conventional multi-port laparoscopic hysterectomy with long-term follow-up evaluation are needed to confirm the initial experience.

Although all TSPLH procedures were successfully performed, further research is still needed to solve many operative difficulties. In TSPLH, all three ports enter into the abdominal cavity from the same incision, and it is difficult to work from two directions as in traditional LH; TSPLH is feasible in practice. No postoperative complication such as incision hernia or wound infection occurred. It seemed that TSPLH showed the same safety as traditional LH. TSPLH achieved a high patient satisfaction score indicating it was attractive, especially to young women. The authors’ experience has shown that single-port laparoscopic hysterectomy with the TriPort system is safe and feasible. Prospective randomized studies comparing single-access and conventional multi-port laparoscopic hysterectomy with long-term follow-up evaluation are needed to confirm the initial experience.

Although all TSPLH procedures were successfully performed, further research is still needed to solve many operative difficulties. In TSPLH, all three ports enter into the abdominal cavity from the same incision, and it is difficult to work from two directions as in traditional LH, without the 3rd assistant grasper aid. The mobile range of the laparoscopic apparatus was also restricted by other ports and the operating field was extremely narrow [18]. A recent article from Korea reports an inventive technique to perform single-incision laparoscopy using standard instrumentation: the authors fitted a self-retaining ring retractor with a surgical glove that had three of the
fingers cut off and replaced by trocars [19]. The latest instruments are designed to dissect, cauterize, and cut, thereby decreasing the number of instrument exchanges necessary. Thus, concurrent manipulations are very important and necessary to work effectively, and to avoid having the apparatus suddenly disappear from view. The surgeon requires considerable experience with laparoscopic operations to overcome these difficulties. The potential benefits of single access include decreased pain, a shorter recovery period, lower morbidity, reduced cost, and superior cosmesis. Careful case selection and a low threshold of conversion to conventional laparoscopic surgery are essential. Multicenter, randomized, prospective studies are needed to compare short- and long-term outcome measures against those of conventional laparoscopic surgery.

Acknowledgment(s)

The authors sincerely thank Professor Hong Bing Wang PH.D, who offered us time and effort during his revision of this paper.

References

Transumbilical single-port laparoscopic hysterectomy using traditional laparoscopic instruments: a report of 20 cases


Address reprint requests to:
Y. HAN, M.D.
Division of Minimally invasive Gynecology
Central Hospital of Fengxian
District of Shanghai
No. 9588 Nanfeng Road
Nanqiao Town, Fengxian District
Shanghai (China) 201499
e-mail: xjhy0519@163.com
Doppler examination in the evaluation of outcomes in pregnancies complicated by gestational hypertension and fetal intrauterine growth retardation - is it enough?

I. Babovic¹, S. Plesinac²

¹Institute of Obstetrics & Gynecology, Clinical Center of Serbia, Belgrade
²School of Medicine, University of Belgrade, Belgrade (Serbia)

Summary

Aim: The relations between abnormal umbilical and cerebral Doppler, cerebral-umbilical (C/U) ratio, and outcomes in pregnancies complicated by gestational hypertension and fetal intrauterine growth retardation were evaluated. Materials and Methods: A retrospective study of 53 monofetal pregnancies in 2010 was conducted at the Institute of Gynecology and Obstetrics, Belgrade. Statistical analysis: chi-square likelihood ratio test, Student’s t-test and Spearman’s coefficient correlation. Results: There was not a significant correlation between the timing of registration of abnormal umbilical Doppler to delivery and outcomes of high-risk pregnancies. There was a significant correlation between C/U ratio and APGAR-5 (p = 0.003). We found a significant correlation between neonatal birth weight and APGAR-5 (p = 0.000), neonatal asphyxia (p = 0.000), intracranial hemorrhage (p = 0.000) and respiratory distress syndrome (p = 0.000). Conclusion: Umbilical and cerebral artery Doppler is a relatively poor predictor of neonatal outcome. It seems that neonatal birth weight is the best predictor of neonatal outcome in high-risk pregnancies.

Key words: Gestational hypertension; Intrauterine growth retardation; Doppler; Fetus; Umbilical and cerebral velocimetry; Neonatal outcome.

Introduction

In 2008, The Society of Obstetricians and Gynecologists of Canada (SOGC) revised guidelines that simplified the classification of hypertension in pregnancy into two categories, preexisting or gestational, with the option of adding “with preeclampsia” to either category if additional maternal or fetal symptoms, signs or test results support this. It occurred in about 5-8% of all pregnancies in Canada [1] and in about 7% of all pregnancies in Yugoslavia in 1996 [2]. Intrauterine growth restriction (IUGR) fetuses have low growth potential, either as a result of genetic disease or environmental damage, or due to reduced placental perfusion and utero-placental insufficiency, and they are at increased risk of perinatal morbidity and mortality and will require close fetomaternal monitoring and probably earlier intervention. IUGR occurs when gas exchange and nutrient delivery to the fetus are not sufficient to allow it to thrive in utero. This process can occur primarily because of maternal disease causing decreased oxygen-carrying capacity (e.g., cyanotic heart disease, smoking, etc.), a dysfunctional oxygen delivery system secondary to maternal vascular disease (e.g., diabetes with vascular disease, hypertension, etc.), or placental damage resulting from maternal disease (e.g. thrombophilia, various autoimmune diseases). In pregnancies complicated by placental dysfunction, there may be a reduction in the number of functional villi and/or small blood vessels with resulting increased impedance, mainly reflected by decrease in end-diastolic velocity.

When the resistance increases even more, there is no diastolic forward velocity (absent end-diastolic velocity - AEDV). Further increase in the resistance causes reversed end-diastolic velocity - REDV and middle cerebral centralization, a late step in the cascade of events leading to poor perinatal outcome [3]. The aim of the study was to assess the accuracy of pathological antepartum Doppler velocimetry AEDV, REDV and middle cerebral artery centralization on prediction of poor perinatal outcome in pregnancies complicated by hypertensive disorders.

Materials and Methods

Fifty-three pregnancies in and after the 28th to 39th gestational week complicated with gestational hypertension and IUGR (below the 10th percentile for estimated gestational age), and treated at the Institute of Obstetrics and Gynecology, Clinical Center of Serbia, in 2010, were retrospectively included in the study. Gestational age was calculated according to date of the last menstrual period and confirmed by first trimester ultrasound. If there was a discrepancy (> 5 days), ultrasound (US) examination was used to determine gestational age. According to the criteria of Xiong and Fraser [4] gestational hypertension was defined as a blood pressure equal to or greater than 130/90 mmHg on more than two occasions more than six hours apart without proteinuria after 21 weeks of gestation in 40 (75.48%) out of 53 patients. Preeclampsia was diagnosed as hypertension of equal or greater than 130/90 mmHg but less than 160/110 mmHg with proteinuria of 1+ or 2+ on dipstick in two samples six hours apart or more than 0.3 g in a 24-hour urine collection in 24.52% of patients (13/53) but severe preeclampsia was diagnosed when preeclampsia was complicated by a systolic pressure of ≥ 160 mmHg or diastolic pres-
Doppler examination in the evaluation of outcomes in pregnancies complicated by gestational hypertension and fetal etc.

The mean age of all women participating in our study was 28.26 ± 6.1 years. Of the women, 18.8% were older than 35 years and 24.5% were younger than 25 years. Most of them were nulliparous - 71.7%. Recurrent IUGR was found in two cases. The majority of women had adequate prenatal care during their pregnancy (94.3%). The most common cause of fetal IUGR might be attributable to pregnancy induced hypertension (PIH), accounting for 62.2% (33/53), preeclampsia - 26.4% (14/53), and severe preeclampsia 11.4% (6/53). PIH and preeclampsia were excluded from studies. Our study showed that gestational hypertension was the most frequent complication of the pregnancies. The mean gestational week at presentation was 35.6 ± 2.41. Cesarean section was performed in 58.5% (31/53) and elective cesarean section was performed in 41.5% (22/53) of cases. Six patients delivered before 32 gestational weeks and four patients delivered before 37 gestational weeks. Mean neonatal weight was 1,925.5 ± 524 g (med 1,850; IQR 1700) Incidence of low birth weight (LBW) neonates under 2,500 g was 15.1% (8/53). Apgar score - 5 min and less than 7 was found in 28.0% (15/53). C/U ratio < 1 and Apgar score - 5 min were significantly correlated (p = 0.003), but the time interval between abnormal UA and cerebral Doppler and gestational week at presentation were not significantly correlated. There was a significant correlation between neonatal birth weight and perinatal asphyxia, (p = 0.000), IVH (p = 0.000), RDS (p = 0.000) as well as between Apgar score - 5 min and neonatal birth weight (p = 0.000) (Figure 1).

Discussion

Of the women, 24.5% were younger than 25 years and most were nulliparous (71.7%). PIH and preeclampsia occurs most often in young nulliparous women as documented in studies. Our study showed that gestational hypertension was the most frequent complication of the pregnancies. PIH, preeclampsia and IUGR might have similar determinants and consequences. Attempting to identify the cause of fetal growth restriction is important because it may have an influence on estimating recurrence and future pre-conception counseling, pregnancy management, prenatal diagnostic procedures and neonatal management [5]. The majority of women (94.3%), had adequate prenatal care during pregnancy.

When primary surveillance with UA Doppler is found to be abnormal, the biophysical profile is likely to be use-
ful given its good negative predictive value in high-risk populations [6]. The combination of small abdominal circumference, normal anatomy, low BPP score values and abnormal UA Doppler recording is strongly suggestive of fetal IUGR due to placental insufficiency [7]. Kurkinen-Rati et al. [8] concluded in their study that early AEDV or REDV (before 34th gestational week) in the UA was a serious warning signal of probable fetal distress. In such cases, the rates of perinatal morbidity and mortality are very high, which is a reflection of the severity of the condition. Six fetuses had no structural and chromosomal abnormalities in which the development of AEDV was evidenced by Doppler imaging. Sallout et al. evaluated the time interval between incidence of AEDV in the umbilical artery and either the development of abnormal fetal heart-rate patterns or delivery. The median time interval between AEDV and the onset of late decelerations was 12 days (range 0-49 days) [7]. In our study, this time interval was approximately 48 hours. The study revealed a significant reduction in elective cesarean section for fetal distress and most deliveries were performed before 37 gestational weeks. In the management of the preterm (before 32 gw) IUGR fetuses there is uncertainty whether iatrogenic preterm delivery should be undertaken before the development of signs of severe hypoxemia, with a consequent risk of prematurity-related neonatal complications, or whether delivery should be delayed but with the risks of prolonged exposure to hypoxia. An Apgar score < 1 and Apgar score < 5 min were significantly correlated (p = 0.003). The last calculated C/U ratio could not predict the development of brain lesions and only repeated measurements of fetal Doppler indices over a longer period before delivery could be useful [9]. Resnik and Creasy [10] reported that birth weight after 29 weeks appeared to be a better predictor of survival than Doppler, as documented in our study as well (Figure 1).

Conclusion

A weekly biophysical profile or fetal heart rate testing as well as umbilical and cerebral artery Doppler is a relatively poor predictor of neonatal outcome (especially brain damage) due to placental dysfunction. It seems that neonatal birth weight is the best predictor of neonatal outcomes in pregnancy complicated by hypertension and fetal IUGR.

References

Association of first trimester low PAPP-A levels with adverse pregnancy outcomes

Z. Saruhan, M. Ozekinci, M. Simsek, I. Mendilcioglu

Obstetrics and Gynaecology Department, Akdeniz University Faculty of Medicine, Antalya (Turkey)

Summary

Objective: To investigate whether first trimester low PAPP-A levels are associated with adverse pregnancy outcomes. Methods: A case control retrospective study including 663 pregnant women whose gestational age ranged between 11 and 14 weeks attending prenatal care at Akdeniz University Hospital was carried out. Chromosomal abnormalities, spontaneous abortions, and multiple pregnancies were excluded from the study. Finally 318 singleton pregnancies were included in this study. Pregnant women whose PAPP-A levels were ≤ 10th percentile were compared with PAPP-A levels > 10th percentile for the frequency of pregnancy complications such as SGA, preeclampsia, preterm delivery, gestational diabetes mellitus and gestational hypertension. Results: The most common complication of pregnancy was SGA (9.4%, n = 30). There was no significant association between low PAPP-A levels and incidence of subsequent pregnancy outcomes. Maternal age was found to be a risk factor for gestational diabetes (p = 0.00). Small for gestational age was significantly associated with nulliparity and smoking during pregnancy (p = 0.03 and p = 0.01, respectively). Conclusion: First trimester of low PAPP-A level (≤ 10th percentile) was not associated with SGA, preeclampsia, preterm delivery, gestational hypertension or gestational diabetes mellitus.

Key words: First trimester serum screening; PAPP-A; Pregnancy complications.

Introduction

Pregnancy-associated plasma protein-A (PAPP-A) and free human chorionic gonadotropin (β-hCG) are increasingly being used as a part of screening programs for the detection of fetal aneuploidy in early pregnancy [1-7]. Recent studies have focused on the relation between PAPP-A and low levels of this marker with negative obstetrics results. These negative results are related to intrauterine growth restriction (IUGR), gestational hypertension, gestational diabetes, preeclampsia, fetal death, spontaneous abortus and preterm delivery [8-13]. PAPP-A is produced by trophoblasts, thereby abnormal levels of this protein detected as early as possible in the first trimester are considered to be related to abnormal placentaion and to cause the mentioned pathological situations [10-14]. The relation of fetal growth with PAPP-A is based on insulin-like growth hormone activity which is considered to have an important role in trophoblast invasion [15]. PAPP-A is a protease produced from syncytiotrophoblasts for insulin-like growth factor binding protein-4, and its protease activity divides complex growth factor binding protein and release growth factors in mitogenic signal channels [16, 17]. High concentrations of PAPP-A release more insulin-like growth hormones for bioactivity, and this could result in advanced growth. On the other hand, low concentrations of PAPP-A results in a limited amount of growth factor which causes reduced development. Growth retardation was detected in homozygote mice in which the PAPP-A gene was affected [18]. A number of screening studies reported the relation between PAPP-A and reduced fetal development [9, 19, 20].

The present study aimed to investigate the relation between PAPP-A values observed in the first trimester and birth weight, as well as the predictability of pregnancy complications like IUGR, preeclampsia, gestational hypertension, gestational diabetes, and preterm labor by using low PAPP-A values.

Materials and Methods

In the study, the files of 663 pregnant women who underwent first trimester screening between December 2008 and September 2009 at the Akdeniz University Obstetrics Unit were retrospectively investigated. Subjects with no record, multiple pregnancies, fetal anomaly detection in the follow-up period and pregnancies resulting in abortus were excluded from the study. A total of 318 pregnant women were included.

PAPP-A multiple of median (MoM) values used in the study were taken from the records of Biochemistry Clinics. Immunoassays were performed according to the manufacturer’s protocol (Roche Diagnostics GmbH, Mannheim).

The PAPP-A MoM values were reported routinely after adjusting for maternal weight, smoking status, ethnicity and diabetes.

Obstetric outcomes were obtained from file records or by reaching them via phone contact. Gestational hypertension was diagnosed in cases with >140/90 mm Hg blood pressure of two measurements at 6-hour intervals, no chronic hypertension and no significant sign of proteinuria. Preeclampsia was defined as the existence of gestational hypertension developing proteinuria. Proteinuria was defined as 0.1 g/l (> 2+ at dipstick) in at least two samples taken at an interval ≥ 6 h or ≥ 300 mg total protein on 24 h urine collection. Preterm birth was defined as...
delivery before the 37th week of gestation. Small for gestational age (SGA) was defined as birth weights below the ≤ 5th and ≤ 10th percentile birth weights. Gestational diabetes is diagnosed when at least two values exceed the limits in a 100 g oral glucose tolerance test.

Pregnant women with PAPP-A > 10th percentile and PAPP-A ≤ 10th percentile were compared for pregnancy outcomes. The differences between the two groups were analyzed by chi-square or Fisher exact tests. The Mann-Whitney U test was used in the comparison of birth weight, age, and PAPP-A ≤ 10th percentile at delivery week. Multiple logistic regression models were fit to estimate the risk association between PAPP-A ≤ 10th percentile MoM and the risk for adverse pregnancy outcomes (SGA, preeclampsia, gestational diabetes, gestational hypertension, preterm birth) while adjusting for potentially confounding factors. Receiver-operator analyses were performed, including significant variables identified in the regression models, and the area under the curve (AUC) was used to estimate the ability of PAPP-A ≤ 10th percentile to predict adverse obstetric outcomes. SPSS (18.0 version) software was used in the analysis of the obtained data and p < 0.05 was accepted as significance level.

Results
Between December 2008 and September 2009, 663 pregnant women underwent first trimester screening tests for aneuploidy. A total of 318 pregnant women met inclusion criteria for the study analysis. The mean age of the 318 pregnant women was 29.1 ± 3.8, while mean crown-rump-length (CRL) was 59.3 ± 8.4 mm. The mean maternal transabdominal (NT) value was 1.45 ± 0.4 mm.

Among 318 pregnant women, 11 (3.5%) pregnant women had a history of smoking during pregnancy, 151 (47.5%) were nulliparous, and three (0.94%) had Type1 diabetes mellitus (DM), while six (1.8%) had thalassemia trait. Among 318 deliveries, there were six preterm deliveries < 35 weeks and 15 preterm deliveries < 37 weeks. The median PAPP-A MoM in the patient NT ≤ 10th percentile group was 1.53 versus 1.40 in the NT > 10th percentile group.

The 5th, 10th, 90th and 95th percentiles of PAPP-A MoM values were 0.36, 0.45, 2.05 and 2.55 respectively. The PAPP-A MoM values of 35 patients were ≤ 10th percentile.

The median PAPP-A MoM values in the SGA and control group were 0.74 and 0.99, respectively (p = 0.055). The median PAPP-A MoM in preterm birth (PTB) < 35 weeks was 0.81 versus 0.97 in the group who delivered > 35 weeks (p = 0.441). The median PAPP-A MoM in PTB < 37 weeks was 1.04 versus 0.97 in the group who delivered > 37 weeks.

Compared to 35 women with a PAPP-A ≤ 10th percentile MoM (0.45), the 283 women with PAPP-A > 10th percentile MoM had similar age, parity and smoking status (Table 1). No significant difference was determined between patients with ≤ 10th percentile MoM value and with PAPP-A > 10th percentile in terms of pregnancy complications and characteristics (Table 1).

Table 1. — Pregnancy complications and characteristics of patients with ≤ 10 percentile MoM value and with PAPP-A > 10 percentile.

<table>
<thead>
<tr>
<th></th>
<th>PAPP-A ≤ 10th percentile MoM (n = 35)</th>
<th>PAPP-A &gt; 10th percentile MoM (n = 283)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>29.4 ± 3.8</td>
<td>29.0 ± 5.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>57.1% (20)</td>
<td>46.3% (131)</td>
<td>0.22</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.9% (1)</td>
<td>3.5% (10)</td>
<td>0.99</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>–</td>
<td>1.8% (5)</td>
<td>0.99</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>2.9% (1)</td>
<td>1.1% (3)</td>
<td>0.37</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>2.9% (1)</td>
<td>4.9% (14)</td>
<td>0.99</td>
</tr>
<tr>
<td>Preterm &lt; 35 wks</td>
<td>2.9% (1)</td>
<td>1.8% (5)</td>
<td>0.50</td>
</tr>
<tr>
<td>Preterm &lt; 37 wks</td>
<td>2.9% (1)</td>
<td>4.9% (14)</td>
<td>0.99</td>
</tr>
<tr>
<td>SGA &lt; 10th percentile</td>
<td>17.1% (6)</td>
<td>8.5% (24)</td>
<td>0.12</td>
</tr>
<tr>
<td>Polyhydramnios</td>
<td>5.7% (2)</td>
<td>2.1% (6)</td>
<td>0.21</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>5.7% (2)</td>
<td>1.8% (5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3206 (± 495)</td>
<td>3261 (± 551)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Age was determined to be an independent predictive factor for gestational diabetes (p = 0.00), while no relation was detected with PAPP-A ≤ 10th percentile (AUC 0.55, 95% CI 0.45-0.69). PAPP-A ≤ 10th percentile was also not predictive of preeclampsia (AUC 0.58, 95% CI 0.29-0.86), SGA (AUC 0.60, 95% CI 0.50-0.71), preterm delivery (≤ 37 weeks) (AUC 0.52 95% CI 0.39-0.64) and preterm delivery (≤ 35 weeks) (AUC 0.59 95% CI 0.38-0.80). Nulliparity and smoking were predictive for SGA (p = 0.03 and p = 0.01, respectively).

Discussion
In our study, no significant relation was determined between low PAPP-A values and pregnancy complications (SGA, preeclampsia, preterm birth, gestational hypertension, gestational diabetes, oligohydramnios, polyhydramnios). Similarly, in a retrospective case-control study evaluating the role of serum free β-hCG and PAPP-A levels in SGA and preterm birth, no statistically significant difference was reported between case and control groups [21]. Conversely, in a study investigating the relation between gestational diabetes, gestational hypertension, preeclampsia, spontaneous abortus, PTB, and IUGR in patients with low maternal serum levels of PAPP-A and β-hCG measured between 10-14 weeks, the authors determined a significant relation between PAPP-A MoM values and gestational hypertension, spontaneous abortus, SGA < 10th, < 5th, < 3rd percentile, preeclampsia and gestational diabetes mellitus, while no significant relation was detected with preterm labor [22]. They concluded that first trimester low PAPP-A and free β-hCG had a weak sensitivity for pregnancy complications and this was similar to findings with abnormal serum biochemistry in the second trimester. Low levels of PAPP-A at 10-14 weeks may be a marker of impaired placentation and a smaller placental mass [22]. The paracrine effects of insulin-like growth factors (IGF) are thought to control the invasion of trophoblasts into the decidua. As PAPP-A is
a protease for IGF-binding proteins (IGFBP), low PAPP-A is associated with high levels of IGFBP, which consequently causes lowering of free IGF, leading to impaired invasion of the trophoblasts into the maternal decidua. This process may lead to adverse pregnancy outcomes. In another retrospective study, first trimester low PAPP-A level (≤ 0.25 MoM) was found to be a risk factor for IUGR, preeclampsia and spontaneous abortus but this study could not demonstrate any single cutoff value below [23].

In a prospective multicenter study a significant relation was found between PAPP-A and β-hCG levels measured at 8–14 weeks and pregnancy complications [20]. A significant relation was revealed between PAPP-A < 5th percentile and SGA < 5th percentile, preterm birth 33–36 weeks, preterm birth 24–32 weeks, preeclampsia and the death of placental origin, while β-hCG < 5th percentile was related significantly only with SGA < 5th percentile.

In another prospective multicenter study, low PAPP-A levels were determined as the most relative marker with pregnancy complications [10]. However, no relation was detected between high PAPP-A levels and adverse pregnancy results. A significant relation was determined in patients with PAPP-A level ≤ 5th percentile between SGA < 10th percentile, SGA ≤ 5th percentile, preterm birth < 37 weeks, preterm birth ≤ 32 weeks, preeclampsia, gestational hypertension, and < 24 weeks spontaneous abortus.

As stated by the authors of the aforementioned study, if PAPP-A is an important factor in fetal development, a relation could be expected between its high levels and macrosomia. The fact that it could not be demonstrated with such a large patient population indicates that PAPP-A is not the only factor that controls fetal growth and there should be other factors with important roles.

A new model for early prediction of SGA has recently been published [24]. The authors used maternal characteristics, NT thickness, PAPP-A, free β-hCG, mean arterial pressure, uterine artery pulsatility index, placental growth factor, placental protein 13, and A disintegrin and metallopeptase to predict SGA. They concluded that combining maternal factors and biophysical and biochemical markers at 11–13 weeks could detect half of pregnancies with SGA neonates in the absence of preeclampsia.

In our study, calculated 5th and 10th percentile PAPP-A MoM values were lower than most previous studies reported, but similar to the one of the study reported by Marttalal et al. [25]. They also could not find any explanation for this situation and suspected the genetic background of the region was a factor.

As a result of our study, we could not find any significant relation between PAPP-A ≤ 10th percentile values and such pregnancy complications as SGA, preterm birth, preeclampsia, gestational hypertension, and gestational diabetes. Our study has several limitations, including its retrospective design and small sample size which could affect the inconsistency between previous results. Therefore, further prospective studies should be implemented, especially on larger populations.

References


Address reprint requests to:
M. OZEKINCI, M.D.
Obstetrics and Gynaecology Department
Akdeniz University Faculty of Medicine
07058, Antalya (Turkey)
e-mail: mozekinci@akdeniz.edu.tr
Effects of estrogen and progestin on expression of MMP-2 and TIMP-2 in a nude mouse model of endometriosis

J. Wang¹, X. Ma²

¹Department of Clinical Laboratory, The Dezhou People’s Hospital, Dezhou
²Department of Blood Transfusion, Qilu Hospital of Shandong University, Jinan (China)

Summary

Objective: The study endeavored to observe the effects of estrogen and progestin on expression of matrixmetalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in a nude mouse model of endometriosis (EMT) and to explore the roles of MMP-2/TIMP-2 in the pathogenesis of EMT. Methods: Sixty nude mice were injected in the abdominopelvic cavity with human endometrial tissue and randomly divided into four hormone treatment groups (estrogen, progestin, estrogen+progestin, and saline control; n = 15 per group). Implantation rates and gross morphological characteristics were assessed. Further, expression of MMP-2 and TIMP-2 in ectopic endometrial lesions was detected by immunohistochemistry. Results: The overall implantation rate of endometrial samples was 87.5% (50/60) in injected nude mice. Although there was no statistically significant difference in implantation rates between groups, the number of lesions in the progestin group was higher than that in other groups, and the size of lesions in the progestin and estrogen+progestin groups was larger than in the estrogen and control groups (p < 0.05). Further, expression levels of MMP-2 in the estrogen and estrogen+progestin groups were higher than in the progestin and control groups (p < 0.05). In contrast, expression levels of TIMP-2 in estrogen, progestin, and estrogen+progestin groups were lower than in the control group; additionally, the expression level of TIMP-2 in the progestin group was lower than in the estrogen group (p < 0.05). Finally, the ratios of MMP-2/TIMP-2 expression in the estrogen, progestin, and estrogen+progestin groups were higher than for the control group; in fact, this ratio was highest in the estrogen+progestin group (p < 0.05). Conclusions: The nude mouse is an appropriate model for early clinical studies of EMT, specifically in the detection of MMP-2 and TIMP-2. These proteins appear important in the pathogenesis of EMT. Specifically, estrogen can raise the expression level of MMP-2 to promote ectopic implantation of endometrial tissue. Meanwhile, progestin can inhibit the expression of TIMP-2 to raise the MMP-2/TIMP-2 ratio, which can enhance invasiveness of ectopic endometrium to promote implantation.

Key words: Endometriosis; Nude mouse; Matrixmetalloproteinase-2; Tissue inhibitor of metalloproteinase-2; Estrogen; Progestin.

Introduction

Endometriosis (EMT) occurs when functional endometrial glands and stroma exist outside the uterine cavity and affect normal physiological functions. This chronic disease is common in women of childbearing age, often leads to pelvic and abdominal pain and infertility, and affects women’s quality of life [1]. Recently, studies have suggested that ectopic implantation of active endometrial histocytes, which have entered the abdominal cavity, must be achieved through adhesion, invasion, and angiogenesis [2]. Matrixmetalloproteinases (MMPs) play important roles in ectopic implantation. A key member of this family of proteins, MMP-2, is regulated by tissue inhibitor of metalloproteinase-2 (TIMP-2); the dynamic balance between MMP-2 and TIMP-2 in the body is important [3]. Additionally, some evidence indicates that the female sex hormones estrogen and progesterone affect expression of MMP-2 and related proteins, and that this dysregulation may promote pathogenesis of endometriosis [4-8]. Here, we establish a nude mouse model for EMT and determine the effect of estrogen and progesterone on expression of matrixmetalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in early lesions of endometriosis (EMT).

Materials and Methods

Human endometrium specimens. Specimens were collected from a patient who underwent a hysterectomy because of benign lesions (non-endometriosis) at the Affiliated Hospital, School of Medicine, Anhui University of Science and Technology in September 2009. The patient was 43 years old with regular menstruation, no medical complications, and no hormone use for six months preceding the procedure. The endometrium was scraped in the late secretory phase, rinsed repeatedly with cold PBS, cut into 0.5 mm-1 mm³ pieces, and placed in D-Hanks solution with 200 U/ml penicillin and 200 U/ml streptomycin. Postoperative pathological biopsy confirmed that the endometrium was normal. Transfer to animals was conducted within one hour after specimen preparation.

Experimental animals. Sixty female nude mice 6-10 weeks old and weighing 18-24 g were purchased from SLACAS and raised in SPF conditions. Animal protocols were approved by the university.

Endometrial transplantation. Nude mice were anesthetized via intraperitoneal injection (10% chloral hydrate 0.5 mL/20 g body weight). D-Hanks solution with endometrium samples was injected into the abdominal cavity at the puncture point under the navel on the medioventral line at a volume of 1 ml for each nude mouse (containing about 20 pieces of endometrial tissue). Mice were returned to cages for recovery. Penicillin and streptomycin were administered for three days to prevent infection. Mice were killed 18 days after operation for assessment of lesion formation in the abdominopelvic cavity after celiotomy. Ectopic lesions were removed and immediately placed in formaldehyde solution with 10% phosphate buffer for fixing, dehydration, paraffin embedding, slicing, and staining.
were administered the following hormones on the 7th and 14th days after operation: Group A, 0.02 mg estradiol benzoate; Group B, 5 mg progesterone; Group C, 0.02 mg estradiol benzoate + 5 mg progesterone; and Group D, 0.05 ml normal saline.

**Immunohistochemistry.** Rabbit anti-human MMP-2 polyclonal antibody and mouse anti-human TIMP-2 monoclonal antibody were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Three-micron serial sections were made from the removed ectopic lesions. For each sample, antibody were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Three-micron serial sections were stained excised lesions were examined under light microscopy. Single-layer columnar, cubic, or flattened epithelial cells surrounded uterine glands in samples from all groups and were infiltrated by a large number of inflammatory cells. At their edges, fiber cells proliferated, connective tissues increased, and hyaline degeneration was apparent; a large number of capillaries could also be seen. Implants from estrogen-treated mice showed proliferative changes: the number of glands were significantly greater than those in the estrogen group and the estrogen+progestin group were significantly greater than those in the estrogen group and the control group ($p < 0.05$).

**Histological characteristics.** Hematoxylin and eosin-stained excised lesions were examined under light microscopy. Single-layer columnar, cubic, or flattened epithelial cells surrounded uterine glands in samples from all groups and were infiltrated by a large number of inflammatory cells. At their edges, fiber cells proliferated, connective tissues increased, and hyaline degeneration was apparent; a large number of capillaries could also be seen. Implants from estrogen-treated mice showed proliferative changes: the number of glands were small, the glandular cavity was regular with a round or oval shape, glandular epithelial cells were single-layer columnar, glandular secretion was absent, and stroma was dense (Figure 2A). Implants from estrogen+progestin-treated mice had typical secretory changes. Further, the mean diameters of the lesions in the progestin group and the estrogen+progestin group were significantly greater than those in the estrogen group and the control group ($p < 0.05$).

**Results**

**Morphology of endometrial transplants.** To develop a model of endometriosis, human endometrial samples were implanted into the abdominal cavities of 60 nude mice that were divided into four hormone treatment categories. Transplants were assessed 18 days after implantation; gross assessment of mice showed adhesions in the abdominopelvic cavities and fusion of transplanted tissue with native tissues. Further, lesion texture was firm, and the surface was covered with small blood vessels (Figure 1). Implantation occurred mainly in the abdominal wall, mesentery, intestinal tubes, and liver in the abdominal cavity, and the adhesion belt was mostly in the adipose tissues of the abdominal wall, intestinal tubes, and omentum.

Endometrial transplants survived in 50 mice (87.5%). No difference in implantation rate was detected among the four treatment groups (estrogen, progestin, estrogen+progestin, or saline control; Table 1). However, the number of ectopic lesions in mice receiving progestin was significantly greater than in other groups ($p < 0.05$). Further, the mean diameters of the lesions in the progestin group and the estrogen+progestin group were significantly greater than those in the estrogen group and the control group ($p < 0.05$).

Table 1. — Ectopic implantation of human endometrium in nude mice administered sex hormones or saline control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Implantation ratio (%)</th>
<th>Number of lesions ($x \pm s$)</th>
<th>Size ($x \pm s$, cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10/15 (66.7)</td>
<td>1.40 ± 0.52</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>Estrogen</td>
<td>12/15 (80.0)</td>
<td>1.33 ± 0.49</td>
<td>0.33 ± 0.09</td>
</tr>
<tr>
<td>Progestin</td>
<td>15/15 (100)</td>
<td>2.87 ± 0.83*</td>
<td>0.73 ± 0.20*</td>
</tr>
<tr>
<td>Estrigen+Progestin</td>
<td>13/15 (86.7)</td>
<td>1.62 ± 0.51+</td>
<td>0.65 ± 0.16+</td>
</tr>
<tr>
<td>Total</td>
<td>50/60 (87.5)</td>
<td>1.88 ± 0.90</td>
<td>0.52 ± 0.25</td>
</tr>
</tbody>
</table>

$\chi^2$ F = 6.240, 18.358, 31.796, p < 0.05, * Control group; # p < 0.05, vs Estrogen group; # p < 0.05, vs Progestin group.

Table 2. — MMP-2 and TIMP-2 expression in ectopic endometrium in nude mice administered sex hormones or saline control (AOD, $x \pm s$, cm).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MMP-2</th>
<th>TIMP-2</th>
<th>MMP-2/TIMP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.225 ± 0.015</td>
<td>0.286 ± 0.043</td>
<td>0.797 ± 0.095</td>
</tr>
<tr>
<td>Estrogen</td>
<td>12</td>
<td>0.273 ± 0.032*</td>
<td>0.241 ± 0.019*</td>
<td>1.141 ± 0.190*</td>
</tr>
<tr>
<td>Progestin</td>
<td>15</td>
<td>0.237 ± 0.013+</td>
<td>0.211 ± 0.014+</td>
<td>1.126 ± 0.086+</td>
</tr>
<tr>
<td>Estrigen+Progestin</td>
<td>13</td>
<td>0.298 ± 0.019*#</td>
<td>0.225 ± 0.016*#</td>
<td>1.334 ± 0.139*#</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>0.259 ± 0.035</td>
<td>0.237 ± 0.036</td>
<td>1.119 ± 0.224</td>
</tr>
</tbody>
</table>

F = 31.369, 21.262, 31.026, p < 0.001, * vs Estrigen group; # p < 0.05, vs Progestin group.

Figure 1. — Lesions of ectopic human endometrium implanted in nude mice.
treated mice also expressed secretory features, compared with the estrogen+progestin group these changes were less apparent and atypical (Figure 2C). Finally, lesions from the control group had proliferative changes, but the glands were fewer, thin, and short, glandular epithelium was mostly single-layer flattened, some glands were incomplete, stroma was less dense, and connective tissue proliferation significantly infiltrated the implants (Figure 2D).

**Immunohistochemistry of ectopic endometrium.** MMP-2 and TIMP-2 are typically detected in cytoplasm of normal and ectopic endometrial glands and stroma; the glands are generally highly positive [8]. MMP-2 and TIMP-2 were both detected in our ectopic endometrial samples by IHC with anti-MMP-2 and anti-TIMP-2 antibodies, visualized as brownish-yellow staining (Figure 3). MMP-2 and TIMP-2 staining was compared between groups using a software that analyzes staining intensity. The difference in MMP-2 and TIMP-2 protein expression between treatment groups was statistically significant ($p < 0.05$). Pairwise comparisons between groups indicated that MMP-2 expression in ectopic endometrium from
mice in the estrogen and estrogen+progestin groups was significantly higher than in the control group and progestin groups ($p < 0.05$). The difference in MMP-2 expression between lesions from mice in the progestin and control groups was not statistically significant. Conversely, TIMP-2 expression in ectopic endometrium from mice in the estrogen, progestin, and estrogen+progestin groups was significantly lower than in the control group. Further, TIPM-2 expression was significantly lower in lesions from the progestin group than in the estrogen group ($p < 0.05$). The differences in TIPM-2 expression in ectopic endometrium between the estrogen+progestin, estrogen, and progestin groups were not statistically significant. Additionally, the ratio of MMP-2 expression to TIPM-2 expression also differed. Pairwise comparisons between groups indicated a significantly higher MMP-2/TIPM-2 ratio in ectopic endometrium from mice in the estrogen, progestin, and estrogen+progestin groups compared to controls; further, the MMP-2/TIPM-2 ratio of lesions from the estrogen+progestin group was significantly higher than for the estrogen and progestin groups ($p < 0.05$). In contrast, there was no significant difference in the MMP-2/TIPM-2 ratios between the estrogen group and the progestin group.

**Discussion**

**EMT mouse model.** Although EMT is a benign disease, some characteristics resemble those of malignant tumors, such as invasion ability, which is capable of local or remote metastasis and invasion and damage to other tissues [9]. To study the pathogenesis of EMT and its influencing factors, an animal model must be established. Similar to a previously reported study in rats [10], we injected human late-secretory endometrial samples into the abdominal cavity of nude mice. Implanted mice were treated with estrogen and/or progestin to determine the effects of female sex hormones on implantation/EMT pathogenesis and MMP-2 and TIPM-2 expression. We obtained surviving ectopic endometrial implants in 87.5% of injected mice. These implants displayed typical endometrial glands and stroma, with proliferative and secretory morphological characteristics. However, 12.5% of the ectopic endometrial implantation failed, perhaps because endometrial samples were inadequate.

While there was no difference in implantation success between different hormone treatment groups, the mean size of implanted lesions in the progestin group was significantly larger than that of any other group, and the mean lesion size in the progestin group and the estrogen+progestin group was significantly larger than that in the estrogen group and the control group. Thus, progestin can promote the growth of ectopic endometrium. Studies have shown that progestin can induce secretion of ectopic endometrium, resulting in glandular cavity expansion and thus increasing the number of ectopic implants and implantation area [11]. Therefore, progestin stimulation may aid in the development of appropriate EMT animal models.

**Estrogen and progestin influence MMP-2 and TIPM-2 expression.** MMPs are important for the degradation of extracellular matrix (ECM), with MMP-2 playing a significant role in many current studies. MMP-2 is secreted by a variety of cells, such as fibroblasts, macrophages, endothelial cells, and malignant cells [12]. Through the degradation of ECM and basement membrane, MMP-2 provides space for new blood vessels and promotes tumor cell invasion and metastasis [13]. The studies on human EMT [14] and mouse EMT models [15] find that, compared with normal endometrium, ectopic endometrium has a stronger ECM degradation activity. Indeed, studies have shown that MMP-2 is highly expressed in stroma and epithelial cells in ectopic endometrium of patients with endometriosis, suggesting that MMP-2 may promote EMT occurrence and development through the degradation of ECM and basement membrane [16]. Additionally, ectopic endometrium has enhanced MMP-2 production, which increases the hydrolytic ability of this tissue. In turn, increased hydrolysis degrades the surrounding basement membrane and damages the connection of peritoneal stromal cells, causing the ectopic endometrium to grow and expand [17]. We found that MMP-2 protein expression in human endometrium ectopically implanted to nude mice was higher in animals treated with estrogen than in the control group; concurrently, TIPM-2 protein expression decreased, resulting in the increase of the ratio of MMP-2/TIPM-2 in the lesions. Thus, estrogen can promote the expression of ectopic endometrial MMPs and inhibit TIMPs, promoting ectopic endometrial invasion and growth. This effect on MMP-2/TIPM-2 may be an important factor for estrogen to promote EMT occurrence and development [18]. We also found that MMP-2 protein expression in ectopic endometrium in the progestin group was not significantly different from that in the control group. However, in these lesions TIPM-2 expression significantly decreased, resulting in a higher MMP-2/TIPM-2 ratio and increased ectopic endometrial invasion. Progestin is generally considered an inhibitor of MMPs; this hormone reduces estrogen production and directly or indirectly inhibits MMP gene expression and protein secretion [19, 20]. These results indicate that the increase in the MMP-2/TIPM-2 expression ratio regulates endometrium growth and expansion, not the activity of a specific hormone.

In summary, estrogen and progestin can regulate MMP-2 and TIPM-2 expression in human endometrium ectopically implanted in nude mice. An increase in the MMP-2/TIPM-2 ratio may lead to extracellular matrix degradation and angiogenesis, promote implantation and growth, and facilitate remote implantation and metastasis. Therefore, MMP-2 and TIPM-2 expression in ectopic endometrium may be used clinically to determine EMT invasion and metastasis and evaluate prognosis. In addition, estrogen and progestin can regulate EMT expression, which has important significance for exploring new methods of clinical diagnosis and treatment.
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References

Address reprint requests to:
X. MA, M.D.
Department of Blood transfusion
Qilu Hospital of Shandong University
Jinan Culture Road 107
Jinan 250012, Shandong Province (P. R. China)
e-mail: mxj19710612@126.com
Exaggerated placental site reaction detected during caesarean delivery: a case report

O. Akbayır¹, I. Alkis¹, A. Corbacioglu¹, A. Ekiz¹, A. Akca¹, S. Cekic²
¹Department of Obstetrics and Gynecology, Kanuni Sultan Suleyman Research and Teaching Hospital
²Department of Pathology, Bakirkoy Women’s and Children’s Research and Teaching Hospital, Istanbul (Turkey)

Introduction
In the human placenta, the trophoblasts that grow associated with chorionic villi are referred to as villous trophoblasts, whereas the trophoblasts in all other locations are termed as extravillous trophoblasts. The extravillous trophoblast that infiltrates the decidua, myometrium and spiral arteries of the placental site is almost exclusively composed of intermediate trophoblasts [1, 2].

In addition to the trophoblastic neoplasms such as hydatiform mole and choriocarcinoma, benign and malignant proliferations of intermediate trophoblasts have been identified recently. A spectrum of lesions derived from the intermediate trophoblasts have been described as placental site nodule or plaque, exaggerated placental site (EPS) reaction, placental site trophoblastic tumour, and epithelioid trophoblastic tumour [1, 3]. The definition of the lesions of intermediate trophoblasts is relatively new, therefore, their behaviour has not been well characterised [1].

EPS reaction is an exuberant physiologic process in which intermediate trophoblasts infiltrate the underlying endometrium and myometrium at the implantation site. During a caesarean section, we noted a polypoid well shaped smooth lesion, about 3 cm in diameter on the anterior wall of the uterus apart from the placenta. The histopathologic examination revealed an exuberant proliferation of trophoblastic cells in the placental site, a low Ki-67 labelling index and the absence of mitotic activity. Distinguishing EPS reaction from the other intermediate trophoblastic tumours is critical, as the latter may likely involve surgical intervention and/or chemotherapy, although no specific treatment and follow-up is required for EPS reaction. It is necessary to be aware of this pathology and take biopsies from suspicious lesions in the placental site for pathologic examination.

Key words: Exaggerated site tumour; Caesarean section; Intermediate trophoblast.

Case Report
A 24-year-old woman, gravida 2, para 1, underwent a caesarean section at 39 weeks’ gestation in elective conditions because of a previous caesarean delivery. After the removal of the placenta, a polypoid well-shaped smooth lesion, about 3 cm in diameter was noticed on the anterior wall of the uterus apart from the placenta. The lesion was removed and sent for histopathologic examination (Figure 1). There were no intraoperative or postoperative complications, especially related to haemorrhage.

The pathologic findings after examination of the specimen revealed an intermediate trophoblastic cell aggregation among the superficial myometrium and desidua. At the cytologic level, the cells had large eosinophilic cytoplasm and hyperchromatic nuclei with variable shape and size. Intermediate trophoblasts were surrounded by calcification areas and hyaline matrix. Any mitotic activity or necrotic change was not recognised. These microscopic findings were not adequate for the differential diagnosis of EPS reaction from a placental site trophoblastic tumour. Immunohistochemical examination with Ki-67 showed that there was no staining with Ki-67 on intermediate trophoblastic cells.

Summary
Exaggerated placental site (EPS) reaction is an exuberant physiologic process in which intermediate trophoblasts infiltrate the underlying endometrium and myometrium at the implantation site. During a caesarean section, we noted a polypoid well shaped smooth lesion, about 3 cm in diameter on the anterior wall of the uterus apart from the placenta. The histopathologic examination revealed an exuberant proliferation of trophoblastic cells in the placental site, a low Ki-67 labelling index and the absence of mitotic activity. Distinguishing EPS reaction from the other intermediate trophoblastic tumours is critical, as the latter may likely involve surgical intervention and/or chemotherapy, although no specific treatment and follow-up is required for EPS reaction. It is necessary to be aware of this pathology and take biopsies from suspicious lesions in the placental site for pathologic examination.

Key words: Exaggerated site tumour; Caesarean section; Intermediate trophoblast.
The exuberant proliferation of trophoblastic cells in the placental site with a low Ki-67 labelling index and the absence of mitotic activity indicated the diagnosis of EPS reaction (Figure 2).

Discussion

EPS is an exuberant infiltration of endometrium and myometrium by intermediate trophoblasts at the implantation site [5]. However, the overall architecture of the placental site is not disturbed. Endometrial glands and spiral arteries may be completely engulfed by trophoblastic cells, but there is no necrosis or mitotic activity. Also, the associated placentas are unremarkable [1].

On microscopic examination, EPS has an infiltrative border and is composed predominantly of mononucleate and a variable number of multinucleated intermediate trophoblastic cells. These cells are large with pleomorphic nuclei and abundant eosinophilic cytoplasm that diffusely infiltrate the endomyometrium and invade the spiral arteries. Immunohistochemical studies show that the trophoblastic cells in EPS are diffusely positive for human placental lactogen (hPL), the Ki-67 labeling index is nearly zero [1, 6].

On microscopic examination, EPS has an infiltrative border and is composed predominantly of mononucleate and a variable number of multinucleated intermediate trophoblastic cells. These cells are large with pleomorphic nuclei and abundant eosinophilic cytoplasm that diffusely infiltrate the endomyometrium and invade the spiral arteries. Immunohistochemical studies show that the trophoblastic cells in EPS are diffusely positive for human placental lactogen (hPL), and the Ki-67 labeling index is nearly zero [1, 6].

The distinction between a normal placental-site trophoblastic tumour (PSTT) and EPS is somewhat arbitrary, because there is no reliable data quantifying the amount and extent of trophoblastic infiltration during different stages of normal gestation [5]. Furthermore, EPS can be confused with the other intermediate trophoblastic tumours and tumour-like lesions [1, 4, 7]. Distinguishing EPS reaction from the other intermediate tumours is critical, as the latter may likely involve surgical intervention and/or chemotherapy. A PSTT is the most important differential diagnosis of EPS as both lesions are characterised by an exuberant infiltration of implantation site by intermediate trophoblastic cells. The microscopic features that support the diagnosis of PSTT include confluent masses of trophoblastic cells, unequivocal mitotic figures, the absence of chorionic villi, and elevated Ki-67 labeling index (14% ± 6.9%) [1, 5]. On the contrary, as with the histologic findings in our case, the exuberant proliferation of trophoblastic cells in a placental site with no mitotic activity and a low Ki-67 labelling index supports the diagnosis of EPS reaction. In cases where the diagnosis in not certain by pathologic examination, a follow-up with serum β-hCG concentration should be performed. A plateau or elevation in the β-hCG level require further evaluation, even a hysterectomy [1, 5, 7].

EPS reaction may develop secondary to a normal pregnancy, abortion, ectopic pregnancy, and hydatiform mole [5, 8]. It is a physiological process that resolves spontaneously after curettage or removal from the uterus. No specific treatment or follow-up is required. The reports describing clinical course of EPS are uncommon, but it is shown that an EPS which is not associated with hydatiform mole does not carry an increased risk of persistent gestational trophoblastic disease [8]. We followed up our case with β-hCG and ultrasound during the six postoperative months, and there was no evidence of recurrence or persistence.

In recent years, in spite of many articles published by pathologists, only a few case reports have been written by clinicians evaluating this subject. This is the first case report in the literature in which an EPS reaction is detected during a caesarean delivery.

More reports and studies are required regarding EPS reaction because of the awareness of the pathophysiology and clinical course of this lesion is important, especially in reproductive-age women who desire further pregnancies. We strongly advise all gynecologists to inspect the placental site and take biopsies from suspicious lesions for pathologic examination in order to obtain more information about this pathology.

References


Address reprint requests to: I.. ALKIS, M.D.
Istanbul Kamuni Sultan Suleyman Research and Teaching Hospital Turgut Ozal Caddesi No. 1
Halkali/Istanbul (Turkey)
e-mail: ismetalkis@hotmail.com
A case of polycystic ovary syndrome conceived by intracytoplasmic sperm injection following laparoscopic ovarian drilling

S. Sugaya, R. Honma

Department of Obstetrics and Gynecology, Joetsu General Hospital, Joetsu City (Japan)

Summary

Polycystic ovary syndrome (PCOS) is a disease in which an ovulation disorder is the main cause of infertility. Clomifene citrate (CC) is the treatment of first choice for ovulation induction in PCOS. If ovulation cannot be induced by CC, then either laparoscopic ovarian drilling (LOD) or gonadotropin therapy is selected as a subsequent treatment. Assisted reproductive technology (ART) is indicated for women with PCOS, similar to other infertility patients, when pregnancy is not achieved by intrauterine insemination (IUI). In this study, we experienced a case of PCOS in which pregnancy was achieved by ART following LOD. The case pertains to a 26-year-old patient. She consulted our hospital with a chief complaint of primary infertility. IUI with administration of CC plus recombinant follicle-stimulating hormone (rFSH) was carried out; however, pregnancy was not achieved. Subsequently, ART was carried out. In the first attempt, the development of several follicles was observed under the gonadotropin releasing hormone (GnRH) agonist long protocol. However, a fertilized oocyte was not obtained. In the second attempt, an ovum could not be collected after CC-rFSH ovarian stimulation. In the third attempt, a good quality embryo could not be obtained under the GnRH antagonist protocol, and therefore pregnancy could not be achieved. We performed LOD using a harmonic scalpel for the purpose of preventing severe OHSS and improving the quality of embryos. The operation, ovarian drilling was performed under the GnRH agonist protocol. Eighteen follicles were aspirated, six oocytes were picked-up, and five oocytes were normally fertilized. As a result, four embryos from day 2 culture were cryopreserved. Cryopreserved-thawed embryo transfer was thereafter performed, and a single pregnancy was achieved. LOD is a clinically effective treatment for PCOS requiring ART.

Key words: Polycystic ovary syndrome; Assisted reproductive technology; Ovarian hyperstimulation syndrome; Laparoscopic ovarian drilling; Quality of embryos.

Introduction

Polycystic ovary syndrome (PCOS) is a disease in which an ovulation disorder is the main cause of infertility. Ovulation induction using clomifene citrate (CC) is the first-line treatment for PCOS. Laparoscopic ovarian surgery (LOS) is often carried out when ovulation cannot be induced by CC treatment. Multiple ovarian puncture using either diathermy or laser is known as “ovarian drilling” [1]. This treatment is a method by which an improvement in hormone abnormalities is anticipated, and has been reported to be an effective therapy for infertility from PCOS [2-5].

PCOS patients are indicated to undergo assisted reproductive technology (ART), similar to other infertility patients, when pregnancy is not achieved by intrauterine insemination (IUI). In addition, ART is indicated for cases of tubal damage and male factor infertility. Moreover, ART is selected when there is a risk of ovarian hyperstimulation syndrome (OHSS) by gonadotropin therapy. Dor et al. [6] reported that the fertilization rate was low in PCOS compared to patients with tubal diseases, although the pregnancy rate was equal. They mentioned that the elevated serum LH level in PCOS might affect their oocyte potential for fertilization. On the other hand, a previous report observed an improved prognosis, such as a decrease in the rate of OHSS, when LOS was performed prior to ART [7].

We herein report a case of PCOS in which pregnancy was achieved by ART following laparoscopic ovarian drilling (LOD). The patient could not conceive even after ART by means of three types of ovarian stimulation. We performed LOD with the purpose of preventing severe OHSS and improving the quality of embryos, and pregnancy was subsequently achieved by ART following LOD.

Case Report

A 26-year-old female and her 30-year-old husband visited our hospital due to primary infertility of two years duration. Her menstruation was oligomenorrhea. Her hormonal testing showed increased secretion of early follicular phase serum LH (13.6 mIU/ml), and normal secretion of serum FSH (4.7 mIU/ml). The serum testosterone level was 100.1 ng/dl. Ultrasoundography showed the typical appearance of polycystic ovaries. At hysterosalpingography, her uterine cavity was normal and both tubes were patent. The semen analysis was normal (density: 40x10^6/ml, motility: 71%). The patient underwent ovarian stimulation with 100 mg of CC on days 4-8 of her menstrual cycle plus 75 IU of recombinant follicle-stimulating hormone (rFSH) (Follistim; Organon, Osaka, Japan) on days 8 and 10 of the cycle, and IUI. The number of developing follicles were one or two in each CC-rFSH stimulation cycles. A
A case of polycystic ovary syndrome conceived by intracytoplasmic sperm injection following laparoscopic ovarian drilling

In the first ICSI attempt, in April 2009, the patient received three weeks of oral contraceptives (planovar; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) from day 3 of the pretreatment cycle. Next, she received 900 µg of gonadotropin releasing hormone (GnRH) agonist (Supreecur; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) daily, starting on day 21 of the pretreatment cycle and ending at the time of hCG injection. The patient received 150 IU of rFSH daily from day 3 of the treatment cycle until the day before the administration of hCG (HCG Mochida; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). On day 8 of the treatment cycle (day 17 of rFSH), five follicles reached a diameter of ≥15 mm and more than 30 follicles reached a diameter of ≥11 mm. HCG administration was intended when at least two follicles reached a diameter of ≥18 mm. However, the administration of 5,000 IU of hCG was performed, since she was considered to be at too high a risk of OHSS to continue rFSH ovarian stimulation. Transvaginal follicular aspiration was performed approximately 34 hr after hCG injection. Only one oocyte was obtained, although more than ten follicles were aspirated. The sperm density was 8 x 10⁶/ml, and the sperm motility was 67.0%. Motile sperm were injected into two metaphase II oocytes using the routine ICSI procedure. However, the oocyte did not become normally fertilized.

In the second attempt, in November 2009, the patient underwent ovarian stimulation with 100 mg of CC on days 4-8 and 150 IU of rFSH injection on day 8, day 10 and day 12 of her menstrual cycle. One follicle was developed. Transvaginal follicular aspiration was performed approximately 34 hr after 5,000 IU of hCG injection. However, an oocyte could not be retrieved.

In the third attempt, in January 2010, GnRH antagonist protocol in controlled ovarian hyperstimulation (COH) was elected. From day 3 of her menstrual cycle, ovarian stimulation was commenced with 150 IU of rFSH daily for ten days. The administration of 0.25 mg of GnRH antagonist (ganirel; Schering-Plough Corp., Osaka, Japan) was performed on days 11 and 12 of her menstrual cycle. Five follicles were developed. Transvaginal follicular aspiration was performed approximately 34 hr after 5,000 IU of hCG injection. Two oocytes were retrieved. Transvaginal follicular aspiration was performed until the treatment cycle (day 7 of rFSH), five follicles reached a diameter of ≥15 mm, and the administration of 0.25 mg of GnRH antagonist was started. The administration of 150 IU of rFSH and 0.25 mg of GnRH antagonist were performed on day 13, day 14 and day 15 of the cycle. Eighteen follicles were developed. Transvaginal follicular aspiration was performed approximately 34 hr after 5,000 IU of hCG injection. Six oocytes were retrieved. The sperm density was 13 x 10⁶/ml, and the sperm motility was 67.0%. Motile sperm were injected into six metaphase II oocytes using the routine ICSI procedure. Five oocytes became normally fertilized. Four good quality embryos were obtained in day 2 culture. All four embryos were then cryopreserved by a vitrification method [8]. Cryopreserved-thawed embryo transfer was performed with an artificial cycle using transdermal estradiol (Estrana Tape; Hisamitsu Pharmaceutical Co., Inc., Japan) and vaginal progesterone in November 2010. The patient started transdermal estradiol administration every other day from day 3 of the treatment cycle. She received transdermal estradiol at a dose of 1 mg from day 3 to day 9 of the cycle. This dose was sequentially increased from day 11, to a maximum dose of 4.32 mg on day 15 of the treatment cycle. After adequate endometrial proliferation was documented by transvaginal ultrasonography on day 16, administration of vaginal progesterone suppository (400 mg/day) was initiated. At the same time, the dose of transdermal estradiol was decreased to 2.2 mg. After three days of progesterone administration, two embryos (4G, 4G) were thawed [8], and single embryo (morula) was transferred two days later. After ten days of transfer, the patient had a positive pregnancy test. Transdermal estradiol and vaginal progesterone administration was continued until eight weeks of gestation. This pregnancy is now ongoing at 30 weeks of gestation.

Discussion

In this case, pregnancy was not achieved although ART by three types of ovarian stimulation was performed. Under the GnRH agonist long protocol, the development of several follicles was observed, accompanied by a risk of severe OHSS. On the other hand, under the CC-rFSH protocol, an oocyte could not be obtained although it was of a single follicular growth. Under the GnRH antagonist protocol, a good quality embryo could not be obtained and embryo transfer was cancelled. Subsequently, LOD was carried out, and good quality embryos were obtained by ART, which was thereafter performed, and pregnancy was achieved.

In PCOS, ART is indicated, (1) when pregnancy is not achieved even though the timing of sexual intercourse or IUI was performed following ovulation induction; (2) when male factor such as oligozoospermia or tubal disease exists; (3) when there is a high possibility of the onset of OHSS at ovarian stimulation by gonadotropin treatment; (4) when there is a high possibility of multiple pregnancy of triplets or more.

For cases in which ART is performed on PCOS as well, if several follicle growths are observed, the risk of onset of severe OHSS can be decreased by carrying out cryopreservation of all embryos. On the other hand, a reduced incidence rate of OHSS by performing LOS prior to ART has been observed [7]. Moreover, a reduction in the ART cancellation rate by LOS has been reported [9]. Even when showing onset of OHSS, there are often cases in

Discussion

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which long-term treatments can be avoided, although in-hospital treatment may be necessary, because embryo transfer is not performed by cryopreservation of all embryos, as mentioned previously. From this, it can be said that the necessity of LOD for patients who require ART is decreasing. However, when the psychological damage from the onset of OHSS and the psychological state of patients for whom ART was cancelled were considered, it is believed that LOD will, even then, play an important therapeutic role.

The effect of LOD on follicular growth is not apparent. However, there have been reports that serum levels of LH and testosterone were reduced after surgery [2, 4, 5, 10]. In this case, we performed LOD using a harmonic scalpel. A harmonic scalpel is a mechanical cutting and coagulating energy source causing little tissue damage, and which also obtains hemostasis at relatively low temperatures. Takeuchi et al. [10] performed LOD on CC-resistant PCOS using a harmonic scalpel, and reported that this procedure was safe and also clinically effective.

As mentioned above, regarding the dynamics of hormones following LOD, serum levels of LH and androgen were decreased in many cases. LH is involved in the production of androgen at theca cells, and the production of androgen increases in patients with PCOS due to the increased secretion of serum LH. It has been reported that the increased production of androgens reduces oocyte quality [11]. On the other hand, a decline in the miscarriage rate has been reported to be observed for PCOS following surgery [7]. From these facts, it can be believed that the increased secretions of LH and androgen in PCOS may thus have a negative effect on embryo quality and the subsequent course of pregnancy. Therefore, for PCOS in which an elevated serum LH level is observed, even in CC-responsive cases, it is believed that performing LOD in order to improve the quality of embryos is a clinically useful treatment modality.

ART outcome following the use of GnRH antagonist protocol in COH has been reported to be as good as that of the GnRH agonist long protocol in PCOS [12]. In our case, ART was first carried out under the GnRH agonist long protocol in COH. Several follicle developments were observed, and there was a risk of OHSS. Subsequently, ART was performed under CC-rFSH stimulation. In the third attempt, the GnRH antagonist protocol in COH was applied. As a result, no oocyte could be obtained under the CC-rFSH protocol. In the GnRH antagonist protocol, good quality embryos could not be obtained. As a result, LOD was carried out to reduce the risk of OHSS, and to improve the quality of embryos following surgery. Five fertilized ova of six were acquired at the subsequent ART, and four good quality embryos were obtained. Pregnancy was achieved by the subsequent cryopreserved-thawed embryo transfer, and the patient is progressing very well.

In conclusion, we performed LOD to achieve adequate follicular development and also improve the quality of embryos in a case of PCOS, who could not conceive by means of three types of ovarian stimulation. Pregnancy was subsequently achieved by ART following LOD. This treatment is therefore believed to be a clinically effective treatment for PCOS requiring ART.

References


Address reprint requests to:
S. SUGAYA, M.D.
Department of Obstetrics and Gynecology
Joetsu General Hospital
148-1, Daidofukuda, Joetsu City
943-8507 (Japan)
e-mail: mususu@msg.biglobe.ne.jp
Sexual delusion in a case of vaginal aplasia after surgical operation for neovagina

H. Mellieu1, E. Daskalopoulou1, I. Evdokimides2, K. Gourounti1, P. Christopoulos1, G. Kreatsas2, G. Vaslamatzis1

1Department of Psychiatry, Athens University, Medical School, Eginitio Hospital, Athens
2Department of Neurology, Athens University, Medical School, Eginitio Hospital, Athens
3Department of Midwifery, TEI of Athens, Elena Benizelou Hospital, Athens
42nd Department of Obstetrics and Gynecology, Athens University, Medical School, Aretaieion Hospital, Athens (Greece)

Summary

The Mayer-Rokitansky-Kuster-Hauser (MRKH) is a syndrome of unknown etiology characterized by congenital aplasia of the uterus and the upper part (2/3) of the vagina in women showing normal development of secondary sexual characteristics. We report the case of a patient with vaginal aplasia and schizophrenia presenting with sexual delusion. To the authors’ knowledge this is the first case to provide evidence of coexistence between MRKH and sexual delusion in a schizophrenic patient. The core of the patient’s delirium was that she was having sexual intercourse with an eminent person through the big toe of her right foot. We approached this case using a neurological and a psychodynamic hypothesis. The neurological hypothesis suggests that the “deactivation” of the patient’s genitalia led to an expansion of the adjacent big toe cortical area. The psychodynamic hypothesis supports that the sexual function and pleasure was partially expelled from the body image and was stored in a non sexual part of the body (i.e., big toe). Clinicians should be aware of this association and offer patients with MRKH psychological or/and psychiatric evaluation.

Key words: Mayer-Rokitansky-Kuster-Hauser syndrome; Narcissistic trauma; Neovagina; Sexual delusion; Schizophrenia.

Introduction

The Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome is a rare entity (incidence of 1 in 4,000-5,000 female births) characterized by vaginal aplasia and absent uterus or an extremely rudimentary one. Its etiology is unclear [1, 2]. In embryological terms, it is an aplasia or dysplasia of the Müllerian (paramesonephric) ducts. The external genitalia and vestibule, deriving from the urogenital sinus, are normal. The sex chromatin pattern is female, and the endocrine system is not affected. Ovarian function is normal, so that secondary sex characteristics appear on schedule. Although MRKH syndrome can be diagnosed after birth, in the majority of cases, diagnosis is made during puberty, due to primary amenorrhea or inability to complete the sexual act. Sterility also constitutes a major problem in the syndrome [3].

In one study the existence of narcissistic trauma was characteristic in all patients with MRKH syndrome [4]. It is difficult to assess the emotional trauma at diagnosis and its repercussions on these women’s future life [3]. The absence of a vagina and uterus creates a feeling of dissimilarity and doubt about femininity and gender [2]. Patients often refer to the fact that they feel incomplete, present low self-esteem and express fear and concern about their sexual life and their relation to the opposite sex. Patients may show emotional instability and isolation, depression, anxiety and rarely suicidal behavior [2, 5-7].

Treatment involves the development of an operational neovagina [8, 9]. The emotional maturity of the patient seems to be more important than the type of intervention chosen (surgical or not) for the creation of a neovagina [7]. A rehabilitation process does not necessarily take place immediately after the diagnosis. Nevertheless, it has been suggested that after the patients have been informed of their condition, psychiatric evaluation followed by psychiatric consultation and psychotherapy is necessary [10]. The sooner the psychological support is provided to the patient, the better the therapeutic results become, both during and after the surgical intervention [4, 11, 12].

The aim of this case report is to present a case report of a schizophrenic patient with MPKH and to discuss the possible connections between sexual delirium and MPKH in a schizophrenic patient. With the incidence of the MRKH syndrome being 1/4,000 in female births while that of schizophrenia being approximately 1/100, the possibility of both disorders co-occurrence is exceptionally rare (1/400,000). To our knowledge, our case is the first of a simple co-occurrence between schizophrenia and MRKH syndrome.

Case Report

We present the case of a 28-year-old woman (NN) with MRKH which was diagnosed at the age of 17 during the evaluation of primary amenorrhea. After surgical creation of a neovagina following Creatsas vaginoplasty she engaged in a sexual life.

At the age of 20, while a university student, she presented with psychotic symptoms: delusions of persecution, grandeur, reference, passive control, mind reading, and thought broad casting. She believed that she was being trapped by her employers, her brain was opened by electromagnetic waves, her thoughts were withdrawn (thought withdrawal) and broad-
cated. She thought she would become a diplomat and relate to very important political persons. Initial treatment with haloperidol and biperidine failed due to poor compliance. For the next few years, she suffered almost continuously from psychotic symptoms both positive and negative, never being able to return to her studies and subsequently to work. She was hospitalized in a psychiatric clinic (age 25). Since then she has been continuously under psychiatric observation and medication (risperidone, olanzapine, amisulpride, quetiapine and for some time antidepressants, citalopram, venlafaxine, fluoxetine) never resulting in a total remission of symptoms.

In January 2009 she was referred to our psychiatric clinic. She agreed to hospitalization, and she was cooperative. She had some negative-oriented symptoms including a slightly blunted affect, emotionally withdrawn, socially withdrawn; but mainly positive-oriented symptoms: sexual delusions of persecution, reference, thought withdrawal, tactile/kinesthetic hallucinations. One of her delirious experiences was that she had communication with Mr K (an ex-president of the USA) who cared about her and used her as a secret secretary. She believed that Mr K had caused a burning inside her brain. He communicated with her and they made love through the big toe of her right foot. The intercourse with Mr K via the patient’s lower limb represented the core of her delirium, an event about which she spoke exclusively to her psychiatrist. The patient mentioned that often when she was lying down, she was thinking of Mr K and she was fantasizing about making love with him. Specifically, she mentioned that she felt an orgasm by stimulation of the big toe of the lower limb through which she was having sexual intercourse with him.

She was diagnosed with schizophrenia, paranoid type, treatment resistant and she was started on the atypical antipsychotic clozapine. During her hospitalization psychosocial interventions occurred including individual psychotherapy, group psychotherapy and expressive group therapy. Positive symptoms were partially limited and negative symptoms had ameliorated.

Unfortunately, NN denied any further examinations, such as for example somatosensory evoked potentials from the corresponding areas which might have been relevant to details of her delirium.

Discussion

A literature review revealed limited evidence on the psychological impact of MRKH diagnosis [12]. Symptoms of anxiety and depression observed in patients with MRKH are more severe than those in the general population and less severe than those in the psychiatric population [6]. Informing the patient about the diagnosis of the syndrome causes narcissistic trauma, disrupts the self image and leads to a deep feeling of incompleteness. Feelings of insufficiency/deficiency may follow the patient for years due to their sterility [6, 13].

In this case, psychosis followed MRKH and the surgical creation of a neovagina. It is known that a severe corporeal deficit, illness, or amputation may either precede or/and provoke a psychosis. However this putative cause-effect relation is often obscure and vague. In this case, the putative cause (aplasia and then neovagina) is focused on an extremely crucial area-organ for a woman’s sexual life and identity maturation and the phenomenology of the assumed effect (i.e., the psychosis) is dominated by a sexual delirium.

An interesting neurological approach to this case is provided by the remapping, reorganization hypothesis based on the plasticity of the central nervous system (CNS), a hypothesis developed by Merzenich et al. [14]. In the somatosensory cortex and according to the Penfield homunculus, the genitals (vagina and penis) are adjacent to the foot and especially to the big toe [15]. It is well known that extensive training of the thump leads to an enlargement of the corresponding cortical area [14]. The plasticity of the CNS and the reorganization of the cortical representation areas has already been described as the main mechanism for explaining the phantom lower limb when some points in the genitalia are stimulated, in a word “from the phantom leg to the adjacent genitalia” [15]. One wonders whether the “deactivation” of NN’s genitalia led to an expansion of the adjacent big toe area. Consequently, the patient’s delirious replacement is different from that observed by Aglioti et al., i.e., from the phantom vagina to the adjacent big toe. Furthermore, this putative remapping effect appears two to three years after the neovagina operation and only during delirium. NN never reported any kind of vagina and big toe interplay.

In parallel to the neurological hypothesis, a psychoanalytically based hypothesis can be built with regard to the correlation between vaginal aplasia and its surgical correction, and consequent changes in sexual life and sexual delusion. Psychoanalysts working with psychotic adolescents describe that changes of the body image will occasionally lead to threatening the person’s ego cohesion. Laufer and Laufer [16] and Laufer [17] have suggested that overwhelming anxiety is produced from the new physical experiences of the sexual body and the inability, in some cases, to be gradually integrated. The so-called “idealized prepubertal body image” is attacked from within. The ego is forced to defend itself against external reality and the person’s actual body to maintain the fantasy of the idealized body image. This “intrusion” leads to a psychotic core.

In our case the surgical correction led to a violent - for NN’s ego – transformation of body image to sexual body. We suggest that during the preceding period in adolescence, a non-sexual, ideal body image was maintained, which may have allowed NN to feel in unity with the ideal mother. This omnipotence served as an inner vulnerability, so that changes in the body and in the feelings could not be integrated in the internal body image. Therefore, the sexual function and pleasure were partially expelled from the body image and stored in a non sexual part of the body (i.e., big toe). The ego was impoverished, but retained the ability for a phantasized, albeit illusional, sexual activity. The psychotic episode may represent the patient’s inability to embrace the physical change, the appearance of the syndrome and the operation as “demands on the psyche of the change of the body image” [17].

Conclusions

To the authors’ knowledge this is the first case to provide evidence of a correlation between vaginal aplasia...
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(MRKH) and sexual delusion in a schizophrenic patient. Clinicians should be aware of this association and offer patients with MRKH psychiatric evaluation followed by psychotherapy if necessary.

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Address reprint requests to:
K. GOUROUNTI, Ph.D.
Department of Midwifery
TEI of Athens, Elena Benizelou Hospital
Agnoston Martiron 33-37, Nea Smirni
17123, Athens (Greece)
e-mail: clairegourounti@yahoo.gr
Management of genital warts in pregnancy

T. Hamouda, M.A. Freij, M. Saleh

Department of Obstetrics and Gynaecology, Norfolk and Norwich University Hospital NHS Foundation Trust, Norfolk (UK)

Summary
Genital warts are the most prevalent form of viral genital mucosal lesions. In pregnancy they may proliferate and become easily irritated due to the increased vascularity and altered immunity. This case highlights the importance of a multidisciplinary approach and exact planning to ensure good outcome in the management of genital warts in pregnancy.

Key words: Genital warts; Multidisciplinary approach; HPV; Pregnancy.

Introduction
Genital warts are the most prevalent form of viral genital mucosal lesions. They are caused by infection with several types of the double-stranded, one-enveloped HPV [1]. There are more than 80 HPV types which have been identified [2]. The types which affect the genital area are divided into low- and high-risk groups depending on their malignant potential [2, 3]. Approximately 90% of all genital warts are related to HPV types 6 and 11 (low-risk group).

We report a case of a 23-year-old primigravida to highlight the importance of a multidisciplinary approach and accurate planning to ensure a good outcome in the management of genital warts with pregnancy.

Case Report
A 23-year-old primigravida booked under community care noted a small swelling near the anal orifice when she was six weeks pregnant. Two weeks later she was seen by her physician who recognized the presence of a rapidly growing wart near the anal orifice and referred her to the GUM clinic. The patient had started her sexual activity at the age of 16 and had six previous sexual partners. She used to have unprotected sex and was not aware that any of her partners had had a sexually transmitted disease. She had never been treated for any sexually transmitted infections and she used to smoke about ten cigarettes per day.

The patient was reviewed in the GUM (genito-urinary medicine) clinic at 12 weeks of gestation. At that time a significant increase in the size of the wart was noted. She tested negative for sexually transmitted diseases and was treated with cryo-cautery. She was followed up in the GUM clinic after that and cryo-cautery treatment was repeated on another occasion without any notable effect. A decision was taken to refer her to the antenatal clinic for gynaecological assessment. The patient was at 27 weeks of gestation when she was first seen in the clinic. Her main complaints besides the rapidly growing lesion were increasing discomfort and pain, difficulties with defaecation and occasional bleeding from the swelling. Examination showed that the lesion was about 10 x 10 cm in size covering the entire perineum and perianal area. It was difficult to ascertain the exact site of origin because of the marked tenderness. A biopsy was taken from the superficial surface of the wart which confirmed its benign nature. It was decided at this stage that surgical treatment was required because of the rapidly growing nature of the lesion, the increase in the intensity of the symptoms and the need to have a thorough histological examination. The patient was reviewed by a plastic surgeon preoperatively to discuss the possible need for a skin graft if a wide excision of the skin was required. A colorectal surgeon was involved in the planning for the procedure as well.

A joint operation by the gynaecologist, colorectal and plastic surgeons was arranged. Under Epidural anaesthesia and sedation, the lesion was assessed and found to be 15 x 15 cm in size and arising mainly from the perianal and anal skin with no involvement of the anal canal. There was limited extension into the perineum (Figure 1). No evidence was observed suggestive of malignant change. The lesion was excised with unipolar diathermy from outside inwards securing the blood supply of each stem of the complex warty lesion separately. The extent of the skin damage at the end of the procedure did not require a skin graft (Figure 2). The wound was covered by opsite dressing and the patient was kept on oral antibiotics and laxatives postoperatively. The epidural anaesthesia was left for 24 hours for pain control and a urinary catheter was kept in for the same duration to care for the bladder. The welfare of the baby was monitored preoperatively by ultrasound scan examination to assess growth and fluid volume, and cardiotocography was performed before the operation. In the postoperative period routine midwifery care was found to be sufficient in monitoring the baby. The patient was discharged home on day 3 under the care of her physician, and the district nurse was informed to care for the wound.

Histopathology showed multiple viral papillomata with florid koilocytosis and foci of low grade dysplasia. The slides were reviewed and discussed at the gyne-oncology MDT meeting. It was agreed that the dysplasia associated with basal cell hyperplasia was very mild and mainly related to the florid HPV. The findings were regarded as benign viral warts.

The patient made a good recovery. She was followed-up after that on three different occasions. It took a few weeks for the complete healing of the perineum. Mode of delivery was discussed with the patient and she preferred to have an elective caesarean section to avoid the chance of damage or injury to the perineum. The caesarean was performed at 38 weeks of gestation.

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Management of genital warts in pregnancy

Discussion

Genital warts are the most prevalent form of viral genital mucosal lesions. They are caused by infection with several types of the double-stranded, one-envelope HPV [1]. There are more than 80 HPV types which have been identified [2]. The types which affect the genital area are divided into low- and high-risk groups depending on their malignant potential [2, 3]. Approximately 90% of all genital warts are related to HPV types 6 and 11 (low-risk group). We report a case of a 23-year-old primagravida to highlight the importance of a multidisciplinary approach and accurate planning to ensure a good outcome in the management of genital warts with pregnancy.

The infection manifests as verrucous fleshy whitish to red papules that may coalesce into plaques, ranging in size from a few millimetres to several centimetres [4]. The warts may be located anywhere in the ano-genital area, including the mucosal surfaces [5]. Lesions typically appear within weeks to months after exposure, and they are generally asymptomatic, but may be painful, friable or pruritic [3].

In the U.K., The highest rate of infection is seen among women aged 19 to 22 and men aged 22 to 26 years [2]. The risk factors for acquiring HPV infection include early age of intercourse [6], high number of sexual partners and high number of partners' sexual partners [7]. This is all due to the increase of the risk of exposure to HPV infection. Spontaneous resolution of HPV infection is less likely in immunocompromised individuals [8].

The diagnosis is usually made based on the clinical presentation of lesions located on the anogenital area or adjacent areas, such as the mons pubis [5]. Biopsy is generally not performed for the diagnosis of genital warts however it may be indicated if the warts appear fixed to underlying structures, refractory to standard therapy, ulcerated or if an individual wart is larger than 1 cm [3].

In pregnancy, genital warts may proliferate and become easily irritated due to the increased vascularity and altered immunity, therefore prophylactic removal might be indicated. The genital warts may be removed with destructive methods including cryotherapy, surgery or laser [3]. Trichloroacetic acid can be also used but this is usually more effective for the treatment of moist warts. Podophyl-
lum resin and podophyllotoxin must be avoided, as they are teratogenic. Imiquimod has not been adequately studied in pregnant patients thus should also be avoided [3].

HPV types 6 and 11 have been associated with laryngeal papillomatosis in infants. However, the presence of genital warts is not an indication for caesarean delivery [9]. A caesarean section is indicated only in the rare circumstance of obstruction or bleeding.

There is no cure for genital warts. The goal of treatment is to eliminate visible lesions. There is no evidence to show that treatment affects the natural course of HPV infection [10].

Conclusion

Early diagnosis and prompt treatment is required for genital warts in pregnancy particularly if it happens in the early stages and shows a tendency for rapid growth.

Surgical treatment was required because the lesion was progressively increasing, more symptomatic and there was a need to verify the histological nature of the deep parts of the lesion.

The multidisciplinary approach and careful planning ensured a good outcome.

The indication for caesarean section was based on maternal request to avoid any possible trauma to this area during delivery.

References


Lithiazis in the periurethral gland of a woman

R.N. Ergin, H. Köseoğlu

Başkent University Züeyde Hanım Practice and Research Center, İzmir (Turkey)

Summary
We present a very rare case of stone formation in the periurethral gland of a 49-year-old woman who was referred to our hospital with suspicion of a malignant or granulomatous soft-tissue lesion in the paraurethral area. The lesion was excised and the histopathological examination revealed cystic dilatation and squamous metaplasia in the lining of the glandular structure and surrounding lymphocytic infiltration. The scanning electron microscope examination of the stone revealed egg shell-like stratified concentric calcifications. The chemical composition revealed by the X-ray diffraction technique was a mixture of calcium oxalate and phosphate similar both at the outer and inner layers.

Key words: Lithiazis; Periurethral gland; Woman; Scanning electron microscope; X-ray diffraction.

Introduction
The formation of a stone in the periurethral gland in women is a very rare clinical situation. In the literature few authors have reported such cases [1-3]. Thus, we report this very rare case of stone formation in the periurethral gland and present the chemical composition of the stone together with the morphological structure examined under scanning electron microscope.

Case Report
A 49-year-old woman was admitted to our university hospital with suspicion of a granulomatous lesion or malignant soft-tissue lesion in the paraurethral area. She was complaining of palpation of firmness around her urethra while wiping after urination during the last few years and intermittency and difficulty in voiding which increased in severity during the last six months. Her medical history was non-specific. She had had no systemic disease nor any operations before. Her physical examination revealed that the urethral meatus was deviated to the right upper side by a firm mobile mass with calcified surface on bimanual vaginal examination located in the left lower side of urethral meatus (Figure 1). Her uroflowmetry study revealed an obstructed and intermittent pattern of voiding with residual urine of approximately 60 cc. Vaginal superficial ultrasonography revealed an approximately 15 mm diameter hyperechoic lesion with a smooth margin that had a posterior acoustic shadow in the periurethral region. Ultrasonographic findings were consistent with calcification. Under sedation and local anesthesia, cystourethroscopy was performed to rule out any lesion related to the urethra and bladder. Lumen of the urethra and bladder was normal and no pathological finding was present. The lesion was excised under microscopic magnification. The macroscopic examination of the excised formation revealed a stone formation in a cystic dilatation of the periurethral gland (Figure 2). The histopathological examination revealed cystic dilatation and squamous metaplasia in the inner lining of the excised glandular structure and surrounding lymphocytic infiltration. The stone was excised and the histopathological examination revealed cystic dilatation and squamous metaplasia in the inner lining of the excised glandular structure and surrounding lymphocytic infiltration.

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phocyte infiltration. Her voiding complaints were healed immediately after the operation and the uroflowmetry performed one month after operation revealed a completely normal voiding pattern with minimal residual urine. The scanning electron microscope examination of the stone revealed egg shell-like stratified concentric calcifications (Figure 3). The chemical composition of the stone revealed by a X-ray diffraction technique was a mixture of calcium oxalate and phosphate similar both at the outer and inner layers.

Discussion

In women, lithiazis in the periurethral gland is a very rare clinical situation [1-3]. The calculus seems to be formed by precipitation of calcium oxalate and phosphate deposits concentrically in the obstructed gland as suggested by the egg shell stratified concentric layered view seen under scanning electron microscope.

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References


Address reprint requests to:
H. KOSEĞLU, M.D., FEBU Valide-I Atik Mah Çimili Mescit Sok Yıldız Apt No: 33 D:16 Üsküdar, İstanbul (Turkey)
e-mail: hikmet.koseoglu@gmail.com
Expectant management of preterm premature rupture of membranes remote from term with exiguous amniotic fluid and a prolonged latency period: report of two cases

M.T. Canda¹, O. Sezer¹, C. Ozturk², N. Demir¹

¹Department of Obstetrics and Gynaecology, Kent Hospital, ²Neonatal Intensive Care Unit, Kent Hospital, Izmir (Turkey)

Summary
Management of preterm premature rupture of membranes (PPROM) is a very challenging issue for the obstetricians. We report two cases of PPROM occurring in early gestation remote from term (both < 26 weeks) with exiguous amniotic fluid (amniotic fluid index of ≤ 2 cm) that were managed successfully by conservative treatment and resulted in a latency period of almost two months. This treatment option might be feasible in carefully selected patients following meticulous evaluation and warrants further research.

Key words: Preterm premature rupture of fetal membranes; Amniotic fluid; Premature birth.

Introduction
Preterm premature rupture of membranes (PPROM) refers to the spontaneous rupture of the membranes before the onset of labour before 37 weeks of gestation. Almost 3% of all pregnancies are complicated by PPROM [1]. PPROM occurs in one-third of all preterm births, and it is the most definitive cause of preterm birth [2]. PPROM can increase the incidence of perinatal infections, prematurity, pulmonary hypoplasia and umbilical cord compression or prolapse all of which can result in perinatal morbidity and mortality [3]. This condition may cause maternal infections and placental abruption [4]. PPROM that occurs at viability (23 weeks) up to 32 weeks of gestation is referred to using the term PPROM remote from term [1].

Herein, we report two patients with PPROM remote from term who were treated successfully with conservative expectant management with for almost two months at our institution.

Case Reports
Case 1
A 32-year-old patient (G2/P1; 23 3/7 weeks of gestation) was referred to our department due to amniotic fluid leakage. Her pregnancy history was uneventful. Some amniotic fluid was seen at the posterior fornix without cervical dilation on sterile speculum examination. The Amnisure test (N-Dia Inc. New York, NY) was positive. An ultrasound (US) scan revealed a 23-week fetus with an estimated weight of 595 g and an amniotic fluid index (AFI) of ≤ 2 cm. Cervical length was measured as 39 mm without evidence of funneling. Upon admission, blood leukocyte count and C-reactive protein (CRP) levels were within normal limits (9.9 *10³/mm³ and 0.35 mg/dl, respectively). No pathogen was detected in cultures or gram staining of urine and cervical-vaginal samples for Group B Streptococcus, Chlamydia and Gonorrhoea. The patient was informed about the benefits and risks of conservative-expectant management and was hospitalised for bed rest. Parenteral antibiotic treatment with ampicillin (2 g every six hours for 48 hours) and intravenous serum (isolyte) were initiated. Thereafter, parenteral antibiotics were replaced with oral amoxicillin (250 mg every 8 hours for 5 days). Daily fetal cardiotocography and daily maternal blood pressure, pulse rate and body temperature were checked. A maternal US scan and blood tests (leukocyte count and CRP) were carried out twice weekly. Amniotic fluid leakage was present every day until birth. After 55 days of conservative-expectant management (31 1/7 weeks gestation), premature contractions began without any sign of amnionitis. Tocolysis and a course of betamethasone were initiated immediately. However, contractions continued and a cervical dilatation of 2 cm was detected. Due to variable decelerations the baby was delivered by caesarean section. A baby girl with a birth weight of 1,610 g (10-50th percentile), length of 41 cm (10-50th percentile), head circumference of 27 cm (10th percentile) and 1- and 5-min Apgar scores of 6 and 8, respectively, was hospitalised in the neonatal intensive care unit (NICU). After 42 days of treatment (3 weeks before the calculated delivery date), the infant was discharged at a weight of 2,050 g (3-10th percentile).

Case 2
A 40-year-old patient (G1/P0; 25 6/7 weeks gestation) was referred to our department due to PPROM. US demonstrated a 26-week foetus with an estimated weight of 858 g and an AFI of ≤ 2 cm. Cervical length was 40 mm without evidence of funnelling. The vagina was wet with amniotic fluid upon speculum examination. Cervical-vaginal cultures were negative. Blood tests and urine analysis were within normal limits. Similarly, the patient was treated with conservative-expectant management as explained above with the same protocol. AFI was detected to be < 2 cm throughout the pregnancy. In the 32nd gestational week, a single course of betamethasone was administered. At the follow-up, the estimated weight of the fetus remained the same with an increased umbilical artery resistance index (RI) of 0.76. At 34 1/7 weeks of gestation (after 59 days of hospital stay with prolonged PPROM), a caesarean section was performed due to breech presentation. A baby boy with a birth weight of 1,760 g (10th percentile), length of 43 cm (10-50th percentile), head circumference of 30 cm (10-50th percentile) and 1- and 5-min Apgar scores of 7 and 9, respectively, was delivered. Likewise, the baby was hospitalised in the NICU. After 28 days of treatment (2 weeks before the calculated delivery date), the infant was discharged with a weight of 2,346 g (3rd percentile).
Discussion

Conservative management of PPROM includes modified bed rest, foetal and maternal monitoring (including nonstress test, body temperature, tachycardia, and the presence of uterine tenderness), evaluation of haematological tests (blood leucocytes and CRP) and US scans. Modified bed rest is necessary to improve the AFI and chance of spontaneous sealing of the membranes. In our protocol, foetal monitoring was performed daily to ensure foetal well-being and to provide knowledge about foetal distress, umbilical cord compression and premature uterine contractions [1]. Nonetheless, continuous external foetal heart rate monitoring does not seem feasible in patients with PPROM undergoing expectant management [5]. US scans should also be performed at least weekly to determine the AFI, Doppler blood flow and foetal weight gain [1]. It is suggested to give broad-spectrum antibiotics starting with IV therapy for 48 hours (ampicillin 2 g IV every 6 hours) followed by five days of oral therapy (amoxicillin 250 mg every 8 hours) [6]. In cases with an allergy to penicillin, macrolide antibiotics should be chosen. Previous studies [7] have shown that antibiotic therapy may increase the latency period by more than one week and reduce the risk of amnionitis, neonatal sepsis, respiratory distress syndrome and intraventricular haemorrhage. If amnionitis is suspected, amniocentesis can help in determining whether an infection is present. The amniotic fluid can be checked for lowered glucose concentrations, elevated interleukin levels [8], a positive Gram stain or a positive amniotic culture. Although elevated CRP is associated with histologic chorioamnionitis, there is not enough data to support its use as an early predictor of chorioamnionitis; however, serial measurements of CRP could be promising [9].

The goal of treatment for PPROM remote from term is to prolong the delivery of the foetus until after 32-34 weeks of gestation, because the risk of neonatal complications noticeably declines thereafter [10]. Unfortunately 50-60% of women with PPROM remote from term deliver within one week of the rupture of the membranes [11]; only a few of these women will continue their pregnancy for more than a week. Studies of parameters that affect the latency period offer conflicting findings. The AFI is one of the most important predictors of latency. Previously, it has been shown that women with a residual AFI of ≤5 cm usually have a shorter interval period between PPROM occurrence and delivery, and they also have an increased risk of chorioamnionitis [12].

A shorter interval of latency for PPROM of < 30 weeks of gestation is related to increased infant mortality [13]. Additionally, if the foetus survives after PPROM, the neonatal outcomes may improve. In this case, elective delivery could be associated with improved neonatal outcome compared to spontaneous labour [14]. In both of our cases, the time interval between PPROM and delivery was almost two months (55 and 59 days) despite the AFI of ≤2 cm. This strategy provides time for foetal maturation and lung development. To avoid the possible complications during the latency period (e.g., spontaneous onset of labour, infection, abruption placenta and foetal distress), close monitoring of both the mother and foetus should be performed.

In summary, we have reported two cases of PPROM occurring in early gestation remote from term with exiguous amniotic fluid that were managed successfully by conservative treatment and resulted in a latency period of almost two months. Although our experience is very limited (i.e., just two patients), our approach includes close follow-up and treatment. This treatment option might be feasible in carefully selected patients following meticulous evaluation and warrants further research.

References


Address reprint requests to:
M.T. CANDA, M.D.
103 Sok. Melekoglu Apt. No: 1/3
Gortep. 35290, Izmir (Turkey)
e-mail: cananduction@yahoo.com
Pulmonary benign metastasizing leiomyoma: case report and review of the literature

Y. Ni¹, G. Shi¹, H. Wan¹, J. Shen¹, X. Jiang², F. Yuan³

¹Department of Pulmonary Medicine, ²Department of Nuclear Medicine, ³Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai (China)

Summary

We present a case of a middle-aged woman who was admitted to the hospital for abdominal pain and was found to have multiple nodules on her CT scan. After CT-guided biopsy, we finally diagnosed PBML case. The patient had a very effective response to bilateral oophorectomy and hysterectomy. A brief review of the literature for PBML is also included.

Key words: Lung; Pulmonary benign metastasizing leiomyoma (PBML); Hormonal manipulation.

Introduction

Pulmonary benign metastasizing leiomyoma (PBML) occurs very rarely and was first described by Steiner in 1939. It occurs more frequently in women of reproductive age, especially those who have had a hysterectomy due to leiomyoma [1]. The diagnosis of BML should be considered when a reproductive-aged women presents with multiple pulmonary nodules and has a prior history of hysterectomy. The prognosis of BML is good if estrogen levels are well controlled.

Case Report

A 46-year-old female was referred to the emergency department in Ruijin Hospital, Shanghai Jiaotong University, with “intermittent right upper abdominal pain”, which had begun two months before. The abdominal pain was relieved after anti-inflammatory and anti-spasmodic treatment. During treatment, multiple pulmonary nodules were observed on chest X-rays. The patient had no pulmonary symptoms. Her past medical history showed that she had undergone a subtotal hysterectomy four years before. The previous postoperative pathological diagnosis was “multiple intramural uterine leiomyoma, with hemorrhage, cell-rich”. To confirm the diagnosis, the patient was admitted to the pulmonary department after being treated in the emergency department.

The patient did not present any infectious symptoms such as cough, fever, night-sweats, and was negative for bacteria, fungi, and tuberculosis in sputum, so we excluded infectious diseases first. Chest computed tomography (CT) results showed multiple, diffuse, bilateral, well-circumscribed pulmonary nodules ranging from 0.5-1.5 cm in diameter, with pleural thickening on both sides (Figure 1). Due to lack of an irregular spiculated edge on CT imaging, metastasizing lung tumor was considered. Considering the abdominal pain and history of uterine leiomyomas, the primary tumor was speculated to originate from the uterus. We had thought that the previous diagnosis of uterine leiomyoma could have been a misdiagnosis of uterine leiomyosarcoma. Moreover positron emission tomography (PET) CT results showed multiple pulmonary nodules, some of which were hypermetabolic (Figure 2), and hypermetabolic lymphadenopathy in front of the abdominal aorta measuring 1.5 cm in diameter (Figure 3), which supported our speculation. Then we did CT-guided lung biopsy of the hypermetabolic nodules. The following pathological diagnosis performed for this biopsy was “leiomyoma” (Figure 4). Due to the typical morphology, no further analysis such as immunohistochemistry was performed.

During hospitalization, the patient experienced intermittent, severe right lower abdominal pain accompanied by vomiting. Anti-spasmodic treatment had no effect. Pelvic CT showed a suspicious cystic lesion in the right adnexa of the uterus. Given the definite diagnosis of pulmonary nodules and the severe abdominal pain, we advised bilateral oophorectomy and hysterectomy. After the operation, the pain disappeared. Seven months later, chest CT showed a significant reduction of the pulmonary nodules (Figure 5). Considering both the pathological diagnosis and the efficacy of the bilateral oophorectomy and hysterectomy, pulmonary benign metastasizing leiomyoma (PBML) was finally confirmed.

Discussion

Benign metastasizing leiomyoma (BML) was first reported by Steiner in 1939 [1]. About 100 cases were reported in the literature up to 2003 by searching Pubmed. BML is more common in women of reproductive age, especially in those who have undergone hysterectomy for leiomyomas. The lung is the most common organ of metastasis. Other organs include the lymph nodes, peritoneum and retroperitoneal structures. Pulmonary nodules can be detected from three months to 20 years after hysterectomy [2]. Patients are usually asymptomatic with incidental discovery of pulmonary lesions. Some patients may manifest with hemoptysis, cough, chest pain, and dyspnea.

Typical radiographic findings include well circumscribed solitary or multiple pulmonary nodules ranging from a few millimetres to several centimetres in diameter. In a few case reports, BML manifests as cavitary lung nodules and interstitial lung disease. Typically, nodules are not calcified and are not contrast-enhanced on CT. Endobronchial and pleural sparing is one of the features [2].
Upon histological analysis, these tumors consist of well differentiated, benign-appearing smooth muscle cells lacking mitotic figures, anaplasia, or vascular invasion [3]. Because some cases have been diagnosed as metastasis of low-grade leiomyosarcomas [4], some authors pointed out that these lesions actually represent metastatic foci from low-grade leiomyosarcomas. Nuovo and Schmittgen [5] found that the expression of microRNA-221 (miR-221) was negatively associated with carcinogenesis of BML and uterine leiomyoma, but positively associated with most cases of leiomyosarcoma. The authors also proposed that increased miR-221 expression may be a molecular marker to differentiate leiomyosarcoma from BML. Other groups demonstrated that smooth muscle tumors in the lung may represent multiple native smooth muscle hamartomas rather than actual metastatic foci in women with a predisposition to hamartomas [3].

In 1983, Martin et al. proposed a classification system for multiple smooth muscle lesions in the lung [6]. They suggested that the lesions can be classified into three categories: BML, metastatic leiomyoma, and multiple fibroleiomyomatous hamartoma. Although these lesions are histologically indistinguishable, distinctions can be made based on the clinical manifestation and the response to certain treatment. BML develops from a primary uterine source in childbearing-age women. These
tumors are typically hormonally responsive, demonstrating progression with estrogen and regression with progesterone. Thus, treatment may include hysterectomy, bilateral oophorectomy and long-term hormone therapy. The second category includes metastatic leiomyomas, which arise in men and children from an extra-uterine primary source. These tumors are not hormonally responsive and may actually represent slow-growing sarcomas that can be treated with surgical resection with mixed success. The third category, multiple fibroleiomyomatous hamartoma, consists of multiple leiomyomas of the lung, without a primary source. These lesions are benign.

In our case, the patient had a history of subtotal hysterectomy. The pulmonary nodules significantly regressed after hysterectomy and bilateral oophorectomy. Therefore, the diagnosis was one of BML, and the hypermetabolic lymph node in front of the abdominal aorta observed on the PET-CT was also considered to be BML.

The pathogenesis of BML is still unclear so far. Although uterine leiomyoma is common, pulmonary metastasis is rare. The mechanism for metastasis may involve the dissemination of tumor cells from surgical operations including uterine curettage, hysteromyectomy and hysterectomy. However, this mechanism cannot explain why pulmonary nodules manifest before a surgical operation. Patton and colleagues [7] analyzed the pathological and immunohistochemical features, clonality, and telomere length of multiple lung and uterine tumors in three patients with benign metastasizing leiomyoma. The results showed that pulmonary nodules had the same characteristics with those observed in uterine leiomyomas, which supports the notion that BML is clonally derived from benign-appearing uterine leiomyomas.

Due to its origin from uterine leiomyoma and the expression of estrogen and progesterone receptors in pulmonary nodules [8], BML is considered to originate from estrogen stimulation. Natural regression of pulmonary nodules after menopause and pregnancy has been observed [9]. Thus, control of estrogen level is the key to successful treatment. Hysterectomy and bilateral oophorectomy are very effective. In our case, the pulmonary nodules showed significant regression seven months after surgery (Figure 5).

Nonetheless, the possibility of medical castration attracts much more interest due to its reversibility, and its potential to obviate the need for a surgical procedure, or to allow symptom control when surgical management is not possible. The most commonly used drugs include luteinizing hormone-releasing hormone analogues (LHRHA), progesterone, aromatase inhibitors (AI) and selective estrogen receptor modulators (SERM).

LHRHA induce tumor regression by suppressing the synthesis of estrogen. However, in some cases, LHRHA alone cannot prevent tumor progression. A combination with other medicines may be a better strategy [10]. The mechanism of progesterone treatment is to suppress the hypothalamic-pituitary-gonadal axis, thereby reducing ovarian estrogen synthesis. Progesterone also acts to increase the rate of enzymatic inactivation of estradiol by increasing its conversion to estrone, through an increase in the activity of an oxidative type 17β-hydroxysteroid dehydrogenase enzyme. In addition, progesterone reduces aromatase activity. Aromatase-P450 is the key enzyme for estrogen synthesis. Over-expression of aromatase-P450 by uterine leiomyomas may contribute to their growth. Aromatase inhibitors, such as anastrozole, lower estradiol concentrations by acting both on the gonads and on the peripheral and tumor tissues. They are effective and increasingly used to treat ER-positive metastatic breast cancer [11].

Raloxifene, a synthetic non-steroidal SERM, acts as an estrogen agonist on the skeleton, cardiovascular system, and central nervous system, but exhibits only weak estrogenic antagonist effects on the breast and uterus. Additional administration of raloxifene to patients treated with an LHRH agonist for uterine leiomyomas results in higher reduction of leiomyoma size. However, there is still little data published regarding the combination of AIs and SERMs to treat BML.

In summary, a diagnosis of BML should be considered when a reproductive-aged women presents with multiple pulmonary nodules and has a prior history of hysterectomy. The prognosis of BML is good if estrogen levels are well controlled.

References


Address reprint requests to:
G. SHI, M.D.
Department of Pulmonary Medicine
Ruijin Hospital, Shanghai Jiaotong University School of Medicine
Shanghai 200025 (China)
e-mail: shi_guochao@yahoo.com
Decompensated cirrhosis and pregnancy: a case report

F. Kouakou, V. Loué, R. Adjohy, K. N’guessan, D. Effoh

Department of Gynaecology Obstetrics, CHU Cocody, Abidjan (Ivory Coast)

Summary

The association of decompensated cirrhosis and pregnancy is rare. Portal hypertension exposure to gastrointestinal bleeding from a ruptured esophageal varix may at any time complicate the course of the disease. We report the case of a 24-year-old patient who delivered at 35 weeks/four days of gestation with decompensated cirrhosis secondary to viral hepatitis B; icterus, oedema, and ascites were present. The postpartum course was uneventful despite the biological disorder of coagulation.

Key words: Pregnancy; Decompensated cirrhosis.

Introduction

Liver diseases in pregnancy may be categorized into liver disorders that occur only in the setting of pregnancy and liver diseases that occur coincidentally with pregnancy [1]. The association of decompensated liver cirrhosis and pregnancy is a rare event [2]. It is a high-risk pregnancy. Portal hypertension and gastrointestinal bleeding mostly by rupture of the oesophageal or gastric varix may at any time complicate the course of the disease. These occur mainly in the third trimester of pregnancy and the postpartum period [3, 4]. We report the case of a young woman with decompensated cirrhosis and ascites secondary to viral hepatitis B who gave birth.

Case Report

A 24-year-old housewife, fourth gravida and primipara, has suffered from hepatitis B virus since 2000. Her history showed a vaginal delivery in 2005 of a living child (birth weight 3000 g). She had had two arrested pregnancies at 11 weeks of amenorrhea and 18 weeks of amenorrhea in 2009. The diagnosis of hepatitis B – endemic in our region – is the most common etiology of chronic hepatopathies in Black Africa [10, 11]. Liver cirrhosis predominates in developed countries [3], viral hepatitis B – endemic in our region – is the most common etiology of chronic hepatopathies in Black Africa [10, 11]. Liver cirrhosis occurs at a relatively advanced age and the association of cirrhosis and pregnancy occurs mainly between 30 and 35 years [12]. However, cases of young patients like our case have been described [5].

Although uncommon, women with cirrhosis may become pregnant and may have a relatively benign course of pregnancy [1], even with the exceptional decompensated cirrhosis. The late age of onset of liver damage and hormonal changes inducing hypo-fertility explain this rarity [3]. Note however that the actual frequency of this association remains unclear, and in the literature only a few cases or a few short series of cases have been described [5-8]. Our observation is the second documented case in our country after the first was published [9].

Although uncommon, women with cirrhosis may become pregnant and may have a relatively benign course of pregnancy [1], even with the exceptional decompensated cirrhosis. The late age of onset of liver damage and hormonal changes inducing hypo-fertility explain this rarity [3]. Note however that the actual frequency of this association remains unclear, and in the literature only a few cases or a few short series of cases have been described [5-8]. Our observation is the second documented case in our country after the first was published in 2007 [9].

The cirrhosis was secondary to viral hepatitis B infection in our patient. Indeed, while the origin of ethyl cirrhosis predominates in developed countries [3], viral hepatitis B – endemic in our region – is the most common etiology of chronic hepatopathies in Black Africa [10, 11]. Liver cirrhosis occurs at a relatively advanced age and the association of cirrhosis and pregnancy occurs mainly between 30 and 35 years [12]. However, cases of young patients like our case have been described [5].

Discussion

The cirrhosis was secondary to viral hepatitis B infection in our patient. Indeed, while the origin of ethyl cirrhosis predominates in developed countries [3], viral hepatitis B – endemic in our region – is the most common etiology of chronic hepatopathies in Black Africa [10, 11]. Liver cirrhosis occurs at a relatively advanced age and the association of cirrhosis and pregnancy occurs mainly between 30 and 35 years [12]. However, cases of young patients like our case have been described [5].
Our patient had two pregnancies which were arrested at the stage of decompensated cirrhosis. She then gave birth to a child at the 36th week that was hypotrophic but otherwise healthy. Indeed, at that stage of cirrhosis, foetal risks are increased and there is more frequent foetal death [5]. Risks also exist even without any real decompensation of liver function such as in our patient [5]. Cases of miscarriage and pregnancies carried to live term birth in the same women with cirrhosis have been published [4, 7].

In general, the delivery route of choice is not influenced by the existence of liver disease; caesarean section always maintaining its traditional obstetric indications [13, 14]. Some argue that in case of acute liver failure, the emergency retrieval of a premature foetus even seems justified [15]. The rate of caesarean section is higher among pregnant women with cirrhosis compared with general obstetric patients [16].

An indication for caesarean section was assumed in our patient due to IUGR. However the caesarean was not performed immediately because of the major disorders of haemostasis. Faced with these major disorders (prothrombin rate = 44.6%, fibrinogen = 1.15 g/l, platelets = 76000/mm³) our patient needed priority of delivery in order to prevent haemorrhage at that placental stage. The patient received fresh frozen plasma, red cell concentrate and vitamin K1. Fibrinogen is not easily available in our pharmacies. Indeed, the vascular filling pre- and intraoperatively must be associated with the transfusion of FFP and/or platelets in case of biological abnormalities that warrant it. Platelet transfusion is recommended immediately prior to caesarean cases of thrombocytopenia less than 50000/mm³ and immediately prior to vaginal delivery in cases of thrombocytopenia less than 30000/mm³ [17]. FFP transfusion in obstetrics is recommended in case of bleeding associated with disseminated intravascular coagulation syndrome and in patients with an impaired hepatocellular condition; FFP transfusion is also indicated in case of bleeding or an invasive procedure [17]. In our case, the transfusion of FFP was justified by the pre-existing coagulation abnormalities and caesarean section indicated due to IUGR. Her platelet count did not justify a platelet transfusion.

The woman gave birth spontaneously vaginally with a quick and easy expulsion. The instrumental delivery might be justified in difficult cases of expulsion to minimise the patient’s expulsive efforts that could increase the risk of bleeding [5]. The placental stage was made by cord traction control followed by continuous infusion of
syntocinon. The postpartum course was uneventful. However, it is worth noting that during the postpartum period maternal risk remains dominated by major postpartum haemorrhage due to the cumulative effect of thrombocytopenia because of hypersplenism and bleeding disorders resulting from alterations in hepatic synthesis of certain coagulation factors [4]. It is an almost constant risk that must be rigorously prevented.

Conclusion
The association of cirrhosis and pregnancy is a high-risk combination. However, cirrhosis is not a contraindication, as pregnancy may be tolerated if cirrhosis is well compensated. The maternal risk is directly related to the existence of portal hypertension and oesophageal varix which should be systematically investigated in early pregnancy. In the postpartum period, the fear of postpartum haemorrhage requires close monitoring of blood coagulation.

References

Address reprint requests to:
F. KOUKOU, M.D.
Department of Gynaecology Obstetrics
Chu Cocody
03 BP V13 Abidjan (Ivory Coast)
e-mail:kouakof2000@yahoo.fr
Uterine tamponade balloon for the management of massive hemorrhage during cesarean section due to placenta previa/increta

N. Vrachnis, C. Iavazzo, N. Salakos, E. Papanargaritis, I. Boutas, G. Creatsas
2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens (Greece)

Summary
Objective: Uterine tamponade with the Bakri catheter is effectively used as a treatment in postpartum hemorrhage and as a means to prevent fertility. Case: We present a case of a 40-year-old pregnant woman who had a massive hemorrhage during cesarean section who was successfully treated with a tamponade balloon. Furthermore, we comment on a similar technique – Logothetopoulos pack – which was first developed and used in our department in the early years of the previous century. Conclusion: A conservative technique such as the Bakri catheter is an alternative intermediate step to control postpartum hemorrhage when pharmaceutical methods fail and before proceeding to obstetric hysterectomy.

Key words: Bakri catheter; Tamponade balloon; Postpartum hemorrhage; Obstetrical bleeding; Placenta previa; Placenta increta; Logothetopoulos pack.

Introduction
Postpartum hemorrhage (PPH) is a life-threatening complication and is defined as blood loss greater than 500 ml in a vaginal delivery or than 1000 ml in a cesarean section [1]. It leads to obstetric hysterectomy in 0.35 per 1,000 births [2]. It accounts for 24% or more of maternal deaths [3] and is mainly caused by uterine atony, traumas, retained placenta and coagulation disorders [4].

Placenta accreta is an obstetrical pathology characterized by morbid adherence of the placenta through the endometrium and into the myometrium [5]. Placenta accreta and placenta previa increase the risk of PPH [6]. The first line of PPH treatment embraces pharmaceutical methods that include oxytocin, ergometrin or prostaglandins [7-10]. Further steps that conserve the uterus include embolization of the uterine vessels, uterine tamponade, the B-Lynch suture and internal iliac artery ligation [11-13]. When conservative methods fail PPH is treated with the rather undesirable obstetric hysterectomy [14]. However, conservative approaches are being gradually more used.

One of the earliest methods of achieving intraoperative hemostasis with tamponade was by uterine or pelvic packing applied in our institution, the 2nd Department of Obstetrics and Gynecology of the University of Athens that was founded in 1906 at the Aretaieion Hospital of Athens [15]. K. Logothetopoulos (1878-1961) was professor and head of the department in the early 20th century [16]. He was the first to describe, in 1926, a method using a pelvic package to control pelvic hemorrhage in heavy gynecologic surgery [16]. He noted that “this method never failed” [16]. His method included a pack that was constructed by filling a bag with gauze tied rolls and with the tail of the gauze protruding from the neck of the pack [17]. This pack was introduced from the pelvis to the vagina via the abdominal route and the neck was grasped through the vagina and pulled down off the foot of the bed by using a heavy weight [17]. The concept of pelvic packing in gynecologic patients introduced by Logothetopoulos was later applied successfully in obstetrics, with uterine gauze packing and intrauterine balloons (e.g., Sengstaken-Blakemore balloon), which are used to control bleeding in cases of atonic uterus or low-lying placenta.

Conservative techniques of postpartum hemorrhage including the uterine gauze tamponade have been used in our hospital since the era of Logothetopoulos, however, the use of a novel intrauterine tamponade catheter, the Bakri catheter, in a massive hemorrhage is an alternative conservative agent.

We present the case of a pregnant woman with severe postpartum hemorrhage who was successfully conservatively managed with the use of the Bakri catheter.

Case Report
A 40-year-old woman (para 2, gravida 2) presented at 24 weeks and four days of gestation with vaginal bleeding. An ultrasound (US) scan showed that the placenta was low. Urine and vaginal fluid cultures were sterile. The bleeding stopped and she was discharged. The patient was readmitted at 28 weeks and one day of gestation with chorioamnionitis (temperature up to 39°C and abdominal pain). The abdominal US scan showed hydramnios and a low lying placenta. The white blood count was increased from 12.7 x 10³/mm³ to 24.5 x 10³/mm³ within 24 hours. Blood cultures, urine culture and vaginal swabs were taken and they were negative. The patient was started on antibiotics. After balancing the pros and cons and as there was no improvement in her condition and per vaginam
bleeding was noted, she underwent a cesarean section with a low segment uterine incision. A male newborn was delivered weighing 1,250 g with an Apgar score of 7 at the 5th min.

The placenta was anterior, and covering the cervical os. There was difficulty in separating and delivering the placenta. Subsequently, increased bleeding was noticed from the placental site. Due to the difficulty in placental separation a diagnosis of placenta increta was made. Thus, manual dissection of the placenta was tried and the placenta came out in pieces, as no cleavage plane was found. Ergometrine and oxytocin intravenously were already used to control hemorrhage. As the bleeding continued, hemostatic sutures were placed at the bleeding placental bed.

As the above was not effective, a Bakri tamponade balloon was tried. The total blood loss prior to application was estimated to be 1800 ml and the patient showed signs and symptoms of hypovolemia (tachycardia and oliguria). The patient was resuscitated with fluids and transfused with four units of blood. The balloon was introduced from the uterus to the cervix, via the abdominal route. The shaft of the balloon was pulled through the vaginal canal until the deflated balloon base reached the internal cervical os. The uterus was closed in two layers and the balloon was filled with 400 ml sterile saline through the stopcock. We used 400 ml of sterile normal saline to inflate the balloon as the inflation volume should not exceed 500 ml. The above volume was chosen on the basis of the size of the uterus (28 weeks) prior and after the removal of the placenta. The oxytocin and ergometrine infusion continued and misoprostol was given. The choice to increase the balloon size if the bleeding continued was available. Postoperatively the patient was transferred to the intensive care unit for one day and placed on antibiotics. The balloon stayed in place for 20 hours and the hemorrhage was controlled after the first few hours of placement. It should be mentioned that the balloon was deflated but it remained in the uterus for 30 min in order to have the option to reinsufflate in case of hemorrhage. A Foley catheter was introduced from the uterus to the cervix, but it remained in the uterus for 30 min in order to have the option to reinsufflate in case of hemorrhage. A Foley catheter remained in the bladder for the above period (Figure 1).

Postoperatively the patient was afebrile. Concerning the drainage of the Bakri balloon, 200 ml of blood was collected in the first 12 hours into the bag while in the next ten hours 50 ml was collected. No cramping and/or vaginal bleeding were mentioned. Blood pressure, pulse and urine output were within normal levels. The catheter was slowly deflated prior to gentle removal. The patient was released from the ward without any other problems. The baby was transferred to the neonatal intensive care unit.

Histopathological analysis revealed placenta accreta measuring 15 x 10 x 2 cm with chorioamnionitis, angiitis and local infarcts.

It is worth noting that the woman developed postnatal depression on the 15th postpartum day, but she recovered within two weeks.

Discussion

The diagnosis of placenta increta by US scan is difficult, although magnetic resonance imaging can be a useful modality in such pathology. Usually, the diagnosis is made during the third stage of labor as in our case. Nevertheless placenta increta may be part of the spectrum of placenta previa. Placenta previa increases the relative risk for endometritis or sepsis 5.5 times [18].

The Bakri catheter (Cook Medical, IN, USA) is made of 100% silicone and has a ductile shape in a way to match the inside of the uterine cavity [19]. Its length is 58 cm and the tested capacity of the balloon reaches the 800 ml. Bakri et al. were the first to publish the idea of an intrauterine balloon catheter in the management of postpartum hemorrhage in 1992 [19]. The Bakri balloon works in a similar way to the Sengstaken-Blakemore catheter which is used for esophageal varicocele tamponade [20].

Transabdominal placement of a Bakri catheter has been proposed in cases of hemorrhage during cesarean section [8]. The uterus should be clear of any retained placental fragments, arterial bleeding or lacerations. It should be mentioned that although there was a history of chorioamnionitis in our case, no purulence was detected during the operation, as such a finding would prevent the team from placing the catheter [21, 22]. Other contraindications include cervical bleeding due to trauma or cancer, untreated uterine anomaly, and coagulopathies [21, 22].

In a case series [23], 15 patients who underwent a Bakri catheter tamponade were retrospectively reviewed. The method was effective in all the cases of vaginal delivery and 80% of cesarean sections. It is proposed that special attention should be taken to avoid balloon rupture secondary to damage by the suturing needle. In another review, it was shown that 97/106 cases of postpartum hemorrhage were successfully managed by intrauterine tamponade balloons [22]. Combination of the Bakri catheter with a B-Lynch suture in controlling postpartum hemorrhage due to uterine atony has been described with excellent results (bleeding controlled in 5 out of 5 patients) [24]. This is the first time that a Bakri catheter was used by our team for placenta previa/accreta. It is a promising tool that can be used for conservative treatment of postpartum hemorrhage as a means to preserve
female fertility. Teaching the effective application of Bakri in emergency obstetric practical courses is important, and in Greece the Advanced Life Support in Obstetrics program has run a relevant training station on balloon tamponade from 2009 onwards.

Conclusion

The Bakri tamponade catheter effectively achieved hemostasis in this case of severe postpartum bleeding during cesarean section that was caused by placenta previa/increta.

References


Address reprint requests to: Nea Ionia, 41231 Athens (Greece)
e-mail: christosiavazzo@hotmail.com
Giant omphalocele - prenatal diagnostics, pregnancy evaluation and postnatal treatment

M. Zamurovic¹, A. Jurisic¹, S. Brankovic¹
¹University Clinic for Obstetrics and Gynecology “Narodni front”, Medical Faculty University of Belgrade, Belgrade, Serbia

Introduction

Omphalocele is a defect in the umbilical ring from which a sac protrudes covered with amnion and peritoneum and into which abdominal contents have typically herniated. The incidence of omphalocele is approximately one in every 3,000-4,000 births [1].

The exact etiology of omphalocele is still not well known [2]. The most interesting particularity about this malformation is that it is associated with other structural anomalies in a high percentage (ranging from 25-70%). Congenital heart defects, urogenital tract anomalies and central nervous system anomalies are among the most frequent. References even cite a case of omphalocele associated with dextrocardia, a very rare anomaly [3]. A case of omphalocele associated with alveolar capillary dysplasia was also described [4]. Genetic anomalies, i.e., chromosome aberrations, are not rare either, trisomy 13 and 18 being the most frequent. As early as 1987, Gilbert and Nicolaides performed karyotyping in 35 fetuses with omphalocele at 16 to 36 weeks. They reported that 54% had chromosomal abnormalities [5]. Getachew and associates reported in 1991 that 87% of fetuses with an omphalocele containing only intestine also had abnormal karyotypes, compared to only 95% of cases where the sac contained liver as well [6].

Ultrasonographic diagnosis is made in the early part of the second trimester by ultrasound (US) visualization of an ovoid mass beginning at the front abdominal wall and representing visceral hernia. The size of this centrally located defect ranges from 2 to 12 cm.

Omphalocele prognosis depends on anomalies associated with it. Considering that associated abnormalities are present in a high percentage, additional examinations (invasive diagnostics, most frequently amniocentesis with karyotype determination, fetal echocardiography, 3D/4D color Doppler, etc.) are necessary. Only after all examinations and detection of eventual presence or absence of other associated anomalies are performed can an opinion be given to parents.

Preterm labor and delivery complicates over half of all pregnancies associated with a fetal abdominal-wall defect. Corresponding mortality rate is 60% for omphalocele. The prognosis is good for the fetus weighing more than 1,500 g and has no associated anomalies, provided surgical correction is achieved rapidly. There is no evidence that cesarean delivery improves survival. Fitzsimmons and colleagues emphasized that elective timing of delivery optimizes neonatal surgical care [7].

Even though postnatal corrective surgery is not complicated and most frequently has a positive outcome, it poses a challenge for every surgeon not only when it comes to selecting the right surgical treatment but especially in selecting the optimal moment for surgical treatment and postoperative care of the newborn.

Case Report

A case of pregnancy in which prenatal US examination at 18 weeks gestation detected an omphalocele is described.
The patient, age 38, achieved pregnancy through a spontaneous cycle after a five-year long period of infertility examinations and treatment. She previously had had one miscarriage at eight weeks of gestation.

Monitoring of fetal development and pregnancy control according to the standard protocol was continued after US diagnostics of pregnancy. At 16 weeks of gestation invasive diagnostics was performed by amniocentesis, because of the pregnant woman’s age. Fetal karyotype 46XY confirmed a genetically healthy male fetus. At 18 weeks of gestation an omphalocele, paraumbilical defect through which intestinal convolutes protruded into the umbilical sac, was visualized by US. It contained convolutions of small intestine, colon and mesenterium.

The parents were informed that, at that time, pregnancy became high-risk and they were given necessary explanations about childbirth, early neonatal outcome as well as the necessity of subjecting the infant to surgery in the early neonatal period. Regardless of unpredictable development and growth of the omphalocele during the course of pregnancy, i.e., growth of the fetus, considering the possibility of surgical correction in the early postnatal period, it was decided not to terminate the pregnancy but instead to continue monitoring until term, and to deliver by elective cesarean section, while at the same time planning the surgical treatment of the infant after birth.

US examinations that followed indicated normal development of the fetus. Fetal echocardiography verified regular development of all cardiac chambers and normal position of the large vessels. Development of fetal lungs was regular. Morphology of abdominal organs was also regular, with normoposition of the liver, spleen, and stomach. Fetal kidneys were without hydronephrosis, i.e., normal in structure and normal renal vascularization. Visualization of the fetal bladder showed it filled and discharged normally. US visualization showed the spinal column to be normal in structure. 3D morphological analysis of the fetal head and brain was regular, with a face of normal morphology. Considering the length of gestation, fetal extremities were also normal in morphology and biometry. Fetal biometry and the biophysical profile showed that development of the fetus was proceeding regularly. Hemodynamic parameters in both uteroplacental and fetal circulation were normal. Three blood vessels with normal flow velocity and vascular indices were visualized in the umbilical cord.

Dimensions of the omphalocele at 28 weeks of gestation were 60 x 50 x 60 mm. The paraumbilical defect on the front abdominal wall was 25 mm in size. At 38 weeks, omphalocele dimensions were 66 x 55 x 60 mm while the paraumbilical defect was 29 mm in size.

Cesarean section was performed at 40 weeks of gestation. A live male infant was born weighing 3,550 g and 52 cm in length. The paraumbilical defect in the abdominal wall at birth was approximately 5 cm in diameter.

The infant was subjected to surgery on the first day of life. Initial treatment included gentle compression of the hernial sac and slow repositioning of hernial contents back into the infant’s abdomen. The paraumbilical defect of the front abdominal wall was surgically closed 12 hours after delivery. After surgery, the newborn was monitored in the intensive care unit; he spent seven days under assisted mechanical ventilation in order to prevent variations, i.e., increase in intraabdominal pressure when crying, breast-feeding, etc. The postoperative period went by without complications. The infant was released from hospital 14 days after surgery completely adapted and ready for breastfeeding. The rest of the neonatal period was without complications.
days in an Intensive Care Unit (11-85 days). However, more than half of the babies had postoperative courses complicated by development of sepsis (9 out of 14 children).

Even though the infant described in this study had a giant omphalocele with hernial opening of approximately 5 cm, it was subjected to surgery on the first day of life. Compression of hernial contents and the gradual repositioning into the abdominal cavity was started immediately after birth. The child was operated on under general anesthesia after 12 hours. The paraumbilical defect was sutured in layers – fascia and skin. Problems in initial repositioning can be related to the size of paraumbilical defect; it is easier to perform when paraumbilical defects are larger while it is more difficult and more complicated when defects are narrow. Closing of the abdominal wall defect, however, is easier if the opening is smaller and more complicated if the opening is large. Large paraumbilical hernial openings sometimes require closing by skin grafts [11]. Temporary increase of intraabdominal pressure due to crying, feeding, etc., represents a particular problem related to repositioning of hernial contents into the abdomen and closing of the abdominal defect. That is why the infant described in this study had to be under assisted mechanical ventilation accompanied by adequate sedation by medication. Antibiotic prophylaxis for infection had to be administered during the postoperative period. During this 7-day period, the infant was on parenteral nutrition. After seven days, when it was assessed that the front abdominal wall defect was adequately treated and that the skin wound was healing per primam, the child was extubated and adaptation to enteral feeding started. Full enteral feeding by mother’s milk was possible starting from the 14th day after birth.

Discussions are often found in references about the type of delivery, i.e., is it indicated to do elective cesarean section in cases of a fetus with an omphalocele without other visible morphological and genetic malformations? Although opinions are divided, there is a real danger of hernial sac rupture in spontaneous births regardless of the mode of delivery [11]. References also cite discussions about the use of povidone-iodine, i.e., iodine solution, for conservative treatment of omphalceles, for epithelialization of an omphalocele sac as well as even the use of povidone iodine for disinfection of a surgical lesion on the front abdominal wall. This is due to resorption of iodine through the skin and mucous membranes and effects this might have on thyroid gland function. Whitehouse et al. believe that topical povidone-iodine promotes epithelialization of the omphalocele sac; systemic effects of iodine are minimal and thyroid supplementation is not necessary. Topical povidone-iodine is an effective initial strategy for giant omphalceles and does not produce clinically significant hypothyroidism [12].

Advances in US diagnostics of fetal anomalies and their early detection have made it possible to monitor pregnancies with diagnosed anomalies, to start fetal therapy as early as inside the uterus, and to perform birth planning...
as well as planning of surgical correction of anomalies after birth. Thus the number of terminations of pregnancy due to anomalies that can be surgically corrected, either prenatally or postnatally, is diminished even though, until recently, such anomalies represented an indication for pregnancy termination.

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Address reprint requests to:
M. ZAMUROVIC, M.D., Ph.D.
University Clinic for Obstetrics and Gynecology “Narodni front”
62, Kraljice Natalije St.
11000 Belgrade (Serbia)
e-mail: mzamurovic@gmail.com
Complete hydatidiform mole coexisting with a live fetus

M.A. Unsal, S. Guven

Department of Obstetrics and Gynecology, Faculty of Medicine, Karadeniz Technical University, Trabzon (Turkey)

Summary

The co-existence of a hydatidiform mole with a living fetus is a rare phenomenon. The condition is a dilemma with respect to the diagnosis and management of associated maternal (a risk of maternal complications, such as preeclampsia, hyperthyroidism, and a risk of malignancy) and fetal (elevated risk of spontaneous abortion, neonatal thyrotoxicosis) complications. A 27-year-old woman was referred to our hospital with a diagnosis of hydatidiform mole and live fetus. The pregnancy was unremarkable except for the complaints of excessive nausea and vomiting. Successive ultrasound examinations demonstrated a normally growing live fetus (14 weeks) alongside a normal placenta and an additional intrauterine echogenic mass with features of hydatidiform mole. Genetic amniocentesis at 18 weeks of gestation showed normal diploid fetal karyotype. At 20 weeks of pregnancy, a control prenatal visit revealed intrauterine fetal death. The follow-up period for two years was unremarkable. In the case of a normal fetal karyotype and the absence of serious signs of maternal pathology, waiting until fetal viability is achieved can justifiably be proposed, however there is still a risk of prenatal complications such as intrauterine death.

Key words: Mole hydatidiform; Complications; Intrauterine exitus; Twin pregnancy.

Introduction

Gestational trophoblastic disease is a spectrum of cellular proliferations arising from the placental villous trophoblast. Hydatidiform mole (HM) is the most common form of gestational trophoblastic disease. HM refers to an abnormal pregnancy characterized by varying degrees of trophoblastic proliferation and vesicular swelling of placental villi associated with an absent or an abnormal fetus/embryo [1]. Two types are described: partial hydatidiform mole (in which there is a triploid karyotype, and complete hydatidiform mole (CHM), in which the karyotype is diploid. CHMs undergo early and uniform hydatid enlargement of villi in the absence of an ascertained fetus or embryo [1].

The co-existence of hydatidiform mole with a living fetus is a rare phenomenon, with an incidence of one in 22,000 to one in 100,000 [2]. The condition is a dilemma with respect to the diagnosis and management of associated maternal (a risk of maternal complications, such as preeclampsia, hyperthyroidism, and a risk of malignancy) and fetal (elevated risk of spontaneous abortion, neonatal thyrotoxicosis) complications [3]. The aim of this case report was to describe a case of complete hydatidiform mole with a live fetus and uneventful pregnancy course.

Case Report

A 27-year-old woman with gravida 1, para 0, was referred to our hospital with a diagnosis of hydatidiform mole and live fetus. The pregnancy was unremarkable except for the complaints of excessive nausea and vomiting. She had a history of spontaneous conception.

Successive ultrasound (US) examinations demonstrated a normally growing live fetus (14 weeks according to CRL measurement) alongside a normal placenta and an additional intrauterine echogenic mass with features of hydatidiform mole. US evaluation of this mass revealed a central heterogeneous mass with numerous discrete anechoic spaces, which corresponded to diffuse hydatidiform swelling of the hydropic chorionic villi “snowstorm pattern” (Figure 1A-B). Based on these findings, a preliminary diagnosis of hydatidiform mole coexisting with a live fetus was suggested.

Chest X-ray, liver function tests, and 24-hour urine assessment did not suggest any abnormality. Laboratory evaluation revealed high HCG (> 200,000 mIU/ml), and low TSH (0.01 mIU/ml, range 0.27-4.2), elevated levels of free T3 (12.46 mIU/ml, range 2.5-5.8 mIU/ml) and free T4 (38.40 mIU/ml, range 11.5-23.0 mIU/ml). Propylthiouracil (Propicil manufactured by Dr. F. Frik 50 mg, 150 mg/day) treatment was instituted to relieve hyperthyroidism symptoms. After three weeks of therapy the thyroid function tests were near normal levels.

Genetic amniocentesis at 18 weeks of gestation showed normal diploid fetal karyotype. Even though the risk of subsequent fetal complications and the potential of malignant transformation were discussed with the patient and her family, she was insistent on continuing the pregnancy.

Two weeks later (at 20 weeks of pregnancy) the control prenatal visit revealed intrauterine fetal death. Induction of abortion was performed with the delivery of a stillborn normal infant (450 g, grossly normal female fetus via normal vaginal route) and two adjoining placentas. The two parts were clearly distinguishable. The normal looking placenta weighed 160 g, while the other placenta which had a multicyctic appearance weighed 332 g. On microscopic examination, the appearance of the multicystic placenta was consistent with a complete hydatidiform mole with mild trophoblastic hyperplasia and atypias. Unfortunately, attempted culture of molar tissue was unsuccessful. The follow-up period of two years was unremarkable.
Complete hydatidiform mole coexisting with a live fetus

Discussion

Hydatidiform mole with a live co-twin fetus is a rare obstetric occurrence. When clinicians suspect a hydatidiform mole and a live co-twin fetus, they should keep in mind the following four possible associations: (a) dizygotic twin pregnancy having a normal fetus and hydatidiform mole; (b) dizygotic twin pregnancy having a normal fetus and a blighted ovum with normal constitutional karyotype and microscopic findings compatible with hydatidiform mole; (c) partial hydatidiform mole; and (d) mesenchymal dysplasia of the placenta [4]. Herein, we have reported a case of complete hydatidiform mole with an initially live fetus which was highly correlated with category a above.

A hydatidiform mole coexisting with a twin live fetus is a different entity from a partial mole in that there is independent fertilization of two eggs as the origin of mole and normal fetuses. This rare condition also named as twin gestation syndrome (discordant diandric diploidy) in which it is theorized that one gestation develops normally while the other undergoes molar degeneration [5]. There are two types of spontaneous evolution during the second trimester of pregnancy: either the molar part becomes quiescent, allowing the pregnancy to continue, or it goes on growing extensively, leading to fetal death and maternal complications [3].

Based on recent guidelines, women with a complete hydatidiform mole and live fetus could be followed if certain conditions are made. When the fetus is sonographically normal, an amniocentesis or a fetal blood sampling should be carried out after 15 weeks. If the fetus is euploid and the mother is clinically well, she should be followed fortnightly with US assessment of fetal anatomy and growth, cervical length to assess risk of preterm labor. Women should be counseled about the risk of both maternal complications such as pre-eclampsia, hyperthyroidism and theca lutein cysts and fetal ones such as spontaneous miscarriage, intrauterine death and preterm labor [6]. Malignant trophoblastic disease may also possible in 55% of complete hydatidiform mole and fetus cases [7].

According to Sebire et al. data, viable fetuses can be expected in 40% of hydatidiform mole pregnancies that continue beyond 14 weeks of gestational age, while 60% of complete hydatidiform mole and healthy co-twin pregnancies, which are not electively terminated, will result in either intrauterine death of the co-twin or spontaneous pregnancy loss. Most of these events happen in the second trimester, before fetal viability (mostly before 24 weeks of gestational age) [8]. About 50 cases of viable fetuses have been reported up to now, although an increased risk of preterm delivery and of cesarean section is associated with molar complications [3, 8]. Complete hydatidiform mole and healthy co-twin pregnancies can also be associated with potentially life threatening maternal complications such as thromboembolic disease and severe pre-eclampsia. The risk of persistent gestational trophoblastic disease is similar to that after a singleton complete hydatidiform mole (16%) [8].

In conclusion the management of each such pregnancy must be discussed individually with the mother, who must be made aware of the risks of medical complications such as severe vaginal bleeding, preeclampsia and hyperthyroidism. In the case of a normal fetal karyotype and the absence of serious signs of maternal pathology, waiting until fetal viability is achieved can justifiably be proposed, however there is still a risk of prenatal complications such as intrauterine death.

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Address reprint requests to:
M.A. UNSAL, M.D.
Karadeniz Teknik Üniversitesi Tıp Fakültesi
Kadın Hastalıkları ve Doğum Anabilim Dalı
61080, Trabzon (Turkey)
e-mail: mesutunsal@gmail.com
A rare case of intrinsic ureteral endometriosis causing hydronephrosis in a 40-year-old woman.

A case report and literature review

E. Papakonstantinou1, F. Orfanos1, Th. Mariolis-Sapsakos1, D. Vlahodimitropoulos2, A. Kondi-Pafiti3

1Department of Surgery, Evgenideion Hospital University of Athens; 2Pathology, Evgenideion Hospital University of Athens and 3Department of Pathology, Areteion Hospital, Athens (Greece)

Summary

Endometriosis is a multifactorial disease with unclear pathogenesis. Urinary tract endometriosis occurs in about 1% of all endometriotic lesions while isolated ureteral endometriosis is extremely rare. We present a case of intrinsic ureteral endometriosis causing ureteral stenosis in a 40-year’s old woman, in combination with intestinal, extensive peritoneal and ovarian endometriosis. The clinicopathological features and investigation methods used, as well as the treatment approach are discussed. An individual therapy plan depending mainly on the patient’s age, desire for children and the extent of the endometriotic foci should always be attempted. Collaboration between gynecologists and urologists was essential in our cases.

Key words: Ureter; Endometriosis; Ureteral endometriosis; Intestinal endometriosis; Ovaries.

Introduction

Endometriosis is a multifactorial disease with unclear pathogenesis affecting around 5-15% of women of reproductive age. It is characterized by the presence of uterine endometrial tissue outside its normal location, mainly in the ovaries, the pelvic peritoneum, and the recto-vaginal septum and rarely in the pericardium, pleura and even brain [1-3].

Although endometriosis was first described in 1960, it is a very controversial and enigmatic disease, and many theories regarding its pathogenesis have been advanced. Most studies of pelvic endometriosis support the implantation theory of Sampson, which proposes that during menstruation, endometrial tissue flows back via the Fallopian tubes into the abdominal cavity where it can implant [4, 5]. The theory of Mullerian metaplasia of coelomic epithelium was initially introduced by Mayer [6], which was extended later to the induction theory. The development of endometriosis to distant sites may be explained by dissemination through lymphatic and venous vessels and implantation [7-11].

Current investigation focuses on the factors favoring the growth and development of endometrial tissue in the peritoneal cavity in some women and its regression in others. Genetic predisposition, the presence of a familial tendency and the influence of environmental factors suggest a polygenic/multifactorial manner of development [9-12].

There are two histological types of the rare form of ureteral endometriosis: intrinsic and extrinsic. Intrinsic is defined when the lesions infiltrate the muscularis of the ureteral wall, while it is considered as extrinsic when the infiltrating lesions are responsible for significant ureteral obstruction but without involvement of the ureteral muscularis. Severe ureteral endometriosis causes significant obstruction to urinary flow with ureteral stenosis resulting in hydronephrosis [13-16].

In this case report, the clinicopathological findings of a rare case of intrinsic ureteral endometriosis in combination with extensive peritoneal, intestinal and ovarian endometriosis are presented, and the therapeutic approach is discussed.

Case Report

A 40-year-old woman visited the Gynecological Clinic of Evgenideion Hospital complaining of severe lower abdominal pain that usually presented during the third and fourth day of her menstrual cycle.

Clinical examination revealed mild abdominal tenderness, especially in the hypogastrium. No masses were palpated. Rectal examination showed a painful mass at the Douglas space. Menstrual irregularities were not referred. The patient had two children by cesarean section and the history of a surgical removal of a uterine mass that after histological examination was reported to be leiomyoma.

Urination was normal with greater frequency during menstrual periods. Constipation is also reported with occasional episodes of bloody and mucus diarrhea. Mild blood hypertension was noticed.

The patient reported that the symptoms appeared during the previous five months, thus she underwent CT scan and MRI of the lower abdomen which revealed: a cyst of the left ovary, 13 mm in diameter with hemorrhagic content; a similar cyst 18 mm in diameter on the left side of the Douglas space; a non-homogeneous intense nodule close to the left ovary and at the rectosigmoid junction; and a thickening of the left side wall of the uterus (consistent with the history of the removed leiomyoma).
A colonoscopy was performed and abnormal mucosa of the rectosigmoid colon with intense erythematous lesions was revealed. Biopsy specimens obtained from the lesion showed benign glandular formations inside the thickening wall of mucosa similar to endometriotic glands, suggesting endometriosis of the rectum (Figure 1). The rest of the colon was normal.

When the patient was admitted to our clinic, a transabdominal US was performed to obtain a more detailed description of the anatomical structure and it revealed a left unilateral stricture of the ureteral pelvic tract with secondary, severe dilatation of the upstream tract of the left ureter and hydrenephrosis of the left kidney, with no conclusive radiological evidence about the nature of the stenosis. After ureteral involvement was revealed, renal function was checked by kidney scintigraphy which revealed partial loss of left renal function.

During open laparotomy, extensive endometriosis of the peritoneum was observed, as well as cystic structures of the left adnexa, infiltration of the left adnexa and the left ureter from endometriotic lesions, as well as endometriotic infiltrates of the right side of the Douglas pouch, firmly attached to pelvic peritoneum and the rectal wall. A left salpingo-oophorectomy was performed along with excision of a segment of the left ureter en bloc with the periureteral mass that caused dilatation of the left ureter and insertion of a pigtail stent (7 fr). Finally, diathermocoagulation ablation was performed for all the endometriotic foci of the peritoneum. Histological examination revealed a tumor-like form of ureteral endometriosis engulfing the left ureter, while the left adnexa including the ovary contained abundant endometrial glands and supporting stroma (Figure 2).

Immediately, after the surgery, all the patient’s abdominal symptoms abated and hydronephrosis was alleviated. Follow-up investigation revealed complete recovery and no relapses of the ureteral disease were highlighted. Medical hormone suppression was used as adjuvant therapy to surgery and as a preventive therapy for relapses.

Discussion

Endometriosis can be classified according to the revised American Fertility Society classification (rAFS) into minimal, mild, moderate or severe endometriosis, based on a number of points given for the presence of ovarian or peritoneal endometriosis (subdivided into superficial or deep), the presence of adhesions and posterior cul de sac obliteration [2, 3].

The prevalence of extra pelvic endometriosis is unknown because of a lack of epidemiologically well designed studies. The variety of symptoms, signs and locations and the difficulty in establishing the diagnosis of the disease are the main difficulties in estimating the prevalence of the disease [1-3].

Endometriosis, which is biologically much similar to benign neoplasia, is a pathological entity marked by high local invasiveness and high recurrence. It mainly affects women of fertile age, and low or lack of parity, hormone therapies, previous gynecological surgery and cesarean section are regarded as risk factors [1-4].

Urinary tract endometriosis is a rare event, observed in about 1% of all endometriotic lesions, and about 30% of patients suffer from reduced kidney function at the time of diagnosis [13-16]. The most frequently affected side is the left one and, according to Vercellini et al. this fact may be ascribed to the sigma creating locally favorable conditions for cell seeding in a retrograde manner from the uterine cavity [14].

The response of the ectopic endometrial tissue to hormone stimulation results in cyclical bleeding of the lesion and its subsequent desquamation, necrosis and fibrosis, all contributing significantly to the development of ureteral stenosis. Severe ureteral endometriosis causes significant obstruction to urinary flow with ureteral stenosis resulting in hydrenephrosis during radiologic examination [15-18].

Isolated ureteral endometriosis is rare. It is usually considered as a multifocal pathology [16]. Patients usually present with associated histologically proven endometriotic lesions mainly in the intestine [19-23] and our case seems to confirm this finding.

It is essential to consider this tendency for multifocal development of endometriosis when considering the surgical modalities for these patients. An interdisciplinary approach is necessary to detect and excise all the endometriotic lesions in a single operation. The optimum...
A rare case of intrinsic ureteral endometriosis causing hydronephrosis in a 40-year-old woman. A case report and literature review

method of surgical management requires the diagnosis be made preoperatively and not fortuitously during surgery. The difficulty is that the diagnosis of ureteral endometriosis is challenging because the clinical presentation is nonspecific [17]. Indeed, clinical symptoms are often silent and abdominal pain is probably the cardinal symptom of endometriosis. Infertility is another commonly associated complaint. The diagnosis of endometriosis still presents several problems resulting from similarities in clinical symptoms to other benign or malignant diseases. Diagnosis is usually aided by the symptoms chronology linked to the menstrual cycle but, most of all by the patient’s positive medical history for endometriosis [18].

The gastrointestinal tract is the most common site of extra pelvic endometriosis, affecting 5-15% of women with pelvic endometriosis. Among women with intestinal endometriosis, the rectum and sigmoid colon are the most commonly involved areas (75-90%). Other parts of the bowel commonly affected are the distal ileum (2-16%) and appendix (3-18%). Superficial intestinal endometriosis is often characterized by visible serosal implants [19, 20]. It seems that intestinal endometriosis is more specific in the symptoms presented, including abdominal pain, abdominal mass, obstruction, rectal bleeding, infertility, diarrhea and increasing urinary frequency [21]. The classic triad of dysmenorrhea, dyspareunia and infertility, as a result of concomitant pelvic disease, may also exist [22, 23].

Various methods of investigation (intravenous pyelogram, retrograde pyelography, US, kidney scintigraphy) have been proposed in cases of clinical suspicion of ureteral endometriosis [24]. MRI techniques have been increasingly improved during the past ten years and numerous recent studies have underlined the advantages of MRI for the diagnosis of endometriotic lesions [25]. Transabdominal US of the pelvis is the initial method of choice to identify and characterize adnexal structures. Identification of specific locations of deep endometriosis is usually achieved by MRI, which remains the key examination for ureteral and bowel endometriosis [26].

Endometriosis is particularly difficult to treat and the treatment of choice is surgical, medical or a combination of both approaches [23, 27]. Factors to consider in management include the age and reproductive desires of the patients, stage of the disease and most importantly the symptoms. Oral contraceptives, androgenic agents, progestins and gonadotrophin releasing hormone analogs have all been used successfully. Ureteral obstruction develops slowly from periureteral fibrosis and often results in asymptomatic hydronephrosis, loss of renal function and hypertension. Conservative procedures such as ureteral stenting, associated with medical treatment usually lead to favorable outcomes. Surgical management depends on the clinical form of the ureteral endometriosis (minimal or severe). For patients presenting minimal ureteral endometriosis, conservative treatment with excision of endometriotic lesions associated with ureterolysis is an effective approach. However, not all cases of ureteral endometriosis can be treated with simple ureterolysis.

Ureterolysis with possible omentopexy should be considered in case of localized pelvic extrinsic endometriosis with limited ureteral involvement but this method is not adequate in cases of extensive involvement of the ureter or intrinsic endometriosis, which is hard to determine without histological examination. In segmentary ureterectomy the active disease, the diseased part of the ureter and all surrounding fibrosis are removed. Ureteral termino-terminal anastomosis may be performed in less severe cases when the distal ureteral tract shows no signs of endometriosis. However, in many cases the spread of disease in the pelvic area prevents the surgeon from using the distal pelvic ureter and therefore uretero-cystoneostomy is performed [18].

When the endometriosis penetrates through the entire depth of the organ wall, complete resection and re-anastomosis of the ureter or bowel can be safely performed, either laparoscopically or with open surgery [27]. Whereas the intrinsic form of ureteral endometriosis often needs aggressive surgery [28], the extrinsic form may be treated with conservative techniques.

Conclusions

Multifocal development of endometriosis with involvement of the ureter causing severe obstruction, the bowel, the ovaries and the peritoneum is an uncommon condition and the counseling physician’s awareness is important. A detailed study of the urinary tract based on US findings and urographic examinations in combination with MRI for the extent of endometriosis, is absolutely essential for all patients suffering from pelvic endometriosis.

An individual therapy plan depending on the patient’s age, desire for children and the extent of the endometriosis should always be attempted. Collaboration between gynecologists and urologists is essential for the correct therapeutic procedure of these uncommon cases.

References


Address reprint requests to:
A. KONDI-PAFITI, M.D.
Pathology Department
Areteion Hospital
76 Vas. Sofias Ave
Athens, 11528 (Greece)
e-mail: akondi@med.uoa.gr