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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.

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Physicians should be more open-minded about performing in vitro fertilization-embryo transfer in women with diminished oocyte reserve and consider the couple's wishes and desires

J.H. Check - Camden, NJ (USA)

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J.H. Check, D. Kramer, A. Bollendorf, C. Wilson - Camden, NJ (USA)

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J.H. Check, B. Katsoff, C. Wilson, J.K. Choe, D. Brasile - Camden, NJ (USA)

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Physicians should be more open-minded about performing in vitro fertilization-embryo transfer in women with diminished oocyte reserve and consider the couple’s wishes and desires

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Summary

Purpose: By presenting anecdotal cases of women who conceived with IVF-ET despite very poor odds, this editorial hopes to make some of the infertility specialists to be more optimistic about the prognosis for pregnancy in women with diminished oocyte reserve.

Methods: Description of case reports where despite poor odds the couples elected to still attempt IVF-ET which appeared to be needed for conception to be possible.

Results: Five cases are presented where the couple was willing to deplete their financial resources to achieve a pregnancy with IVF-ET using their own gametes and were eventually successful.

Conclusions: Physicians should restrain from being dogmatic and present all options to patients not merely the physicians’ preference. It is embarrassing for a physician to insist that successful pregnancy is impossible only for the patient to prove that physician wrong. After receiving proper data patients should be allowed greater input into their treatment decision.

Key words: Diminished oocyte reserve; Finances; Patients’ rights; In vitro fertilization.

Introduction

There have been several studies in the past suggesting that an increased day 3 serum follicle stimulating hormone (FSH) is associated with extremely poor pregnancy rates [1-5]. However, pregnancy rates have improved considerably in the field of in vitro fertilization (IVF) since these previous publications so the question is do these conclusions still apply in the modern era of IVF. Recently, however, data from one of the foremost IVF centers in the world concluded that if the day 3 serum FSH was > 15 mIU/ml there would be no pregnancies at any age despite the transfer of multiple normal embryos [6].

With all these negative studies concerning the ability to conceive if the serum FSH is elevated even in normal menstruating women, women needing IVF-ET (who are facing an immense expense) might be less willing to undergo the IVF procedure even if that were the only way to achieve a pregnancy.

One might think that if it is difficult to achieve a pregnancy in menstruating women with decreased oocyte reserve it would be a lot more difficult to attain a pregnancy with the egg reserve so low that the woman appears to be in overt menopause (amenorrhea, elevated serum FSH, estrogen deficiency and failure to respond to exogenous gonadotropins). Nevertheless, ovulation induction and pregnancies have been achieved without IVF-ET in women in apparent menopause especially in women aged < 39 [7-12]. However the estimated live delivery rate has been quoted as 8% in aggressively treated patients treated for a maximum of four cycles. Thus the success rate with IVF-ET would be expected to be extremely low. However some women have achieved success despite very low odds and despite lack of third party insurance coverage or being in good financial condition to bear the costs. Nevertheless they sacrificed their life savings in attempt to have a baby with their own genes. Some of these cases are described below.

Case 1

A 32-year-old woman with a tubal problem (bilateral fimbrial agglutination) related to a Chlamydia infection sought to have IVF-ET [13]. She came from Alabama to New Jersey because she was told she was in imminent ovarian failure due to a day 3 serum FSH of 44 mIU/ml and the failure to raise the serum estradiol (E2) level past 52 pg/ml despite several days of high dose exogenous gonadotropins.

Despite the very low odds that were quoted and her limited finances she wanted to try the method of lowering the elevated gonadotropins with ethinyl estradiol followed by low-dose gonadotropins. She had one mature oocyte in two consecutive cycles and she conceived and had a full-term live normal baby.

She tried again after the delivery but seemed to now be in overt ovarian failure and was unable to generate a dominant follicle.
Comment

This couple despite markedly limited finances and poor odds wanted the opportunity to try to achieve a pregnancy with their own gametes. Because of the lack of oocytes and therefore marked work reduction for the embryologist we were able to reduce the cost to one-third of the normal price. Because they had to work they drove 1000 miles back and forth during her stimulation phase.

For personal reasons they did not want to ever consider donor oocyte. They were willing to gamble on a fixed amount of money that they have saved to give them a chance to fulfill their dream.

Case 2

A 41-year-old woman was told that she was nearing ovarian failure. Her serum E2 was 119 pg/ml on day 3 and she was told by another IVF center that serum E2 levels > 50 pg/ml on day 3 prognosticate poor pregnancy rates [2].

She was advised that even though we have had pregnancies in women with elevated day 3 serum FSH and/or high serum E2 levels on day 3 [7-13], the prognosis for women age 40 or greater is markedly reduced [8, 14, 15].

Nevertheless she wanted to try to conceive with her own oocytes. The first cycle she was treated with ethinyl estradiol to lower the elevated serum FSH and to try to increase the length of the follicular phase [16, 17]. Clomiphene citrate was then given and the oocyte retrieved was deemed mature but it did not fertilize [17].

The next cycle the day 3 serum FSH was 17.4 mIU/ml and the serum E2 was 85.2 pg/ml. With ethinyl estradiol therapy the serum E2 dropped to 43.6 pg/ml but the FSH increased to 20.9 mIU/ml. Staying on the ethinyl estradiol the serum E2 was 250 pg/ml. She was given 10,000 units of human chorionic gonadotropin (hCG) and a mature egg was retrieved. A 4-cell embryo with 25% fragmentation was transferred three days later and resulted in a live normal baby [17].

Comment

Advanced reproductive age is much more of a negative factor for conception than the serum FSH. Based on our IVF studies a woman aged 40 has 70% as much of a chance of conception as women ≤ aged 39 and the levels drop to 40% at age 41-43 then plummet to 10% at 44 and are extremely rare at age ≥ 45 [15].

Nevertheless there have been pregnancies recorded without IVF-ET in women aged 45 and even 46 with elevated day 3 serum FSH [18, 19]. These two women still had fairly regular menses. However, there was even one woman aged 45 who was in overt menopause with estrogen deficiency, amenorrhea for six months, and a serum FSH of 43 mIU/ml who had ovulation restored by lowering the high serum FSH with ethinyl estradiol [20]. The interesting thing about this case was that the sperm count was so low (3.0x10^6/ml with 20% motility) that IVF with ICSI was suggested but she did not want to spend the money for such low odds. However, miraculously she successfully conceived after two cycles of intrauterine insemination and delivered a full-term perfectly normal child [20].

Case 3

A 43-year-old woman with primary infertility of five years duration had decreased ovarian egg reserve as manifested by a day 4 serum FSH of 9 mIU/ml and a serum E2 of 55 pg/ml with a total of four antral follicles seen.

She had a previous hysterosalpingogram that showed the right fallopian tube to be patent but the left tube was blocked and showed a hydrosalpinx. She was advised that a unilateral hydrosalpinx could impair fertility even with IVF-ET, and that salpingectomy significantly improves the chance of pregnancy [21-23]. However, she was on medical assistance and the insurance would not cover this procedure for infertility purposes. She was advised that because of the cost to perform the procedure and the risk of laparoscopy considering her bowel resection for colon cancer, that we would suggest doxycyline 200 mg/day from day one of the IVF cycle until embryo transfer.

She had ovulation restored by lowering the high serum FSH with ethinyl estradiol [20]. The interesting thing about this case was that the sperm count was so low (3.0x10^6/ml with 20% motility) that IVF with ICSI was suggested but she did not want to spend the money for such low odds. However, miraculously she successfully conceived after two cycles of intrauterine insemination and delivered a full-term perfectly normal child [20].

Comment

Our regular IVF program the pregnancy rates of 43-year-olds are similar to 42-year-olds with a big plummet at age 44. Our patient was reminded that she had just turned age 43.

Despite the seemingly poor odds not only related to advanced reproductive age but decreased egg reserve and the presence of a hydrosalpinx she decided to try IVF-ET with her own eggs. She of course was advised that even if she used donor eggs, the hydrosalpinx could impair success [22]. Financially, though, she had no choice (plus it was a lot less risky) to hope that a longer course of antibiotics would negate the adverse effect of the hydrosalpinx on embryo implantation.

We charge 50% less for mild stimulation IVF because of considerably less work for the embryologists [26]. In fact, she was stimulated with only five days of highly purified urinary FSH at 75 IU/day. Only one dominant follicle was developed and one metaphase II oocyte was retrieved. The peak serum E2 level was 251 pg/ml.

The egg fertilized and cleaved to an 8-cell embryo that had good symmetry and < 25% fragmentation. She conceived and delivered a healthy full term baby.
Comment

This woman with very meager financial means was willing to become even more impoverished to achieve her dream of a baby with her and her husband’s gametes. She was even willing to undergo even greater expense and risk having surgery for the hydrosalpinx despite the risk of bowel perforation.

Successful cases like this one re-emphasize the importance of allowing patients to make their own choices as long as they are provided the appropriate information to make that choice. The patient was even willing to undergo surgery but we convinced her not to take the risk; however we would have performed the salpingectomy if that was still her final decision. We advised her that she could have three IVF cycles for the price of the one operation and that it was not an absolute fact that the hydrosalpinges would impair embryo implantation especially with a more prolonged course of antibiotics.

Case 4a

A 33-year-old woman presented with a four and a half year history of infertility. She went to one infertility center who upon initial evaluation performed a clomiphene challenge test which she did not pass. On the basis of this test she was advised that she would require donor oocytes.

She went for a second opinion to another infertility center that also suggested the donor oocyte program but was willing to try IVF-ET with her own eggs if she wished. However, despite normal fallopian tubes and normal semen analysis in her husband this center suggested she lose no time and go directly to IVF-ET.

She was placed on a traditional ovarian hyperstimulation protocol and had three failed attempts. She only had three, four and four oocytes retrieved resulting in transfers of two embryos, the first and second time and none on the third. After the third cycle the IVF center said they would do no more IVF with her own oocytes and that she must proceed to donor oocytes.

Since the couple did not want donor oocytes they came to our IVF center knowing that we have no problem in trying IVF-ET with women with diminished egg reserve, especially if they are young. However, we believe it is because of using minimal gonadotropin stimulation [26].

In the first IVF cycle at our facility her peak E2 reached 307 pg/ml and she had one metaphase II oocyte retrieved. She had a transfer on day 3 of just a 4-cell embryo without fragmentation and did not conceive.

She was advised that 4-cell embryos only have a 3.8% chance of implantation but that did not mean that she would not make embryos in subsequent cycles with more blastomeres [24].

In cycle 2 at our center (but number 5 for her) she attained a serum E2 of 443 pg/ml on the day of hCG injection. She had three metaphase II eggs retrieved; three fertilized but only one cleaved to day 3 when an 8-cell embryo with < 25% fragmentation was transferred. She did not conceive.

Encouraged by the improvement in embryo quality she tried again and attained a peak serum E2 of 776 pg/ml. Though two eggs were retrieved only one was mature. One fertilized the first day, the second fertilized on the second day and two embryos with six and five blastomeres with < 25% fragmentation were transferred but she did not conceive.

She still wanted to try again. In cycle 4 with our center (but 7 IVF cycles altogether) her peak serum E2 was only 244 pg/ml and only one metaphase II egg was retrieved. She transferred one 8-cell embryo with ≤ 25% fragmentation. She conceived in this cycle and delivered a full term healthy baby. She was 34.5 years old when she conceived. All four cycles used intracytoplasmic sperm injection in view of failed fertilization in cycle 3 at the previous center.

Comment

The couple had only a moderate income – she was a probation officer and her husband a teacher. Nevertheless, they were willing to sacrifice their money to have their dream of a child with the wife’s and the husband’s genes.

After delivery her menses resumed three months later and they tried on their own for four months without success. They have returned to our IVF center again to try once more a minimal stimulation protocol and IVF with intracytoplasmic sperm injection.

Case 4b

Similar to case 4a there was a 37-year-old woman with secondary infertility who failed to conceive despite ten cycles of follicle maturing drugs and intrauterine insemination. She was advised to do IVF-ET and had a traditional COH protocol. However, only one egg was retrieved and the IVF center told her they would not do another cycle with her own oocytes but would gladly use donor oocytes in her body.

She wanted her own genes so she came to our IVF center. We performed natural to minimal COH and in cycles 2-7 she failed to fertilize any oocytes in three of the six cycles and only transferred four embryos total in the other three cycles.

She still wanted to keep trying with her own oocytes. In her eighth IVF cycle (7 with our center) she transferred one 7-cell embryo without fragmentation. She conceived monochorionic diamniotic twins [27]. She successfully delivered full term twin girls. Though her day 3 serum FSH had been as high as 17 mIU/ml she had a level of 10 mIU/ml on the cycle of conception.
Comment

One does not always have to expect that it will take so many cycles to achieve a successful pregnancy as in cases 4a and 4b. One woman with diminished oocyte reserve achieved three live deliveries (singleton each time) in four IVF attempts over an 8-year time period [28]. However, it would not be fair to merely present to the couple the example of this very fortunate couple without mentioning that some women had to undergo many IVF cycles before achieving a success. Couples must also be advised that they could go through many IVF cycles and still not be successful.

Case 5

A 37-year-old woman with primary infertility traveled 3000 miles from California to New Jersey to see if it was possible to make her ovulate despite the diagnosis of premature menopause [29]. She had not had a menstrual period for one year. After 50 days of amenorrhea her physician diagnosed premature menopause on the basis of a serum E2 of 20 pg/ml, a serum FSH of 120 mIU/ml and failure to have menses following progesterone withdrawal. Furthermore, her failure to stimulate a rise in serum E2 despite gonadotropin therapy convinced the consulting physician that there was complete absence of eggs rather than the production of defective FSH by a possible FSH secreting pituitary tumor.

The woman’s husband, a physician, ran a computer search and found that ovulation induction and pregnancies have been recorded in women in apparent menopause by restoring down-regulated FSH receptors in the few remaining follicles by lowering the chronically elevated serum FSH through the use of ethinyl estradiol [7-12]. They came from California to New Jersey for a consult and she was treated with ethinyl estradiol. Her monitoring was performed in California and we directed her case by telephone. She attained a dominant follicle of 17 mm with a serum E2 of 314 pg/ml (ethinyl estradiol does not cause any increase in serum E2), released the egg and was treated with vaginal progesterone suppositories in the luteal phase at 200 mg twice daily. She did not conceive that cycle. She ovulated again with the same technique but did not conceive in cycle 2.

They requested if she formed a mature follicle on cycle 3 to fly to our center to do single egg retrieval and in vitro fertilization. However, her serum E2 only rose to 32 pg/ml.

The infertility specialists in California who were monitoring her advised them that although they were impressed that she was able to ovulate two times despite apparent menopause, that even if she did ovulate she would not conceive because her oocytes were of poor quality based on previous studies [1-6]. She called and stated that although she believed our data that a pregnancy is possible with even her oocytes she wanted to expedite the process and that she is not uncomfortable with the donor oocyte process. So that was her next step. For convenience she would have the transfer of embryos derived from donor oocytes in California.

Unfortunately she failed to conceive despite the transfer of 12 high quality embryos over four donor egg cycles. She reconsulted us at age 40 stating that she would like to try donor oocyte at our IVF center. She was hoping that another IVF center might identify some correctable factor that would enable her to attain her goal of having a baby with her husband’s sperm. In her case the desire to carry and delivery a baby was more important than her own genes. Surprisingly in the end she was rewarded with both.

Comment

This couple had a better financial situation than the other couples illustrated in this editorial. However, they were feeling the “sting” after spending $120,000 for four failed donor oocyte cycles. Yet they were willing to pursue more financial depletion to attain her goal of having a baby with her husband’s sperm. In her case the desire to carry and delivery a baby was more important than her own genes. Surprisingly in the end she was rewarded with both.

Discussion

All of the reported women were willing to undergo the expense of IVF despite the fact that the number of embryos transferred would be quite low and with the knowledge that in the opinion of the majority of infertility specialists the odds of success were quite small. Some were advised that pregnancy with their own eggs was impossible. However, these anecdotal reports proved the naysayers wrong and strongly suggest that even when the oocyte reserve of a younger woman is comparable to the number of oocytes in women over the age of 45, the quality of the eggs are more analogous to their age peers. Thus these women should not be denied attempts at oocyte retrieval of their own oocytes as long as they have been properly advised of the far greater likelihood of success with donor oocytes.

Case 5 is really fascinating. Here was a woman who was willing to travel 3000 miles to do IVF if a mature follicle could be achieved but yet was able to be convinced to do donor oocytes because of the much greater likelihood of success. However, she found out that donor oocytes are not guaranteed to work either. When faced, though, with the suggestion of using a gestational carrier with transfer of embryos derived from donor oocytes and her husband’s sperm or adoption as her only two options, she chose instead to pursue more donor oocyte cycles despite the immense expense already incurred with her four previous failed donor oocyte cycles.

She was hoping that another IVF center might identify some correctable factor that would enable her to attain her dream of carrying and delivering a baby. Other than the estrogen treatment her only other treatment was progesterone supplementation which she continued through the first trimester.
Physicians should be more open-minded about performing in vitro fertilization-embryo transfer in women with diminished oocyte etc.

Case 5 exemplifies the fact that if a 40-year-old woman with extremely high serum FSH (actually her serum FSH was as high as 180 mIU/ml at some point during her attempts at donor oocyte) and estrogen deficiency can get pregnant with her own oocytes physicians should not advise younger patients with an even greater egg reserve that they should have IVF-ET performed with their own oocytes merely because one day 3 FSH was elevated at least one time.

Cases 4a, 4b, and 5 illustrate that despite repeated failures to conceive despite embryo transfer that successful pregnancy is certainly still possible without changing the gametes or uterus. As long as couples are advised that the repeated failures reduce the odds of success of the next one, and they are re-advised of other options that may be more effective, they should still be given the opportunity to try more attempts under the conditions that they prefer.

It is hard to believe with the plethora of studies dating back to 1984 describing techniques to increase the chance of ovulation even in women in apparent premature ovarian failure, and the demonstration of quite adequate pregnancy rates in women with diminished oocyte reserve, that many physicians only make the couple aware of the negative but not the positive studies. Some of these articles have been written in the same main infertility journals where the negative studies are being quoted [7, 8, 20, 24, 26].

Reproductive endocrinologists/infertility specialists should not be politicians in being able to lure patients into a therapy that better suits the treating physician and not the couple. These physicians should be aware that their reputation as an expert will be marred if a woman advised that only donor oocytes would be successful, goes to another infertility specialist that better suits her needs. These physicians should be aware that their reputation as an expert will be marred if a woman advised that only donor oocytes would be successful, goes to another infertility specialist and conceives with her own oocytes. Even if the woman is unsuccessful she may be very disappointed with the IVF center quoting her studies biased for negative results without presenting other studies with positive results. This is especially important if the positive studies present reasons why the negative studies failed – especially because of the use of a high FSH dosage-controlled ovarian hyperstimulation protocol [26].

References


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“Embryo glue” does not seem to improve chances of subsequent pregnancy in refractory in vitro fertilization cases


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Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology
Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Introduction

Implantation following in vitro fertilization-embryo transfer (IVF-ET) is a multistage process involving opposition and adhesions of the blastocysts to the uterine endometrium followed by invasion of the trophoblast. One of the reasons for implantation failure in what appears to be normal morphologic embryos could be chromosomal abnormalities. Another theoretical cause may be the failure to develop a sufficient “sticky” matrix for the embryos to attach to the endometrial wall.

Embryo glue (Vitrolife, Denver, CO), which is composed of various substances with the active ingredient hyaluronan, has been developed and there are claims that it can improve pregnancy rates (PRs) following embryo transfer (ET) [1-3]. Other studies have found no benefit to using Embryo glue as a transfer medium as evidenced by comparable pregnancy and implantation rates [4-6].

The objective of the present study was to determine if the use of Embryo glue improves implantation and PRs following ET in women who failed to conceive despite at least three previous ETs.

Materials and Methods

A matched controlled study was performed to evaluate the efficacy of Embryo glue in women undergoing IVF-ET. The study population included donor oocyte recipients and women using their own oocytes having fresh or frozen ETs. All women in the study had failed to conceive from at least three prior ETs and had a normal uterine cavity as determined by HSG or saline infusion sonography.

Results

A woman who had used Embryo glue was matched with the very next woman not using glue within six months of age and having the same number of previous failed ETs. Oocyte recipients were paired receiving eggs from the same donor.

Embryo transfer was performed using one of two media; Embryo glue or modified human tubal fluid (HTF) supplemented with 20% serum protein substitute (SPS) (both from Sage BioPharma, Pasadena CA). Both types of media were equilibrated overnight at 37°C, 5.5% CO2 in air. For Embryo glue ETs, embryos were placed in Embryo glue after assisted hatching was performed, between one to three hours prior to ET, and loaded directly into the catheter from the Embryo glue dish. For all other ETs, embryos were moved to the modified HTF solution immediately prior to loading in the catheter. Wallace catheters were used for all ETs.

Comparison of PRs between the two groups (glue or no glue) was made using chi-square analysis with p < 0.05 used to determine significance.

Conclusion

Embryo glue does not improve pregnancy outcome in women failing in previous IVF cycles.

Key words: Embryo glue; Refractory IVF cases.
The only significant difference seen was the delivered pregnancy rate in fresh ETs; significantly more women had a successful delivery when conventional ET media was used, 39.3% (11/28) vs 14.3% (4/28) for Embryo glue (p < .035).

Conclusions

These data suggest that Embryo glue does not improve implantation in women who have had at least three prior failed ETs. In fact, a trend for a better outcome was observed when Embryo glue was not used as the transfer media.

We purposely chose women who had failed to conceive despite three or more failed ETs since the possibility exists that a defect in adhesion molecules may be present in only a minority of cases and therefore would be more likely to be manifested in a group with several previous failures to establish a live pregnancy.

Table 1. — Comparison of outcome by type of ET (fresh or frozen or donor egg) and by use or non-use of Embryo glue.

<table>
<thead>
<tr>
<th></th>
<th>Fresh ETs</th>
<th>Recipient ETs</th>
<th>Frozen ETs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Glue</td>
<td>No Glue</td>
<td>No Glue</td>
</tr>
<tr>
<td># Transfers</td>
<td>28</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td># ET</td>
<td>2.3 ± .8</td>
<td>2.4 ± .8</td>
<td>2.8 ± .4</td>
</tr>
<tr>
<td>Clinical PR (%)</td>
<td>25%</td>
<td>39.3%</td>
<td>50%</td>
</tr>
<tr>
<td>Delivered PR (%)</td>
<td>(7/28)</td>
<td>(11/28)</td>
<td>(3/6)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>15.3%</td>
<td>23.5%</td>
<td>16.7%</td>
</tr>
</tbody>
</table>

References


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Predicting ovarian reserve and reproductive outcome using antimüllerian hormone (AMH) and antral follicle count (AFC) in patients with previous assisted reproduction technique (ART) failure

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Summary

Purpose of investigation: The main objective of our prospective, observational, analytical research work was to determine whether Anti-Müllerian hormone (AMH) and antral follicle count (AFC) could be effectively used as predictors of ovarian reserve and, possibly, of reproductive outcome with ART. Methods: We studied 143 IVF/ET cycles in patients with a previous history of ART failure, all of them supposed to be of poor prognosis, who had agreed to another ART attempt after knowing their AMH, AFC, and base hormone (FSH, LH, 17β-estradiol) levels. Results: AMH and AFC showed a positive correlation with the number of oocytes retrieved (p = 0.0016) and (p < 0.0001), respectively and with percentage of MII oocytes, (p = 0.00756) and (p < 0.001). The combined use of these markers showed an area under the curve of 82.2% for oocytes retrieved. Our results shows a very high cancelation (22% of started cycles) and very low pregnancy rates (6.7% and 9.8%) in low and normoresponders, respectively. Conclusions: AMH levels and AFC are reliable indicators of ovarian reserve. Patients with ovarian reserve levels that predict a very low probability of success should be informed that the poor prognosis associated with these values may not justify the expense of IVF/ET.

Key words: Anti-Müllerian hormone (AMH); Antral follicle count (AFC); 3D ultrasound; AVC; VOCAL; Inverse mode; Low responders.

Introduction

Advanced age is currently the main cause of female sterility in Spain. This is a troubling problem since Spain is the country with the lowest birthrate in the world [1]. We now face older patients desirous of procreation who have a low probability of success due to low ovarian reserve along with poor quality of remaining oocytes. Starting at 37.5 years of age, it is estimated that 70 to 80% of oocytes are bearers of chromosomal or genetic defects [2-4], since the best oocytes are recruited in the early reproductive years [5].

Menopause appears when there are about one thousand follicles left. However, it is estimated that the decline in fecundity precedes menopause by about 13.5 years [5]. Data from the onset of reproductive biology indicate that about 10% of the female population experience an accelerated reduction in the oocyte pool before age 32 [6]. By age 37 this accelerated reduction increases to 25%. The probability of spontaneous gestation after 40 years of age is less than 4% [6].

Ovarian reserve is more of a biological than a chronological function and because of this, the onset of accelerated decline can occur at an early age [6]. Numerous hormones (FSH, LH, estradiol, inhibin B) and dynamic tests (clomiphene challenge test, gonadotropin challenge test, GnRh agonist stimulation test, etc.), upon which there were high expectations that they would serve as markers of ovarian reserve, have turned out to be tests with little or no predictive value [7].

At present anti-Müllerian hormone (AMH) is considered to be an excellent marker of ovarian reserve, response to gonadotropin stimulation, in vitro fertilization (IVF) reproductive success, and even of approaching menopause [8-21]. Determinations of AMH levels along with antral follicle count (AFC) with last-generation ultrasound (US) modes seem to open new avenues to avoid, or at the very least to reduce, the number of patients with no possibility or very low probability of reproductive success who are subjected to artificial reproduction therapy (ART) [22].

The cost of the medications used in ART, the associated psychological discomfort, the risk of complications, and the avoidance of predictable failures justify the need to obtain prognostic information prior to the initiation of therapy [23]. At present, when safety and cost/benefit are very important considerations in ART, excessive and defi- cient responses to ovarian stimulation should be avoided.

Material and Method

The study consisted of 143 IVF/ICSI patients, all of whom had a history of ART failure (i.e., ovulation induction, IVF/ICSI), normal menses, both ovaries present, no previous history of major diseases, endocrinopathies, chemotherapy and/or radiotherapy; and whom in spite of past failures, and with knowledge about their AMH levels, agreed to undergo
another cycle of IVF/ICSI. Couples with severe masculine factor (< 5 million sperm/ml) were eliminated.

Once the stimulation cycle results were obtained, the following subgroup analyses were carried out, taking into account the number of oocytes recovered:

**Group 1. Cycles cancelled or ≤ 5 oocytes recovered**
- A) Cancelled cycles: n = 34 (23.7%)
- B) Low response not cancelled: n = 44 (30.7%)

**Group 2. Normal responders > 5 and < 15 oocytes recovered:**
- n = 65 (45.45%)

We determined basal hormonal levels (FSH, LH, AMH, and 17β-E2), on day 3 of the previous cycle (enzyme-linked immunosorbent assay (ELISA) - Human Gesellschaft für Biochemica und Diagnostica MbH, Wiesbaden, Germany) with an analytical sensitivity of 0.4 μIU/ml for FSH, 0.5 μIU/ml for LH, 3 pg/ml for 17β-E2. For AMH we used the AMH/MIS immunosassay (Laboratory Instrument & Beckman-Coulter, Vienna, Austria). Estimated analytic sensitivity (ELISA) of 0.1 ng/ml (0.7 pmol/l).

AFC was carried out with transvaginal 2D/3D US (Voluson E8, GE, equipped with a RAB 4-8D transvaginal probe) at the onset of the stimulation cycle [24].

Ovarian induction was carried out using 225-300 IU of rFSH (Puregon, MSD, Madrid, Spain) during six days, and continued depending on follicle size and 17β-E2 levels. On day 5 (or follicles of 15 mm), a GnRH antagonist 0.25 mg (Orgalutran, MSD Madrid, Spain) was given daily until the administration of hCG (Ovitrelle, Serono, Madrid).

The following data were taken into account.
- Age
- History of ART
- Total oocytes recovered, percentage of mature oocytes obtained and fertilized
- Total number of GI, GII, and other quality embryos obtained. Number and quality of embryos transferred. Number of gestations achieved and clinical evolution (number of abortions, number of ectopic pregnancies, and number of gestations in evolution).
- Seventy-eight (67.5%) were low responders, and the remaining 65 (32.5%) were normal responders. All data were included in a Filemaker program.

**US modes used for AFC**

AFC was carried out using 2D and 3D vaginal US in surface, inverse, VOCAL, and AVC modes [22, 25, 26] (Figure 1).

**Statistical studies**

The InfoStat (2008) statistical package (InfoStat Group, FCA, National University of Cordoba, Argentina) was employed using ANOVA (analysis of variance) as the parametric test. To verify its significance we used the Kruskal-Wallis non-parametric test to a < 0.05 level of significance.

To accept the normality of data we used the Kolmogorov-Smirnov test (also known as K-S test), a non-parametric test used to determine the adjustment compatibility between the standardized residues of two distributions of probability.

**Results**

**AFC vaginal 2D US versus 3D US using AVC.** From the onset of the study, clear counting differences were appreciated since observations with 2D US are only in two planes. The images observed with 3D (AVC mode) (Figure 1) were superior, and quantification of AFC was faster. Use of these modes saved time, and, as others have reported [21, 22, 24], they eliminate intra- and inter-observer differences.

AVC modes quantified and measured diameters and volumes automatically (Figure 1). Due to the advantages of 3D AFC, no statistical comparison analysis with 2D were performed. Our results are based on 3D US AFC.

**General results of ovarian aging markers:** Table 1 shows the median and p values for age and determined hormones.

<table>
<thead>
<tr>
<th></th>
<th>Low (mean ± sd)</th>
<th>Normal (mean ± sd)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.6 ± 3.5</td>
<td>33.9 ± 4.6</td>
<td>0.0002</td>
</tr>
<tr>
<td>FSH</td>
<td>9.1 ± 8.9</td>
<td>7.4 ± 3.5</td>
<td>ns</td>
</tr>
<tr>
<td>AMH</td>
<td>9.9 ± 9.5</td>
<td>15.7 ± 12.0</td>
<td>0.0016</td>
</tr>
<tr>
<td>LH</td>
<td>6.6 ± 7.6</td>
<td>6.8 ± 4.1</td>
<td>ns</td>
</tr>
<tr>
<td>E2</td>
<td>61.3 ± 56.5</td>
<td>51.2 ± 32.5</td>
<td>ns</td>
</tr>
<tr>
<td>AFC</td>
<td>5.7 ± 4.4</td>
<td>11.2 ± 7.3</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

(AMH in low and normal responders indicates a statistically significant correlation (Table 1) of clinical interest. An AMH value of 9.28 pmol/l predicted a low response with a sensitivity and specificity of 69% and 65% respectively (Figure 2).)

**Reproductive outcome:** Our definitive reproductive outcome showed a high cancellation rate (22%) of initiated cycles, and very low pregnancy rates for low (6.7%) and (Figure 1)
normo-responders (9.8%). There were 24 (16.7%) gestations out of 143 initiated cycles, and only nine (6.2%) were ongoing pregnancies.

Discussion

Several parameters for evaluation of ovarian reserve have been proposed during the past decade. Age is included as a basic criterion in all protocols, but several authors [8-22] have reported that AMH levels seem to be more predictive of ovarian reserve than age alone.

A recent report [26] concludes that many of the hormonal and laboratory tests (i.e., FSH, LH, inhibin B, basal estradiol, ovarian reserve (EFORT), and clomiphene tests), are of limited value, and indicates that the most predictive tests and the ones with best clinical application are AFC, AMH, and stimulation tests with agonists (GAST) [8, 25]. Our results are in agreement with these conclusions.

The main goal is to identify younger women with an already reduced ovarian reserve, as well as older women who still have adequate ovarian reserve. If this goal can be achieved, we can then optimize treatment by identifying patients who have already experienced ovarian ageing and offer them appropriate ART, such as oocyte donation.

The basal determination of FSH and 17β-estradiol have generated interest due to the clinical repercussions. Our results showed no significant differences between low and normal responders, and poor prediction power.

Our results also showed that AMH was predictive of oocyte maturity which is of clinical interest. The more AMH values the more mature the oocytes recovered. Knowledge about the remaining oocyte pool is impossible, but AFC is closely related to it. Numerous US param-
eters have been used, which have different sensitivities [7, 27-30].

Less than 3 cm³ of ovarian volume has been associated with low response and a high cancellation index [31-33]. Ovarian volume has proven to be a good predictor of low response when excessively small, but there are better US parameters [7, 20].

AFC with vaginal 2D US provides excellent results, however vaginal 3D US with inverse and AVC provide even better results [7, 22, 27, 29, 34-40] allowing the observation of follicles from 2 to 3 mm [29, 34-40]. We observed a relationship between AFC and oocytes recovered, which provides useful prognostic information for low responders [5, 29, 34, 36, 37]. A number of AFC from 1-6 is a poor prognostic index [5, 29, 33, 41-43]. Results of AFC with 3D US modes have proven to be highly reproducible [7, 22, 24, 29, 44, 45]. Observation of the number of AF with 3D orthogonal planes is more precise than with 2D US. Comparing low with normal responders, we observed that in low responders, AFC was diminished and the existing follicles were somewhat larger (between 5 and 7 mm instead of between 2 and 3 mm), which is a manifestation of reduced recruitment and of precocious growth due to the effect of circulating levels of FSH in the higher ranges of normality [27].

Using AVC and inverse modes the number and volume of any structure can be calculated with great precision as previously mentioned [7, 22, 44]. Tomographic US image and inverse mode allow storage in cine loop and observation of follicles in all spatial angles [46-48]. In our opinion, these modes along with AVC are the most promising [7, 22, 24, 25, 43, 47-49].

A comparison between vaginal 2D and these 3D modes for AFC revealed that 3D modes were superior, saved time, and reduced inter- and intra-observer differences [22, 25, 49].

Angio-power Doppler has been used for vascular evaluation of follicular development, ovulatory follicle, corpus luteum, hyperstimulation, PCOS and to identify anovulatory patients, however, it cannot predict low reserve [7, 26].

Anti-Müllerian hormone (AMH). The relationship between AMH and AFC was the best ageing parameter. Lower levels of AMH are associated with lower number of AFC observed with all 3D US modes. This association indicates that both parameters reflect existing ovarian reserve. Differences in AMH levels and in AFC between low responders and normal responders were statistically significant.

AMH and AFC were better predictors of ovarian reserve than age. The observed stability of AMH throughout the cycle, regardless of age, allows its use as a marker of ovarian reserve with much more confidence than other hormones [12, 13].

The associations of AMH and AFC with FSH, LH, and 17β-estradiol lack statistical significance and should be eliminated from ovarian reserve protocols. Regarding FSH, if it is true that with values above 10 IU there were only two false-positives, it is also true that the number of women with normal values who did not conceive is high. Levels of 17β-estradiol, which have received so much attention in the medical literature, have no relationship with AMH levels or with AFC.

A comparison of mean values of AMH and AFC in groups with AFC of ≤ 5 vs > 5 and oocyte maturity reveals that both parameters are predictive of ovarian reserve.

Results of adjusted lineal regression comparing AMH levels and AFC with the quality of G1 and G2 embryos showed an almost horizontal line. The model was incapable of detecting any association.

The relationship between AMH, AFC, and reproductive outcome showed no predictive value.
Predicting ovarian reserve and reproductive outcome using antimüllerian hormone (AMH) and antral follicle count (AFC) in etc.

Figure 3. — Area under the curve for AMH and AFC.

Conclusion

We propose AMH levels and AFC as the only reliable parameters to determine ovarian reserve. Very low levels of AMH (1.15 ng/ml = 8.9 pmol/l) are predictive of minimal possibilities of success, and patients should be so informed and recommended alternative techniques such as oocyte donation.

The fact that we have had a few successful pregnancies in women with very low AMH and AFC values does not allow us, as we had wished, to deny IVF services to women with low values if, as will most certainly happen, they request this service having been informed of, and knowing about, the very low probability of success. Cost/benefit considerations hardly justify the economic expense when there are such dismal probabilities of success. Results of AMH level determinations and their association with the probability of success with ART would offer excellent and reliable support when faced with a decision not to recommend IVF/ET, or to recommend consideration of other alternatives with a better prognosis, such as oocyte donation or adoption.

References


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Frequency of subnormal hypoosmotic swelling tests increase with advancing age of the male

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Introduction

Despite the demonstration that when evaluating various semen parameters a subnormal HOS test score of < 50% far better predicts low implantation potential of embryos achieved by conventional insemination compared to low motile density and morphology, this simple inexpensive test is rarely evaluated by most physicians treating infertility [1, 2]. This is probably related to the fact that some studies found the HOS test to be the least valuable semen parameter to predict poor fertilization rates [3-6]. However most physicians treating infertility are not aware that certain male factor abnormalities, e.g., a low HOS test score can produce normal appearing embryos that rarely implant [7-10]. The aim of the present study was to see if the percentage of abnormal HOS test scores, presumably related to a toxic factor possibly in the ejaculatory system, may increase with advancing age.

Materials and Methods

A retrospective evaluation of the HOS test scores was performed on all initial semen analyses performed in our office over a 10-year time period. The HOS scores were sorted into six groups based on ranges of age: ≤ 29.9 years, 30.0-34.9 years, 35.0-39.9 years, 40.0-44.9 years, 45.0-49.9 years and ≥ 50 years.

The HOS test was performed by combining 0.1 ml of ejaculate with 1.0 ml hypoosmotic solution (fructose/sodium citrate) following precisely the technique described by Jeyendran et al [11]. After incubation of the mixture for at least 30 min at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail swelling changes typical of a reaction in the HOS test. The HOS tests were performed on unprepared specimens during standard semen analysis. An HOS test score < 50% was considered abnormal.

Results

A total of 4,309 patients were evaluated. The frequency of low HOS test scores according to age is seen in Table 1. The percentage of subnormal levels gradually increase with each age group with an acceleration in the two oldest groups such that abnormal HOS test scores were twice as high in males age 45-49.5 as males < 35 and were more than 4-fold higher in males aged ≥ 50.

Conclusions

The HOS abnormality is correctable by IVF with ICSI [12] and to a lesser extent by first treating the sperm with the protein digestive enzyme chymotrypsin [13, 14]. Though we perform this simple inexpensive test routinely irrespective of male age, it behooves the treating infertility specialist to evaluate HOS especially in males of advanced reproductive age. It is not clear what the specific etiologic factor is for developing the hypothesized toxic factor that leads to a subnormal HOS test. Whatever this toxic factor is, it seems to increase in frequency with age.

Table 1. — Frequency of low HOS tests according to age.

<table>
<thead>
<tr>
<th>Age range</th>
<th>n 29.9</th>
<th>30-34.9</th>
<th>35-39.9</th>
<th>40-44.9</th>
<th>45-49.9</th>
<th>≥ 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. evaluated</td>
<td>481</td>
<td>1173</td>
<td>1288</td>
<td>835</td>
<td>357</td>
<td>175</td>
</tr>
<tr>
<td>No. with low HOS score</td>
<td>26</td>
<td>77</td>
<td>103</td>
<td>81</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>% with low HOS score</td>
<td>5.41</td>
<td>6.56</td>
<td>8.00</td>
<td>9.7</td>
<td>12.9</td>
<td>25.1</td>
</tr>
</tbody>
</table>

Key words: Advanced reproductive age; Hypoosmotic swelling test; Embryo implantation defect.
References


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Intracytoplasmic sperm injection completely negates the implantation problem associated with conventional fertilization with sperm with low hypo-osmotic swelling test scores as evidenced by evaluating donor-recipient pairs

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Summary

Purpose: To corroborate or refute the claim that intracytoplasmic sperm injection (ICSI) can overcome the problem found after conventional insemination of oocytes with sperm with low hypoosmotic swelling (HOS) tests of forming embryos with low implantation potential. Methods: Matched couple pairs sharing one pool of oocytes were identified where one of the male partners had a low HOS test score and the other one with a normal one. Intracytoplasmic sperm injection was always used in those with low HOS test scores (i.e., < 50%) vs ICSI only used for semen abnormalities in the normal HOS group. Results: There were no differences found in either fertilization rates or clinical or live delivered pregnancy rates or implantation rates between these groups. Conclusions: Intracytoplasmic sperm injection can completely negate the adverse effect that fertilization with sperm with subnormal HOS scores has on embryo implantation potential.

Key words: Hypoosmotic swelling test; Conventional oocyte insemination; Intracytoplasmic sperm injection.

Introduction

Previous studies have shown normal fertilization rates but low pregnancy rates for couples whose male partner has a low score of < 50% tail swelling following a hypoosmotic swelling (HOS) test [1, 2]. The low pregnancy rate has been hypothesized to be related to the transfer of an unknown toxic substance from the sperm to the zona pellucida [3].

Intracytoplasmic sperm injection (ICSI) can be used to overcome low pregnancy rates [4, 5]. One uncontrolled study revealed a clinical pregnancy rate per transfer > 40% when using ICSI [5].

The present study was conducted to corroborate or refute the previous conclusions that ICSI can be used to achieve higher pregnancy rates by performing a matched controlled study, and to determine pregnancy rates following ICSI in couples sharing one pool of eggs with one woman having a male partner with HOS test score < 50% and the other woman a male partner with an HOS score of ≥ 50%.

Materials and Methods

A retrospective review of donor-recipient pairs was conducted over a 7-year time period in which one of the two couples had a male partner with a low HOS test score. Pregnancy outcome was evaluated for fertilization with sperm with low vs normal HOS test scores: only pairs having one male partner with a low HOS test score were evaluated. Intracytoplasmic sperm injection was used on all oocytes when using sperm with low HOS test scores. Intracytoplasmic sperm injection was only used in the normal group when other abnormal semen parameters were present.

Only cycles with at least two embryos transferred on day 3 were evaluated. Two methods of controlled ovarian hyperstimulation were used; either luteal phase leuprolide acetate or antagonist protocols using ganirelix or cetrorelix.

Results

A comparison of pregnancy outcome rates for low HOST and normal HOST groups is seen in Table 1. Seventy-three paired cycles were evaluated, leading to 49 transfers using sperm with low HOS test scores vs 53 with normal HOS test scores. Some fresh transfers were deferred and embryos frozen instead for risk of ovarian hyperstimulation or for inadequate endometrial thickness.

There were no cycles with failure to attain a day 3 embryo. One woman in the normal group transferred only one embryo so the analysis was based upon 49 transfers in the low HOS group vs 52 in the normal HOS test group.

The fertilization rate was 73.1% (483 of 781) in the low HOS test group compared to 65.8% in the normal HOST group. Implantation rate was 29.6% (45/152) for low HOS test group as compared to 27.4% (43/157) for the normal HOS test group (chi-square, p = NS). Clinical pregnancy/transfer rate (evidenced by ultrasound at 8 weeks) was 53.1% (26/49) in the low HOS group as compared to 55.8% (29/52) in the normal HOS test group (p = NS). Delivered pregnancy rate was 49.0% (24/49) for the low HOS test group and 50.0% for the normal HOS test group (p = NS).
This is the first study attempting to corroborate or refute previous claims that ICSI can overcome the HOS test sperm abnormalities. These data confirm previous conclusions and is the first controlled study showing that ICSI fully overcomes the HOS test defect. The fertilization of oocytes by ICSI can completely overcome the embryo implantation problem seen when using conventional insemination methods.

Previous studies have found dismal pregnancy results following conventional oocyte insemination and subsequent embryo transfer when using sperm with low HOS test scores. A matched controlled study found a clinical pregnancy rate of only 3.7% per transfer with low HOS test scores versus 25.9% with normal HOS test scores [1]. There have been no studies refuting this claim of normal fertilization rates but poor pregnancy rates with conventional insemination of oocytes with sperm with low HOST scores.

References

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Table 1. — Shared oocyte pairs were used to determine if intracytoplasmic sperm injection fully corrects the embryo implantation defect caused by sperm with low HOS test scores.

<table>
<thead>
<tr>
<th></th>
<th>Los HOS</th>
<th>Normal HOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cycles</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>No. transfers</td>
<td>49</td>
<td>53</td>
</tr>
<tr>
<td>No. transfers + 2 embryos transferred</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>No. eggs retrieved</td>
<td>781</td>
<td>775</td>
</tr>
<tr>
<td>No. metaphase II oocytes</td>
<td>583</td>
<td>649</td>
</tr>
<tr>
<td>No. inseminated</td>
<td>661</td>
<td>714</td>
</tr>
<tr>
<td>No. fertilized</td>
<td>483</td>
<td>470</td>
</tr>
<tr>
<td>% fertilized</td>
<td>73.1%</td>
<td>65.8%</td>
</tr>
<tr>
<td>No. pregnancies</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>% pregnant/transfers</td>
<td>65.3%</td>
<td>59.6%</td>
</tr>
<tr>
<td>No. clinical pregnancies</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>% clinical pregnancy/transfers</td>
<td>53.1%</td>
<td>55.8%</td>
</tr>
<tr>
<td>No. chemical</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>No. ectopic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No. live deliveries</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>% live delivery/transfers</td>
<td>49.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td># miscarriages</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>% miscarriage/pregnancies</td>
<td>7.7%</td>
<td>20.7%</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>152</td>
<td>157</td>
</tr>
<tr>
<td>Average no. embryos transferred</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>No. sacs implanted</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>29.6%</td>
<td>27.4%</td>
</tr>
</tbody>
</table>

Discussion
This is the first study attempting to corroborate or refute previous claims that ICSI can overcome the HOS test sperm abnormalities. These data confirm previous conclusions and is the first controlled study showing that ICSI fully overcomes the HOS test defect. The fertilization of oocytes by ICSI can completely overcome the embryo implantation problem seen when using conventional insemination methods.

A retrospective study of IVF-ET cycles from 1991 to 1994 using conventional oocyte insemination using sperm with single abnormalities found a 25.7% rate with subnormal motile density, 44.4% with subnormal morphology using strict criteria, 25.7% with all factors normal, but 0% with the HOS test < 50% [6].
Pregnancy outcome following fresh vs frozen embryo transfer into gestational carriers using a simplified slow freeze protocol

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Summary

Purpose: To compare pregnancy rates following fresh vs frozen embryo transfer into gestational carriers. Methods: Choice of deferring fresh embryo transfer and cryopreserving the embryos vs fresh transfers was not randomized but based on circumstances. The cryopreservation protocol used a simplified slow cool technique avoiding the planar programmable freezer and using a one-step removal of the cryoprotectant. Results: The live delivered pregnancy rate was 51.0% (49/96) for fresh embryo transfer vs 34.3% for transfers of frozen thawed embryos in gestational carriers not having a fresh embryo first. Conclusions: Using the simplified slow cool cryopreservation protocol with a one-step removal of cryoprotectants pregnancy rates are comparable to what is found in women of similar ages undergoing controlled ovarian hyperstimulation followed by IVF-ET. However, when transferring to a gestational carrier the live delivered pregnancy rates are 50% higher with fresh embryo transfer.

Key words: Frozen embryo transfer; Fresh embryo transfer; Gestational carrier; Pregnancy rate.

Introduction

There are many IVF centers performing in vitro fertilization-embryo transfer (IVF-ET) that demonstrate very good pregnancy rates with fresh embryo transfer but do not fare as well with the transfer of frozen-thawed embryos. Some believe it is the type of slow freeze technique used that is the problem, especially the programmable freezer [1]. In fact there has been a revival of an old technique of vitrification which is showing promise as to providing comparable pregnancy rates to those with IVF-ET [2].

A slow cool technique has been described that avoids the planar programmable freezer and does provide similar pregnancy rates following transfer of frozen thawed embryos as with IVF-ET [3, 4]. Though indeed pregnancy rates were similar in women undergoing controlled ovarian hyperstimulation having fresh embryo transfer or those having fresh transfer deferred for subsequent frozen-thawed embryo transfer, the pregnancy rates were superior for recipients receiving fresh embryos [4].

Though based on the aforementioned study it would be ideal to synchronize a woman undergoing IVF-ET with a gestational carrier so that fresh embryo transfer could be achieved, sometimes the reason for the need for a gestational carrier is imminent surgical or medical therapy that could also damage ovarian oocyte supply and a gestational carrier cannot be arranged that quickly. Thus there is a need to cryopreserve the embryos for the future.

The present study aimed to determine how much of a sacrifice women are making by delaying fresh embryo transfers but cryopreserving the embryos using the simplified slow cool freezing technique.

Materials and Methods

A retrospective review of pregnancy outcome of all fresh and frozen embryo transfers in gestational carriers over a 6-year time period was performed. Only gestational carriers having previous full-term deliveries were used and with a history of no difficulty in achieving pregnancy.

Intentional freezing of embryos was performed at the pronuclear stage using a simplified freezing protocol and a one-step removal of the cryoprotectant 1,2 propanediol with thawing [3]. Some embryos were frozen on day 3. These were ones selected from fresh transfer. In general twice as many embryos as intended for transfer were allowed to cleave to day 3 and the lesser quality ones were frozen. All fresh and frozen ETs were performed on day 3 and were preceded by assisted embryo hatching.

Most often the luteal phase leuprolide acetate regimen was used for COH especially when there was intention to transfer fresh embryos into the gestational carriers. Sometimes when intentional freezing was to be performed, an antagonist stimulation protocol using either ganirelix or cetrorelix was employed.

Results

There were 96 fresh and 113 frozen ET cycles evaluated. There were 67 gestational carriers having frozen ET who never had a previous fresh ET.

The average age of the woman having COH was 33.6; the average age for the subset of 67 having intentional
freezing was 35.0. The mean number of embryos transferred for fresh ET was 3.1 ± .6 vs 3.1 ± .7 for frozen ET

Clinical (ultrasound evidence of pregnancy) pregnancies were achieved in 56 of 96 (58.3%) gestational carriers having fresh ET vs 45 of 113 (39.8%) in gestational carriers having frozen ET (p = 0.01). To eliminate possible confounding effects of de-selection the fresh ET clinical PRs were also compared to gestational carriers having a frozen ET as their first transfer. Pregnancies were achieved in 28 (41.8%) (p = 0.055).

The live/delivered PRs were 51.0% (49/96) for fresh ET, 33.6% (38/113) for first frozen ETs including gestational carriers failing to conceive in their fresh ET, and 34.3% (23/67) for gestational carriers whose first transfer was with frozen/thawed embryos. The live/delivered PR was significantly higher for fresh ET than either frozen group (p < 0.01 and p < 0.05, respectively).

The implantation rates for fresh ETs was 28.0% (84/300) for fresh embryo transfer vs 20.4% (71/348) and 21.5% (45/209) for the two frozen ET groups (p < 0.01 comparing the fresh ET to either frozen ET group).

Discussion

These data confirm the conclusions of a previous study not using gestational carriers but using the simplified slow freeze protocol that pregnancy rates following frozen-thawed ETs are very respectable but inferior to fresh ET when COH is not used [4].

Thus whenever possible it would be ideal to wait to do oocyte retrieval until a gestational carrier is found. However, if circumstances require the IVF be performed right away it is important to use a cryopreservation technique that is effective.

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The majority of males with subnormal hypoosmotic test scores have normal vitality

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Introduction
Men with low hypoosmotic swelling (HOS) scores (<50%) rarely achieve pregnancy after natural intercourse or intrauterine sperm injection [1]. Sperm with low HOS scores fertilize oocytes at a normal rate; however, the embryos formed have extremely low odds of implanting [2-4].

The HOS test measures the functional integrity of the sperm membrane whereas dye exclusion vitality measures the structural integrity. Nevertheless, some andrologists believe that if you measure viability and it is normal, then there is no reason to perform the HOS test. The sperm vitality test is able to distinguish between live and dead sperm. Sperm that swell under hypoosmotic conditions have intact membranes and will exclude stain. However, dead sperm will uptake the dye and not swell due to some type of damage to the membrane.

The World Health Organization (WHO) laboratory manual (1999) suggests performing a sperm vitality (dye exclusion test) or HOS test every time the sperm motility is abnormal or less than 50% in a semen analysis. The HOS test detects a functional impairment of the sperm membrane which may be related to a toxic factor that transfers to the zona pellucida by the supernumerary sperm that attach and may thus lead to functional impairment of the embryo membrane thus preventing implantation [2-4].

The present study evaluated whether these two tests are indeed interchangeable. Our working hypothesis was that we would find a large percentage of males with subnormal HOS scores who have normal vitality.

Materials and Methods
A retrospective review of initial semen analyses from all patients between May 1999 and May 2008 were included in this study. A subset of those males with HOS scores < 50% was identified with their viability results. To perform the dye exclusion test [5] the reagent was 5% eosin in a sodium chloride solution. The procedure involved mixing one drop of liquefied semen with one drop of 5% eosin solution on a microscope slide and read at 400x. One then counts 200 sperm and calculates the percentage of live sperm (unstained).

The WHO 3rd edition suggested a normal vitality of 75% or more whereas the WHO 4th edition has changed the normal percentage for vitality to 50% or more sperm which exclude dye.

For the HOS test the reagents were 0.735 g sodium citrate, and 1.351 g fructose in 100 ml distilled water [6]. The procedure involved mixing 0.1 ml liquefied semen with 1 ml HOS solution. One then incubates for 30 min at 37°C. After mixing well one drop is placed on a slide. A phase contrast microscope in then used to determine the percentage of sperm with swollen tails in 200 sperm. Then one subtracts the percentage of swollen tails initially seen in the raw sample to obtain the percentage of tails that have swelled from the HOS solution. A result of less than 50% is considered abnormal [6].

Results
There were 361 males with low HOS scores. Only 12.5% (45/361) of these males had a subnormal vitality.

Discussion
Another study did show a correlation with poor motility and low HOS scores [7]. However, there are males with normal motility and low HOS scores. Because of the severity of this implantation defect and the simplicity and lack of expense of this test, we recommend it should be performed routinely.

There were no males with subnormal vitality who had normal HOS tests. Thus we therefore could eliminate the
vitality test and just do the HOS test. By only doing the vitality, 28.8% (104/361) of abnormal males whose infertility could be corrected by either IVF with ICSI or treatment of sperm with chymotrypsin-galactose and IUI would have been missed [8-10].

References

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Immune response and immunotherapy in intraepithelial and invasive lesions of the uterine cervix

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Summary

Infection with the human papillomavirus virus (HPV) induces innate and acquired immune responses in the cervical stroma, which are a delicate, balanced and generally unpredictable immunological defense. Because of the immunological breaks that the HPV virus causes, eradication of infected cells does not occur, potentially leading to development of intraepithelial and invasive lesions. Advances in our understanding of the immune system and in the definition of antigens in tumor cells has led to many new treatment strategies. As a result, immunotherapy has the potential to be the most specific treatment for tumors, and one that requires elaboration. Recently, immunotherapy with interferon and dendritic cells has been used on intraepithelial and invasive cervical lesions with promising results.

Key words: Cervical intraepithelial neoplasia; Human papillomavirus; Immunotherapy; Interferon and dendritic cells.

Introduction

With approximately 500,000 new cases in the world each year, cervical cancer is the second most common cancer among women and is responsible for the death of approximately 230,000 women each year. Its incidence is two times higher in less developed countries than in developed ones [1]. In some developing countries it is the most common form of malignant neoplasia in woman and may comprise up to 25% of all cancers among women [2].

In cervical intraepithelial neoplasia (CINs), the arrangement of the squamous cells of the ectocervix remains disorganized and the cells stay atypical. When disorganization occurs only in the deepest third, there is light dysplasia, or CIN grade 1. When the disorganization involves the two deepest thirds of the epithelium, preserving only the most superficial layers, there is moderate dysplasia, or CIN grade 2. If the disorganization is observed at all levels, involving more than two thirds of the epithelium, there is CIN grade 3 or carcinoma in situ. The high-grade cervical intraepithelial squamous lesion classification includes CIN grades 2 and 3 and carcinoma in situ [3]. In accordance with the Bethesda classification, low-grade squamous intraepithelial lesions (LSILs) correspond to light dysplasia/CIN 1, and cell changes associated with human papilloma virus (HPV) and high-grade squamous intraepithelial lesions (HSILs) correspond to moderate dysplasia/CIN 2, severe dysplasia, carcinoma in situ/CIN3 [4].

Cervical intraepithelial neoplasia and the human papiloma virus

HPV is a double-stranded DNA virus, with approximately 8000 base pairs, belonging to the Papovaviridae family, which is transmitted through sexual relations with an infected partner or by means of fomites, which infect the skin or mucous in the form of warts [5, 6]. To date, 130 subtypes of HPV have been described [7], of which approximately 25 infect a woman’s anogenital area. Based on their association with cervical cancer and pre-cursor lesions, HPVs can be divided into high-risk HPVs (16,18,31,33,34,35,39,45,51,52,56,58,59,66,68 and 70) and low-risk HPVs (6,11,42,43 and 44) [8].

The relationship between HPV and cervical cancer is of greater significance than the relationship between smoking and lung cancer [9]. The four most common types found in cervical cancer cells are 16, 18, 31 and 45, with HPV 16 making up nearly half the cases in the US and Europe and types 18, 31 and 45 accounting for 25-30% of the cases [2]. HPV 16 and 18, the principal high-risk subtypes, have a high correlation with pre-neoplastic lesions that can evolve into cervical cancer, even higher than other types of oncogenic HPV. One study in the United States involving more than 20,000 women demonstrated that the incidence rate of HSILs was 17.2% among those infected with HPV 16 and 13.6% among those women infected with HPV 18, while the incidence of women infected with other oncogenic types of HPV was only 3% [10].

Molecular biology techniques prioritize knowledge about the epidemiological profile of HPV infection and allow the sequencing of HPV-DNA, as well as the recognition of different viral subtypes [11]. Polymerase chain reaction (PCR) is now considered the most sensitive method for detecting HPV infection, since in situ
hybridization is limited by the number of copies of HPV [12, 13]. Studies using molecular biology techniques have suggested that, despite having a normal cytology, 40% of sexually active young women have HPV DNA and that its prevalence diminishes with age [14, 15].

Hybrid capture is an extremely sensitive and easily handled but costly method [16]. Research shows that PCR detects more high-risk HPV than hybrid capture and that both PCR and hybrid capture have a high negative predictive value for high-grade lesions; although the sensitivity of PCR is greater and its cost is lower [17]. In conization material for CIN 3, the use of PCR detects HPV-DNA 16 and/or 18 in most positive cases (87%), and only 9.1% of cases present HPV 6 and 11 together. In this study, HPV 18 was the most common HPV (78.7% of cases) and was frequently associated with HPV 16 but rarely with HPV 6 and 11 [18].

**Immune response in intraepithelial and invasive lesions of the cervix**

Tumor immunology is a promising area of cancer research and is already used in medical therapy [19]. Tumor-specific immune responses are observed in cancer patients, given that in the tumor mass they show infiltration with cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Altered patterns in class I and class II main histocompatibility complex (MHC) molecules are observed on the surface of tumor cells [20, 21].

The first line of the innate defense response begins in the stroma, but is also present in the epithelium or near it [22]. This non-specific resistance occurs when pathogenic agents employ mechanisms to escape the immunology system, evading an adaptive (specific) immune response. Innate immune response is measured in various ways, including interferon (IFN) induction and the activation of macrophages and NK cells, which can lead to the activation of a specific response.

Cell immunity measured by T cells plays an important role in the eradication of cells infected by HPV. Flaws in the induction or maintenance of T-cell response can lead to persistent infection and to the development of malignant neoplasias [23]. T CD4 lymphocytes help in immune response by promoting the secretion of cytokines and are mediators that activate immune response cells, as well as macrophages and B lymphocytes. The T CD8 lymphocytes promote infected or tumor cell death through the action of their toxic granules [24].

Immune responses are moderated by the liberation of different cytokines. Those which are secreted by T CD4+ lymphocytes were originally classified as T helper 1 (Th1) and T helper 2 (Th2). Recently, a new population, T helper 3 (Th3) and T regulatory (Treg), have been discovered; these can indirectly suppress immune response by reducing the expression of co-stimulators in the antigen presenting cells (APCs) or suppress it directly in T cells. The most important Th1-pattern cytokines are IL-2, IL-12, IFN-γ and TNF-α, which are responsible for activating cell immune response. Th 2-patterns, such as IL-4, IL-10 and TGF-β, are directly related to the activation of humoral immune response. The Treg type has already been characterized by the presence of IL-10 and TGF-β. This pattern seems to involve the induction of tolerance.

Regression of HPV infection has been associated with an immune response mediated by Th1-pattern cytokines and the development of CIN seems to be moderated by Th2-pattern cytokines [25]. Inflammation, which plays an important role in innate immunity, is stimulated by cytokines like IL-1 and TNF-α, which are synthesized by keratinocytes following aggression by a viral infection - HPV, for example [26]. These cytokines stimulate changes in the adherence of molecules and their capillary permeability, and also lead to the liberation of other cytokines [27]. Acute inflammation leads to the elimination of infection and tissue repair and is responsible for unleashing acquired immunity. Changes in the Th1 and Th2 cytokine profiles have been demonstrated in humans with neoplastic diseases [28]. In addition, chronic inflammation, which occurs in cases of persistent infection, has been considered to be a risk factor in the activation of carcinogenesis [29] (Table 1).

![Table 1. — Type 1 and type 2 cytokines.](image)

**Table 1. — Type 1 and type 2 cytokines.**

<table>
<thead>
<tr>
<th>Type 1 cytokines</th>
<th>Type 2 cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-helper 1 response</td>
<td>T-helper 2 response</td>
</tr>
<tr>
<td>Immunity mediated by cells</td>
<td>Immune response</td>
</tr>
<tr>
<td>IL-2, IL-12, IL-15</td>
<td>IL-4, IL-5, IL-6, IL-10, IL-13</td>
</tr>
<tr>
<td>Interferon gamma (IFN-γ)</td>
<td>IL-10, IL-13</td>
</tr>
</tbody>
</table>

Various studies have described how HPV interacts with the immune system [30] and how the virus deactivates adaptive immune response [31]. There is a decrease in the expression of class 1 MHC molecules and in T cell receptors (TCRs), impeding the presentation of antigens to T cells. In this way, the immunological recognition of HPV-infected cells on the part of the host’s CD8+ lymphocytes is reduced. Moreover, the virus does not have a blood dissemination phase, or does it cause lysis of keratinocytes, and therefore does not induce an inflammatory immune response. The production and liberation of the virus also occurs in different squamous cells that are far from the immunocompetent and cytokine cells in the submucosa [30, 32, 33].

Systemic and local immune responses play important roles in the progression of CIN. The presence of greater infiltration of T (CD3) lymphocytes in patients with CIN 3 is linked to a greater frequency of recurrence after conization. All women with recurrent CIN 3 showed a high percentage of CD3 lymphocytes in their cell count [34]. The profile of nitrous oxide (NO) cytokines in the evaluation of local immune response in patients with bacterial vaginosis and CIN has already shown an increase in the local production of IL-8, IL-10 and NO, demonstrating an immune response against the tumor or developing tumor from these respective mediators [35]. NO has also
Immune response and immunotherapy in intraepithelial and invasive lesions of the uterine cervix

Table 2. — Immunological transformations in the cervical stroma during carcinogenesis.

<table>
<thead>
<tr>
<th>Inflammation/HPV infection risk factors for carcinogenesis</th>
<th>CIN progression</th>
<th>Invasive cancer progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in macrophages CD4+ reduction in CD8+ CTL or TIL cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in NK cells Reduction in CD8+ NK cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in IFN Reduction in CD4+ Increase in CD3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in type 1 cytokines Reduction in CTL in iNKR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in type 2 cytokines Reduction in IL-10 Progressive loss of type 1 cytokines Low IFN activity MHC I damaged IL-4r 75v HLA polymorphism</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

been evaluated by Fernandes and collaborators, who confirmed its liberation by tumor cells, as well as other soluble circulating mediators [36]. NO can interfere with the initial capacity for neutrophil migration, impeding immune response. Neutrophils can have anti-tumor properties, and their migration is very important for the inflammatory response.

The upper part of the female genital tract has both an innate [37] and acquired [38] immune response, which keeps it in a predominantly aseptic state. There is a delicate balance between pathogenic agents and tolerance to semen [39], pregnancy, and cervical cancer. In the early stages of cervical cancer, HPV-infected cells must bypass the immune system. As the disease progresses, there is a reduction in type Th1 cytokines, and type Th3/Treg cytokines become predominant [40]. Patients with CIN also show a reduction in the expression of receptors for IFN-α in comparison to that in normal tissue. Low levels of IFN-γ mRNA copies are seen in tumor biopsies and are related to prognosis [41].

In studies of the microenvironment of the normal cervix examining CIN 2/3 lesions in immunocompetent women and CIN 2/3 lesions in HPV-infected women, the immunocompetent women with CIN had a mixture of pro-inflammatory cytokines and regulatory cells, characterized by an increase in the production of IFN-γ by CD4+ or CD8+ T cells and NK cells, as well as an increase in the regulation of tumor growth factor beta (TGF-β) [42]. In CIN/HIV+, the numbers of cells and regulatory cytokines were down-regulated. Cervical cancer cells can promote the expression of inhibitory natural killer cell receptors (iNKR) via IL-15 and, possibly via a mechanism mediated by TGF-β and by the antitumor cytotoxic cancellation of TILs (tumor infiltration lymphocytes) [42]. Advances in our understanding of the immune system and in the definition of antigens in tumor cells have encouraged a number of new strategies. Therefore, immunotherapy has the potential to be the most specific treatment that can be developed for tumors.

Practical application of the study of immune response: the role of immunotherapies

The objectives of immunotherapy are activating the immune response, bolstering its natural efficiency, detecting the multiplication of malignant cells, and selectively eradicating tumors without causing lesions in the patient. A vaccine for certain types of HPV that could reduce the frequency of cervical cancer is already on the market. However, this vaccine should not be associated with the prevention of this cancer, but with protection against some types of low- and high-risk HPV [43]. Recently, immunotherapy with interferon (IFN) and dendritic cells has been used on cervical intraepithelial and invasive lesions with promising results [44, 45].

IFN, which has been under study since 1957, was discovered during research on the phenomenon of viral interference. But its mechanism of action is not completely understood. It is known that it encompasses a group of cytokines with important immune system functions, such as inhibition of viral multiplication, immune modulation to stimulate NK cells and monocytes, and an antiproliferative effect, as well as an anti-angiogenic action [46]. There are more than seven types of IFN, which act on the immune system in different ways [47-49]. Recent studies have shown that IFN can be applied to many different kinds of pathologies, including viral hepatitis, multiple sclerosis and cancer. Since the early 1980s, various studies have used IFN in the treatment of gynecological cancer with varying results [50, 51]. Murta and Tavares achieved good results in demonstrating its efficiency, obtaining a complete response in the treatment of a patient with invasive vaginal carcinoma using intraleisional IFNα-2b with complete remission of the lesion [44]. The actions of IFN, both anti-proliferative and immunoregulatory, are today an area of interest to many researchers.

Immune responses can often cease controlling the growth of tumors because these responses are ineffective or because the tumors have ways to escape the immune system. The principal mechanisms by which tumors escape are: reduction in production of tumor antigens; reduction in the expression of MHC molecules; and production of immunosuppressor proteins [52]. These escape mechanisms can act on dendritic cells (DCs), inhibiting their activation and their migration to secondary lymph nodes for activation of T lymphocytes.

The DCs are the main cells that present antigens. They are extremely efficient in activating CD4+ and CD8+ T lymphocytes and can maintain tolerance or unlock immune responses [53]. Because of their role in controlling immune response, interest in studying DCs has surged in recent years in the hope to fully use them as therapeutic tools. Today, the manipulation of in vivo or ex vivo DCs is thought to be one instrument that will lead to progress in the use of immunotherapy in cancers. In fact, DCs are considered an ideal tool for the development of therapeutic vaccines against cancer since numerous clinical studies have shown their efficacy against tumors [45].
Thus, bearing in mind the existence of tumor escape mechanisms that stimulate the persistence or progression of a tumor, the development of immunotherapy to treat tumors must take priority. The principal strategies for cancer immunotherapy mainly aim to prove their anti-tumor effects on patients, actively immunizing these patients against their tumors, and stimulating their own immune responses. Indeed DC therapy is one immunotherapy that seems promising in terms of stimulating anti-tumor immune responses.

With the goal of evaluating the potential of autologous DCs pulsed with the E7 antigen of HPV16 and HPV18 to serve as a therapeutic cell vaccine for patients with recurrent/metastatic cervical cancer resistant to conventional treatments, Santin and collaborators [54] obtained good results: the vaccine was tolerated well by all the patients, and it did not cause local or systemic side-effects. However, patients who developed positive delayed-type hypersensitivity (DTH) showed lower tumor progression (survival beyond 13 months), while the DTH negative patients died within five months after the start of therapy. The number of monocytes/Trx80-activated-monocytes (TAMs) and DCs was positively correlated with the expression levels of growth factor of colonies of granulocytes and macrophages (GM-CSF) and alpha tumor necrosis factor (TNF-α). This finding suggests a role for these cytokines in the differentiation of monocytes in mature DCs, which may constitute an escape mechanism for cancerous cells [55]. The DC vaccine seeks to create the conditions for these cells to develop and to allow for the execution of that which the tumor has not yet permitted—the migration to secondary lymph nodes so that antigens to the T lymphocytes can be presented, inducing their activation and, consequently, the initiation of an anti-tumor immune response.

Many technological developments in clinical immunology for research and diagnosis of HPV infections and their relationship to cervical cancer have been obtained in the last few decades. The techniques that emerged have been a direct result of immunological reactions revealed through markers (immunofluorescence, immunohistochemistry and flow cytometry). The characterization of the structure, gene expression, synthesis of selected genes and their manipulation in cells and animals is made by molecular biology. Through DC vaccine treatment, it should be possible to analyze immune response to treatment, leading to an understanding through flow cytometry of the induction mechanism, the subtype of activated T lymphocyte, the cytokines produced, and the profiles of immunological memory.

Final Considerations

In summary, infection with HPV induces innate and acquired immune responses in the patient’s cervical stroma. This immunological defense is delicately balanced and not always possible to predict. Clinical practice is mainly determined by risk, evolution, and prognosis (Table 2). The study of immune responses is complex, and there is still much to be understood about cell interactions and cytokines. Immunotherapy, by stimulating the immune system through the use of biological response modifying substances, has the potential to offer the most specific treatment for tumors. Developments in the understanding of the immune system and in the definition of tumor cell antigens have led to many new treatment strategies that are based on the mechanisms involved in immunity mediated by cells or their mediators.

Acknowledgements

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References

Immune response and immunotherapy in intraepithelial and invasive lesions of the uterine cervix


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Severe hepatocellular dysfunction in obstetric cholestasis related to combined genetic variation in hepatobiliary transporters

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Summary

Obstetric cholestasis (OC) is a cholestatic disorder with a prominent genetic background including variation in diverse hepatobiliary lipid transporters, such as ABCB4 (phospholipids) and ABCB11 (bile salts). Given a marked hepatocellular dysfunction in an OC patient indicated by > 40-fold rise in alanine aminotransferase activity and minor γ-glutamyl transpeptidase increases, we performed genotyping of candidate gene variants associated with adult cholestatic phenotypes. Genetic analysis revealed the heterozygous ABCB4 mutation p.R590Q, the ABCB11 variant p.V444A and the lithogenic ABCG8 variant p.D19H. Aggregation of multiple hepatobiliary transporter variants is rare in OC, and may cooperate to negatively modulate hepatobiliary transport capacities.

Key words: Obstetric cholestasis; Hepatobiliary transporters; ABCB4; ABCB11; ABCG8; Low phospholipid-associated cholelithiasis; Cholelithiasis.

Introduction

Obstetric cholestasis (OC) is the most common pregnancy-related liver disorder estimated to affect up to 1% of pregnant women in European countries typically in their third trimester. Patients present with troublesome pruritus due to elevated serum bile acid levels (> 11 μM/l) and mild to moderate elevations in aminotransferases. In general, increases in liver function tests do not exceed a 10- to 20-fold rise in alanine aminotransferase (ALT), representing the most specific marker of hepatocellular injury in OC [1]. Maternal prognosis in OC is considered benign, whereas OC may entail a considerable perinatal risk and fetal complication rate, e.g. preterm delivery and fetal distress, in particular, if fasting serum bile acid levels surpass 40 μM/l. As for clinical management, ursodeoxycholic acid (UDCA) has become the therapeutic mainstay, conferring beneficial symptomatic and biochemical effects. Close fetal monitoring with early delivery in severe cases is essential.

Though the precise molecular mechanisms in OC pathogenesis are as yet poorly understood, cholestatic decomposition of the hepatobiliary transport machinery in genetically susceptible individuals challenged by pregnancy-related systemic and hepatic hormonal loads seems to be key to OC development. Indeed, modulation of hepatobiliary transporter expression by sex hormones has been shown in vitro. Specifically, estrogen glucuronides and specific progesterone metabolites negatively regulate expression of canalicular ATP-binding cassette (ABC) transporters, i.e., the bile salt export pump ABCB11 (BSEP) and the phospholipid transporter ABCB4 at the posttranscriptional level.

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morphisms (SNPs), including ABCB11 E297G (rs11568372), ABCB11 D482G (rs72549402), ATP8B1 N45T and ABCB4 R590Q (rs45575636) were detected by direct sequencing using BigDye Terminator Kit (Applied Biosystems, Darmstadt, Germany). Four variants were analyzed using solution-phase hybridization reactions with 5'-nuclease and subsequent fluorescence detection (TaqMan SNP Genotyping Assays, Applied Biosystems, Darmstadt, Germany). The polymorphism V444A (rs2287622) in the ABCB11 gene was genotyped by assay C__16182459_10, while the synonymous ABCB11 variant A1028A (rs497692), previously demonstrated to be associated with exon skipping, was detected by assay C__881357230. The coding variant ATP8B1 E429A (rs34018205) was analyzed by a self-designed assay. Finally, the ATP8B1 mutation I661T (rs2287622) was analyzed by restriction fragment length polymorphism (RFLP) and subsequent polymerase chain reaction (PCR). The PCR product contained 281 bp and was digested with PsI. The major allele was present when the PCR product remained undigested, for the minor allele the PCR product was cut into two fragments of 261 bp and 19 bp. Subsequently, the samples were analyzed on a 4% agarose gel (Biozym, Hess. Oldendorf, Germany). Primer and probe sequences for the respective SNPs can be provided on request.

Results

A 30-year-old primigravida was referred in her 29th gestational week for progressive “pruritus sine materia” with nocturnal exacerbations and markedly elevated liver enzymes. Laboratory data were as follows (maximum values indicated; normal values in parentheses): ALT 1450 U/l (< 35), aspartate aminotransferase (AST) 766 U/l (< 35), γ-GT 46 U/l (< 40), alkaline phosphatase (AP) 207 U/l (35-104), total serum bilirubin 0.7 mg/dl (< 1.2). Fasting serum bile acids were elevated to a maximum of 33 µM/l (pregnancy-specific normal < 11). Abdominal ultrasound revealed an uncomplicated, as yet unknown, cholecytitis and an inconspicuous liver without evidence of chronic liver disease and/or mechanical cholestasis. A dedicated history of medications including recent use of over-the-counter and herbal preparations remained unremarkable. After exclusion of alternative diagnoses, such as viral hepatitis, hereditary, metabolic and/or autoimmune liver disease and ongoing preem-}

Discussion

Pregnancy is an exceptional physiological state challenging the individual’s hepatobiliary transporter capacity by virtue of excessive hormonal levels. Genetic variation in hepatobiliary transporters in association with environmental factors may determine whether cholestatic decompensation occurs, i.e., whether OC manifests clinically. Despite the general appreciation of a complex genetic background in OC, where single gene polymor-
phisms may only have a small impact on clinical phenotype, combined genetic variation in OC-related loci has rarely been reported [5, 6]. The genetic findings in the presented patient are novel as to the co-inheritance of the lithogenic ABCG8 D19H variant with combined variation within ABCB4 and ABCB11 as functionally coupled OC-associated genes.

The non-synonymous ABCB11 SNP p.V444A (c.1331T>C) located in the nucleotide binding fold (NBF) in the intracellular loop of BSEP is a very common variant occurring in up to 50% of the general population, yet a robust association with OC has been established. Though allele-specific functional analyses assessing taurocholate transport capacity yielded similar results under basal conditions, available data demonstrate reduced canalicular membrane BSEP staining and lowered mature BSEP production by the SNP variant 444A, supporting the notion that basal BSEP abundance may be overridden in cholestatic conditions, such as pregnancy where sex hormone levels peak, in genetically susceptible individuals. In our patient, canalicular BSEP deficiency may have contributed to the profoundly elevated liver enzymes as a consequence of hepatocellular bile salt toxicity.

However, the detectable γ-GT elevation in addition raised the possibility of aberrant ABCB4 function underlying her cholestatic phenotype. In fact, she proved heterozygous for the missense mutation p.R590Q in the ABCB4 gene, which has been previously documented in OC [7, 8]. Located in the evolutionary conserved nucleotide binding domain (NBD) 1 engaged in energy transfer from ATP hydrolysis to substrate transport, altered biological function of the variant ABCB4 protein may be assumed. However, as yet, no functional data assessing alterations in transporter activities are available for this specific coding variant. In the report by Ziol et al., liver samples from a single R590Q mutation carrier were available for ABCB4 immunohistochemistry revealing “faint and discontinuous canalicular staining” [8]. In the same direction, potential disruption of targeted protein routing giving rise to functional lack of ABCB4 protein on the canalicular membrane appears plausible, and has been demonstrated previously for another missense mutation in the same NBD. In a study by Tavian et al., the mutation was not detected in 43 parous controls, while previous data from France had indicated that up to 3% of healthy controls may harbor this sequence alteration heterozygously [8, 9]. In the most recent study sequencing the entire ABCB4 coding sequence in 50 OC patients, however, the R590Q variant was identified in a total of five individuals, of interest, in two of them homozygously, compared to one heterozygous carrier among 107 pregnant control women [10]. Of note, some of the patients carrying the R590Q mutation were also homozygous for a distinct haplotype comprising three SNPs (c.175C>T, c.504T>C and c.711A>T), pointing to a possible founder effect of the mutation. The overrepresentation of the R590Q variant in the OC cohort proved statistically significant, and all women carrying the risk allele showed normalization of liver dysfunction after delivery [10]. Likewise, the complete resolution of laboratory evidence for chronic cholestasis postpartum and after subsequent UDCA withdrawal in conjunction with the as yet uncomplicated gall stone disease in the presented patient is confirmative of true OC in its strictest definition. However, as chronic liver disease in the setting of ABCB4 sequence variation representing a spectrum from LPAC syndrome to biliary cirrhosis may occur at a later date, it is mandatory to follow up the patient [8]. However, most of the patients reported so far with co-inheritance of multiple OC risk alleles including ABCB4 mutations exhibited clinical signs of chronic ABCB4 deficiency at the time of OC diagnosis, which does not apply to our patient, suggesting a more subtle effect of the R590Q variant on ABCB4 biological function outside pregnancy. With this perspective, the detection of uncomplicated cholelithiasis, not meeting the diagnostic criteria for LPAC syndrome, may represent a mere coincidence in a pregnant female with an ABCG8 19H carrier status. Conversely, there are no experimental data available up to now to support a pathogenic involvement of the ABCG8 D19G variant in OC, although, given the multitude of complex interaction among the different components of the hepatobiliary transport system, it may be suggested that there might be a potential for modulation of cholestatic phenotypes. Notwithstanding, preliminary data from a small-sized, European-based association study do not indicate an overrepresentation of the ABCG8 risk allele in individuals with advanced OC as indicated by bile acid concentrations in excess of 40 μM/l (unpublished data).

Taken together, considering the unusual extent of OC-related hepatocellular dysfunction, representing one of the most profoundly raised ALT activities reported so far in OC, we conclude that the cholestatic phenotype might have been mediated by combined genetic variations in ABCB4 and ABCB11 synergistically impacting on hepatobiliary transport capacities. To what extent, if any, the acquisition of the heterozygous ABCG8 19H variant may have contributed to the advance clinical phenotype remains yet to be determined. Increased recognition of combined genetic variation in functionally related susceptibility and/or modifier loci is expected to provide further insights into the complex genetic etiology of OC and related cholestatic liver diseases, and may, in the future, guide targeted medical intervention and follow-up strategies.

References
Severe hepatocellular dysfunction in obstetric cholestasis related to combined genetic variation in hepatobiliary transporters


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The effects of benzoylecgonine, oxytocin, ritodrine and atosiban on the contractility of myometrium.

An experimental study

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Summary

Purpose: To investigate the response of pregnant and non pregnant rat myometrium to benzoylecgonine, a cocaine metabolite, and oxytocin and to investigate the efficiency of ritodrine and atosiban to overcome the effects of benzoylecgonine and oxytocin. Methods: Isolation of rat myometrial tissue and recording of contractile activity with isotonic muscle transducer. Results: Benzoylecgonine and oxytocin increase myometrial contractility, while atosiban and ritodrine induce myometrial relaxation. Atosiban was able to revoke the action of oxytocin but not the action of benzoylecgonine. Ritodrine was able to induce muscle relaxation in both oxytocin and benzoylecgonine administration. Conclusion: Cocaine metabolites seem to act on the myometrium through different pathways compared with oxytocin. After comparing two widely used tocolytic agents: atosiban and ritodrine, it is indicated that only ritodrine, a β2 adrenergic receptor agonist, can inhibit the action of cocaine metabolites. This finding indicates that the actions of cocaine on adrenergic mechanisms are responsible to a large part for its effects on myometrium contractility. The use of β2 adrenergic receptor agonists seems to be preferable for the treatment of myometrial contractions induced by cocaine consumption.

Key words: Benzoylecgonine; Oxytocin; Ritodrine; Atosiban; Myometrial contractions.

Introduction

Myometrium is a smooth muscle tissue and its function is controlled by numerous receptors responding to hormonal, chemical or mechanical stimuli [1]. Myometrial contractions play a very important role during pregnancy. In the cases of non terminal gestations, myometrial contractions are undesirable and dangerous for the outcome of pregnancy [2]. Oxytocin and cocaine are both known as factors causing myometrium contraction [3-6]. Atosiban and ritodrine are well known tocolytic agents, causing myometrial relaxation [7, 8].

Cocaine is an alkaloid ester with molecular formula C₁₇H₂₁NO₄ and MW = 303.4. It is extracted from the leaves of the plant Coca (Coca novogranatense). For therapeutic purposes, cocaine is used as local anesthetic in ophthalmologic, ear, nose and throat surgeries [9]. Cocaine is also used as a drug of abuse with amphetamine-like effects [10]. Cocaine consumption as a drug of abuse is a continuously growing global social and economical problem and highly related to health problems of the users [11-13]. The action of cocaine on the myometrium has been shown to increase contractility both in vivo and in vitro [6, 14]. The mechanism of action involves the rise of norepinephrine levels, the rise of intracellular Ca++ levels and the increase of production and concentration of prostaglandin PGF and PGE on the myometrium. Cocaine consumption also causes decline of prosta-cyclin production, decrease and alternation of β-receptors as well as of dopaminergic receptors: D₁ and D₂ [15, 16]. During pregnancy cocaine consumption has been shown to be associated with high risk of spontaneous abortion during first trimester, premature labor and even uterine collapse, premature collapse of embryonic membranes, retarded endometrial development, high possibility of placenta detachments and underweight newborn [14, 17].

Oxytocin is a peptide hormone with molecular formula C₁₉H₃₃N₄O₄S₂ and MW = 1007.19. Oxytocin is produced by neurons of the anterior hypothalamus and is secreted in the anterior pituitary gland. Oxytocin secretion follows a circadian rhythm with the peak secretion during night hours [18-20]. Labor, breastfeeding, situations of anxiety and emotional pressure are all stimuli for the secretion of oxytocin. On its target tissue, oxytocin acts as a muscle contraction agent [19, 21-23]. Oxytocin is also used as a therapeutic agent in cases when induction of myometrial contractions is needed. The oxytocin-receptor coupling initiates a G-protein mediated response, including phospholipase C [2, 24-26]. The increase of inositol triphosphate (IP₃) induces the rise of intracellular and extracellu lar Ca++, which in turn activates calmodulin. Calmodulin phosphorylates the light-chain myosin kinase and results in myometrium constriction [27-29].

Ritodrine is a member of β-agonists with a well established use as a tocolytic agent in preterm labor. Ritodrine has MW: 323,820 and half-life of 6-9 min and total inac-
The effects of benzoylecgonine, oxytocin, ritodrine and atosiban on the contractility of myometrium. An experimental study

The isolated uterine horn of each animal was suspended in the waterbath of the muscle transducer within 10 min of its extraction. The tissue was left on suspension in the waterbath for 20-30 min in order to fully resume handling and to obtain uniform mobility. The waterbath contained 100 ml of Kreb’s medium and was continually supplied with O₂. The agents to be tested were suspended directly in the waterbath. We recorded the changes in the tissue contraction over the first 10 min after the administration of each agent.

To record the myometrial contractions we used an isotonic muscle transducer (Harvard Medical Apparatus, Inc Holeston, MA, USA) which transformed the mechanical movement of the muscle into electric impulse signals and then into graphic charts. The apparatus also included a transducer signal amplifier (Harvard Medical Apparatus, Inc Holeston, MA, USA), a device used to control the strength of electric potential (Attenuator, SanEi/M-1103, Harvard Medical Apparatus, Inc Holeston, MA, USA) and a recording device (Pen Recorder, Kipp&Zonen, Delft, Netherlands). The recording of graphic charts was made on constant recording speed (5 mm/min) and sensitivity (Gain 1 mV). The rate and intensity of myometrial contractions after administration of each specific pharmaceutical agent were evaluated with graphic charts on a procedure similar to electrocardiotocography and the Montevideo recording system.

The rate of contractions was estimated as the mean value of the number of contractions recorded over a time period of 10 min. The intensity of contractions was evaluated by estimating the mean value of the height of contractions that took place the first 10 min after administration of each agent.

Experimental groups

We divided the animals in seven groups, each containing six pregnant and six non-pregnant animals, as follows:

Group 1: to observe changes caused by benzoylecgonine, a cocaine metabolite (Abbott Laboratories, IL, USA), after administration of the first dose of 0.003 ng/ml and the second dose (0.003 ng/ml) after 10 min.

Group 2: to observe changes caused by administration of the first dose of atosiban (Tractocile, Ferring Pharmaceuticals) (0.0038 ng/ml), followed by a second dose 10 min later.

Group 3: to test after one dose of benzoylecgonine (0.003 ng/ml) followed by one dose of ritodrine (Yutopar, Solvay Pharma) at a dose of 0.001 ng/ml, after 10 min.

Group 4: to test benzoylecgonine (0.003 ng/ml) followed by the first dose of atosiban (0.0038 ng/ml) and a second dose of atosiban (0.0038 ng/ml) 10 min after the first.

Group 5: to test oxytocin (Oxytocin, G.A. Pharmaceuticals S.A., Greece) at a dose of 0.50 IU/ml, followed by one dose of ritodrine (0.001 ng/ml) 10 min later.

Group 6: to test oxytocin (0.5 IU/ml) followed by the first dose of atosiban (0.0038 ng/ml) after 10 min and the second dose of atosiban (0.0038 ng/ml) 10 min after the first dose.

Group 7: to test benzoylecgonine (one dose of 0.003 ng/ml) followed by oxytocin (one dose of 0.50 IU/ml) followed by atosiban (first dose of 0.0038 ng/ml and 10 min later a second dose of 0.0038 ng/ml) followed by ritodrine (one dose of 0.001 ng/ml).

Statistical analysis

The normality of studied parameters was evaluated with the Shapiro-Wilks test. The comparisons of the rate and strength of myometrial contractions between animals were made with the Student’s t-test. Analysis of variance for repeated measurements was used to evaluate myometrial contractions after successive administration of agents. The two-tailed significant level was set at p < 0.05.
The effects of benzoylecgonine on myometrial contractions in pregnant and non-pregnant rats.

Table 1. 

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Initial value</th>
<th>1st dose of benzoylecgonine</th>
<th>2nd dose of benzoylecgonine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant (n=6)</td>
<td>0.58 ± 0.08^a</td>
<td>0.75 ± 0.10^a^c</td>
<td>0.77 ± 0.08^a^c</td>
</tr>
<tr>
<td>Pregnant (n=6)</td>
<td>0.78 ± 0.08^a</td>
<td>1.43 ± 0.18^a^c</td>
<td>1.63 ± 0.05^a^c</td>
</tr>
<tr>
<td>Total (n=12)</td>
<td>0.68 ± 0.13^a</td>
<td>1.09 ± 0.38^a^c</td>
<td>1.20 ± 0.46^a^c</td>
</tr>
</tbody>
</table>

Intensity of myometrial contractions (mean height of contractions in cm ± SD) before and after administration of the 1st (0.0030 ng/ml) and 2nd (0.0030 ng/ml) dose of benzoylecgonine with 10 min time intervals, in pregnant and non-pregnant groups. *: p < 0.05 versus initial value, #: p < 0.05, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 2. 

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Initial value</th>
<th>1st dose of atosiban</th>
<th>2nd dose of atosiban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant (n=6)</td>
<td>1.22 ± 0.19^a</td>
<td>4.28 ± 0.21^a^c</td>
<td>4.79 ± 0.27^a^c</td>
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<tr>
<td>Pregnant (n=6)</td>
<td>1.84 ± 0.12^a</td>
<td>5.25 ± 0.87^a^c</td>
<td>5.73 ± 0.74^a^c</td>
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<tr>
<td>Total (n=12)</td>
<td>1.53 ± 0.36^a</td>
<td>4.77 ± 0.79^a^c</td>
<td>5.26 ± 0.73^a^c</td>
</tr>
</tbody>
</table>

Intensity of myometrial contractions (mean height of contractions in cm ± SD) before and after administration of the 1st (0.0003 ng/ml) and 2nd (0.0003 ng/ml) dose of atosiban, with 10 min time intervals, in pregnant and non-pregnant groups. *: p < 0.05 versus initial value, #: p < 0.05, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Results

Benzoylecgonine

After the administration of the first dose of benzoylecgonine (0.0030 ng/ml) the rate of myometrial contractions increased significantly (compared to the initial value) in both pregnant (p < 0.001) and non-pregnant (p = 0.032) experimental groups (Table 1). After the second dose of benzoylecgonine (0.0030 ng/ml) the rate of myometrial contraction was slightly but not significantly increased in the pregnant group (p = 0.098). In the non-pregnant group the rate of contractions was similar before and after the administration of the second dose of benzoylecgonine.

The intensity of myometrial contractions was increased in both pregnant (p < 0.001) and non-pregnant (p < 0.001) experimental groups after the administration of the first dose of benzoylecgonine (0.0030 ng/ml) (Table 2). A further increase in the intensity of contractions was observed after the administration of the second dose of benzoylecgonine (0.003 ng/ml) in both pregnant (p = 0.007) and non-pregnant (p < 0.001) experimental groups.

Atosiban

The administration of the first dose of atosiban induced a decrease in the rate of myometrial contractions in both the pregnant (p = 0.001) and non-pregnant (p = 0.002) group. The second dose of atosiban induced a further decrease in the rate of contractions (p = 0.009 for pregnant, p = 0.018 for non-pregnant) (Table 3).

The intensity of contractions was significantly decreased after the first dose of atosiban in both pregnant (p = 0.001) and non-pregnant groups (p = 0.014), and further decreased by the administration of the second dose of atosiban (p = 0.039 for pregnant and p = 0.039 for non-pregnant). The intensity of contraction after the second dose of atosiban was lower compared to the initial values (p = 0.002 for pregnant, p < 0.001 for non-pregnant). The decrease of the intensity of contractions was greater in the pregnant compared to the non-pregnant group (Table 4).

Benzoylecgonine and ritodrine

The response of myometrium in terms of rate and intensity of contractions, to the administration of benzoylecgonine first and then to ritodrine is shown in Table 5. The rate of contractions significantly increased after benzoylecgonine administration in the pregnant (p < 0.001) and non-pregnant (p = 0.038) group. Administration of ritodrine significantly reduced the number of myometrial contractions by 80% in pregnant (p < 0.001) and by 81.8% in non-pregnant (p < 0.001) rats.
The effects of benzoylecgonine, oxytocin, ritodrine and atosiban on the contractility of myometrium. An experimental study

Table 6.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean height of contractions in cm</th>
<th>Benzoylecgonine</th>
<th>Oxytocin</th>
<th>Ritodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>Initial value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>1.33 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.14 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>2.05 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.28 ± 0.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total n = 12</td>
<td>1.69 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.71 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.24 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of benzoylecgonine (0.003 ng/ml) followed by ritodrine (0.0005 ng/ml). The time intervals between the administrations of each agent were 10 min. *: p < 0.05 versus initial value, #: p < 0.01 versus non-pregnant group, Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 7.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean number of contractions per min</th>
<th>Benzoylecgonine</th>
<th>Oxytocin</th>
<th>Ritodrine</th>
</tr>
</thead>
<tbody>
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<td>Non-pregnant</td>
<td>Initial value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>0.63 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>0.80 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.38 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.32 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total n = 12</td>
<td>0.72 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03 ± 0.38</td>
<td>1.04 ± 0.41</td>
</tr>
</tbody>
</table>

Rate of myometrial contractions after the administration of one dose of benzoylecgonine (0.003 ng/ml), the 1<sup>st</sup> dose of atosiban (0.0008 ng/ml) and the 2<sup>nd</sup> dose of atosiban (0.0038 ng/ml). The time intervals between the administrations of each agent were 10 min. *: p < 0.05 versus initial value, #: p < 0.01, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 8.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean number of contractions (cm / 10 min)</th>
<th>Benzoylecgonine</th>
<th>Oxytocin</th>
<th>Ritodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>Initial value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>1.34 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.40 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.38 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.42 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>1.99 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.08 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.95 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.90 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total n = 12</td>
<td>1.67 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.74 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66 ± 0.43</td>
<td>4.66 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Changes of the intensity of myometrial contractions (mean height of contraction in cm) after the administration of oxytocin (0.50 IU/ml) followed by atosiban (0.0005 ng/ml). The time intervals between the administrations of each agent were 10 min. *: p < 0.05 versus initial value, #: p < 0.01, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 9.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean number of contractions per min</th>
<th>Oxytocin</th>
<th>Ritodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>Initial value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>0.63 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.27 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>1.13 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.65 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total n = 12</td>
<td>0.88 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rate of myometrial contractions (mean number of contractions/10 min ± SD) after the administration of atosiban (0.0016 ng/ml). The time intervals between the administrations of each agent were 10 min. *: p < 0.05 versus initial value, #: p < 0.01, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Benzoylcgonine and atosiban

The effects of benzoylecgonine and atosiban administration on the pregnant and non-pregnant animals are shown in Table 7. Benzoylcgonine administration resulted in a significant increase on the rate of myometrial contractions in both pregnant (p = 0.03) and non-pregnant (p = 0.007) animals. The administration of the first and second dose of atosiban did not affect the rate of contractions at a significant level. The effect of benzoylcgonine was significantly higher in the pregnant compared with non-pregnant group (0.58 ± 0.30 vs 0.15 ± 0.05, p = 0.016). On the contrary, the effect of atosiban did not differ in the two groups.

The intensity of myometrial contractions after the administration of benzoylcgonine and atosiban are shown in Table 8. The administration of benzoylcgonine caused an increase on the intensity of myometrial contractions in the pregnant (p < 0.001) and non-pregnant (p < 0.001) group while the administration of the first and second dose of atosiban did not cause significant changes on the intensity of contractions. The changes on the intensity of myometrial contractions caused by benzoylcgonine and atosiban did not differ between the pregnant and non-pregnant group.

Oxytocin and ritodrine

The response of myometrium of pregnant and non-pregnant groups to the administration of oxytocin and subsequently to ritodrine is presented in Table 9.

After oxytocin administration the rate of myometrial contractions increased in both the pregnant (p = 0.004) and non-pregnant (p = 0.001) group. After the administration of ritodrine, the number of contractions was reduced to levels lower than the initial (those before administration of oxytocin) in both pregnant (p < 0.001) and non-pregnant (p < 0.001) groups. Furthermore, the effect of oxytocin and ritodrine on the rate of myometrial contractions was greater in the pregnant compared to non-pregnant group (-1.37 ± 0.30 vs -1.03 ± 0.14, p = 0.003).

The intensity of myometrial contractions increased after administration of oxytocin in the pregnant (p = 0.008) and non-pregnant (p = 0.001) group (Table 10). After administration of ritodrine the intensity of myometrial contractions was decreased in both pregnant (p = 0.002) and non-pregnant (p < 0.001) groups. There were no significant differences in the strength of the effects of oxytocin and ritodrine between the pregnant and non-pregnant group.

Oxytocin and atosiban

The administration of oxytocin caused a significant increase in the rate of myometrial contractions in pregnant (p < 0.001) and non-pregnant (p < 0.001) groups, as shown in Table 11. The rate of myometrial contractions decreased after the administration of the first dose of atosiban in both pregnant (p < 0.001) and non-pregnant (p < 0.001) experimental groups. The administration of
Oxytocin, benzoylecgonine, atosiban and ritodrine

Administration of benzoylecgonine (0.003 ng/ml) caused a significant rise in the rate of myometrial contractions in both pregnant (p < 0.001) and non-pregnant (p < 0.001) groups. The following oxytocin administration (0.5 IU/dose) further increased the rate of myometrial contractions (p = 0.012 for pregnant, p = 0.001 for non-pregnant). When the first dose of atosiban (0.0038 ng/ml) was administrated, the rate of contractions was significantly decreased (p = 0.009 for pregnant and p = 0.01 for non-pregnant). After the second dose of atosiban (0.0038 ng/ml) a decrease in the rate of contractions was observed but not at a significant level. Noticeably, the rate of contractions reached similar levels to those after administration of benzoylecgonine. After the following administration of ritodrine (0.001 ng/ml), the rate of contractions was significantly decreased to levels lower than the initial, in both pregnant (p = 0.001) and non-pregnant (p < 0.001) groups. The effect of ritodrine was more intense in the pregnant compared to non-pregnant group (-1.25 ± 0.14 vs -0.93 ± 0.14, p = 0.003) (Table 13).

The intensity of myometrial contractions was increased after the administration of benzoylecgonine (p < 0.001) and non-pregnant (p = 0.002) experimental animals. Administration of the first dose (0.0015 ng/ml) of atosiban induced a decrease in the intensity of myometrium in the pregnant (p < 0.001) and non-pregnant (p = 0.004) group. The administration of the second dose of atosiban (0.0015 ng/ml) induced further decrease on the intensity of myometrial contractions on pregnant (p = 0.039) and non-pregnant (0.012) groups. As observed in the rate of myometrial contractions, the effects of oxytocin and atosiban on the intensity of myometrial contractions was significantly stronger in the pregnant compared to non-pregnant group (p < 0.001).

Table 10.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Height of contractions (cm)</th>
<th>Oxytocin</th>
<th>1st dose of atosiban</th>
<th>2nd dose of atosiban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant n = 6</td>
<td>2.33 ± 0.54</td>
<td>0.76 ± 0.90</td>
<td>1.30 ± 0.36 ±</td>
<td></td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>2.85 ± 0.40</td>
<td>7.94 ± 1.98 ±</td>
<td>1.53 ± 0.26 ±</td>
<td></td>
</tr>
<tr>
<td>Total n = 12</td>
<td>2.59 ± 0.53</td>
<td>7.50 ± 1.54 ±</td>
<td>1.42 ± 0.32 ±</td>
<td></td>
</tr>
</tbody>
</table>

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml) followed by one dose of ritodrine (0.0005 ng/ml), in pregnant and non-pregnant groups. *: p < 0.05 versus initial value, #: p < 0.05, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 11.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean number of contractions per min</th>
<th>Initial value</th>
<th>Oxytocin</th>
<th>1st dose of atosiban</th>
<th>2nd dose of atosiban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant n = 6</td>
<td>0.62 ± 0.08 ±</td>
<td>1.22 ± 0.16 ±</td>
<td>0.22 ± 0.10 ±</td>
<td>0.05 ± 0.05 ±</td>
<td></td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>6.08 ± 0.19 ±</td>
<td>2.74 ± 0.19 ±</td>
<td>0.33 ± 0.10 ±</td>
<td>0.22 ± 0.04 ±</td>
<td></td>
</tr>
<tr>
<td>Total n = 12</td>
<td>0.75 ± 0.20 ±</td>
<td>1.98 ± 0.17 ±</td>
<td>0.28 ± 0.11 ±</td>
<td>0.13 ± 0.10 ±</td>
<td></td>
</tr>
</tbody>
</table>

Rate of myometrial contractions (mean number of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml), the 1st dose of atosiban (0.0015 ng/ml) and the 2nd dose of atosiban (0.0015 ng/ml). *: p < 0.05 versus initial value, #: p < 0.05, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 12.

<table>
<thead>
<tr>
<th>Experimental animals</th>
<th>Mean height of contractions</th>
<th>Initial value</th>
<th>Oxytocin</th>
<th>1st dose of atosiban</th>
<th>2nd dose of atosiban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant n = 6</td>
<td>1.89 ± 0.13 ±</td>
<td>4.51 ± 0.79 ±</td>
<td>2.23 ± 0.24 ±</td>
<td>0.50 ± 0.55 ±</td>
<td></td>
</tr>
<tr>
<td>Pregnant n = 6</td>
<td>6.26 ± 0.31 ±</td>
<td>8.80 ± 1.84 ±</td>
<td>3.52 ± 1.16 ±</td>
<td>1.67 ± 0.75 ±</td>
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</tr>
<tr>
<td>Total n = 12</td>
<td>2.94 ± 0.30 ±</td>
<td>6.65 ± 2.62 ±</td>
<td>2.87 ± 1.05 ±</td>
<td>1.08 ± 0.87 ±</td>
<td></td>
</tr>
</tbody>
</table>

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml), the 1st dose of atosiban (0.0015 ng/ml) and the 2nd dose of atosiban (0.0015 ng/ml). *: p < 0.05 versus initial value, #: p < 0.05, non-pregnant versus pregnant. Significant differences (p < 0.05) within each group (non-pregnant or pregnant) over time are shown by letters.
of the first dose of atosiban the intensity of contractions was reduced in both pregnant \( (p = 0.01) \) and non-pregnant rats \( (p < 0.001) \). After the second dose of atosiban the reduction of the intensity of contractions was not significant but it reached levels similar to those after the administration of benzoylecgonine. The administration of ritodrine induced a significant decrease in the intensity of contractions, to levels lower that the initial (before the administration of oxytocin) \( (p < 0.001 \) for pregnant, \( p < 0.001 \) for non-pregnant). The effect of ritodrine was shown to be stronger in the pregnant compared to non-pregnant animals \(-3.76 \pm 0.58 \text{ vs } -3.29 \pm 0.16, p = 0.096\) (Table 14).

Discussion

As shown in our results, the administration of benzoylecgonine, a cocaine metabolite and oxytocin increases myometrial contractility, while the administration of atosiban and ritodrine induce myometrial relaxation. In particular, atosiban was shown to be able to revoke the action of oxytocin but not the action of benzoylecgonine. Ritodrine was shown to be able to induce muscle relaxation in both oxytocin and benzoylecgonine administration.

The effects of oxytocin on myometrial contractility are well known [40-50]. In our experiments the administration of oxytocin caused an increase of the rate and the strength of myometrial contractions, whereas the subsequent administration of benzoylecgonine further increased those parameters. This additional increase of contractility is attributed to the different mechanisms of actions of oxytocin and benzoylecgonine.

Previous studies have shown that cocaine augments myometrial contractility by both adrenergic and non-adrenergic mechanisms [51]. Regarding the adrenergic receptors, it is possible that cocaine acts on a post-receptor level [52]. On the other hand, ritodrine is a \( \beta-2 \) adrenergic receptor agonist. Our results showing that ritodrine is a potent inhibitor of the action of cocaine metabolites on myometrium confirm the opinion that the effects of cocaine on adrenergic mechanisms are responsible to a large part for cocaine actions on myometrium.

In conclusion, cocaine metabolites seem to act on the myometrium through different pathways compared with oxytocin. After comparing two widely used tocolytic agents: atosiban and ritodrine, it is indicated that only ritodrine, a \( \beta-2 \) adrenergic receptor agonist, can inhibit the action of cocaine metabolites on myometrial tissue. This finding indicates that the actions of cocaine on adrenergic mechanisms are responsible to a large part for its effects on myometrium contractility. The use of \( \beta-2 \) adrenergic receptor agonists seems to be preferable for the treatment of myometrial contractions induced by cocaine consumption in non-pregnant \( (p < 0.001) \) rats.

References


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Regulation of interleukin-1α and tumor necrosis factor-α-induced interleukin-8 production by amnion-derived (WISH) cells

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Summary

Purpose of investigation: It has been reported that interleukin (IL)-8 is produced in the amnion and that its production is enhanced by the initiation of labor. The purpose of this study was to clarify the mechanism of IL-8 production by amnion-derived (WISH) cells. Methods: Cells were cultured and treated with various concentrations of interleukin (IL)-1α, IL-1 receptor antagonist (ra), tumor necrosis factor (TNF)-α, C2-, C6-ceramide, mitogen-activated protein (MAP) kinase kinase (MEK) inhibitor (U0126) and pyridinyl imidazole (p38 MAP kinase inhibitor, SB203580). IL-8 in the culture medium was measured by ELISA. Results: The production of IL-8 was significantly increased by IL-1α or TNF-α, and the increase of IL-8 stimulated by IL-1α was suppressed by IL-1 ra in a dose-dependent manner. The increase in IL-8 production by IL-1α or TNF-α was further enhanced by simultaneous addition of C2-ceramide. The increase of IL-8 stimulated by IL-1α or TNF-α was also suppressed by treatment with U0126 or SB203580. The results of this study demonstrate that the production of IL-8 induced by IL-1α and TNF-α is enhanced by C2-ceramide, and suppressed by MEK inhibitor or P38 MAP kinase inhibitor. Conclusion: The results suggest that ceramide-mediated accumulation and MAP kinase-mediated suppression of inflammatory events in the amnion may play an important role in the maintenance of pregnancy and initiation of labor.

Key words: Interleukin-8, MAP kinase; Amnion; Parturition.

Introduction

It has been hypothesized that inflammatory mediators cause the pathophysiological symptoms associated with infection-induced preterm labor. Neutrophils, which are the predominant type of white blood cell, are found in amniotic fluid, chorionic membranes, and placenta affected with chorioamnionitis caused by intraamniotic fluid bacterial infection. However, the detailed mechanism of neutrophil attraction to fetal membrane tissues and amniotic fluid is unclear.

Interleukin (IL)-8, an eight-kilodalton glycoprotein, has been reported to be a chemotactic and activating factor for neutrophils [1]. IL-8 concentration in amniotic fluid has been found to be increased gradually in the third trimester of pregnancy [2]. It has been reported that cultured amnion, chorion, and decidua cells produce IL-8 constitutively and in response to other cytokines [3, 4]. Cherouny et al. demonstrated that spontaneous labor is associated with an increase in the amount of IL-8 in amniotic fluid in women with and without chorioamnionitis [5]. We recently reported that IL-8 concentrations in human cervicovaginal fluid are increased exponentially during the second and third trimesters [6].

Ceramide is the most important of the mediators derived from the sphingolipids produced by the enzymatic breakdown of sphingomyelin by either acidic or neutral sphingomyelins [7]. Studies have shown that ceramide concentrations are elevated in the amniotic fluid of women who suffer from chorioamnionitis and experience preterm labor [8]. It has been demonstrated that mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) – both of which belong to the family of serine/threonin protein kinases – are widely distributed in lower eukaryotes and mammalian cells [9]. It has also been reported that activation of MAP kinase isosforms is caused by a cascade consisting of sequential activation of Ras, Raf-1 [MAP kinase kinase kinase (MEKK)], and MAP kinase or ERK kinase (MEK) [10-12]. In these studies, MAP kinase was activated by cytokines and growth factors, and it was shown to be involved in the proliferation and differentiation of cells through the stimulation of gene expression. Recent studies have shown that p38 mitogen-activated protein (MAP) kinase, which belongs to the MAP kinase superfamily [13], is involved in cytokine expression [14-18]. p38 MAP kinase also plays a role in gene regulation of cytokines.

The purpose of this study was to investigate the effects of ceramide on the production of IL-8 in the enhancement of IL-1α or TNF-α by human amnion-derived cells (WISH cells). In addition, we also examined whether IL-1α - and TNF-α-induced IL-8 production was regulated by MAP kinase in these cells.

Materials and Methods

Reagents

Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Nissui (Tokyo, Japan) and fetal calf serum (FCS) from HyClone (Logan, UT, USA). IL-1α, IL-1 ra and TNF-α...
were purchased from R&D Systems (Minneapolis, MN). N-acetylsphingosine (C2-ceramide) and fumonisin B1 (ceramidase inhibitor) were obtained from Biomol (Plymouth Meeting, PA). U0126 (MEK inhibitor) and pyridinyl imidazole (SB203580) were obtained from Calbiochem (La Jolla, CA).

Cell culturing

The human immortalized amnion epithelial cell line (WISH cells) was maintained in the laboratory and cultured in DMEM supplemented with 10% heat-inactivated FCS, penicillin (100 IU/ml) (Gibco-BRL, Gaithersburg, NY) and streptomycin (100 mg/ml) (Gibco-BRL). WISH cells were grown to confluence in 12-well culture plates (Corning, New York, NY) and in 100 mm diameter wells (Nalge Nunk Int., Naperville, IL) with three replicates per condition. Cells were grown for seven days before each experiment. For the experiments, WISH cells were washed twice with phosphate-buffered saline (PBS) without calcium and magnesium, and then serum-free DMEM was added. Control cells received an equivalent volume of medium alone during incubation. Cultures were incubated at 37°C in air with 5% CO₂.

Stimulation by cytokines, other reagents and measurement of IL-8

To investigate the production of IL-8 by WISH cells, 2 × 10⁵ cells were plated on 12-well culture plates (Corning) in 1 ml of culture medium with 10% FCS and cultured until they were fully confluent. The supernatant was replaced with fresh culture medium containing various concentrations of IL-1α, TNF-α, C2-ceramide, fumonis B1, U0126 and SB203580 for the desired length of time. The culture medium and cells were removed at specific intervals to establish the time course of IL-8 production. At the end of the culture period, the medium was stored at -80°C until assayed. The vitality of cells was tested and not changed. Experiments were performed in triplicate and were repeated three times. The levels of IL-8 in the supernatant were determined by means of a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems). The sensitivity of the assay for IL-8 was 10 pg/ml. The inter- and intra-assay coefficient of variance for the ELISA were 9.6% and 5.2%, respectively. Experiments were performed in triplicate and were repeated three times.

Statistical analysis

Data are presented as the mean ± SD. Statistical evaluations were performed using the Student’s t-test and Bonferroni/Dunn test with StatView 4.5 software (Abacus Concepts, Berkeley, CA). A level of $p < 0.05$ was accepted as statistically significant.

Results

IL-8 production following treatment with IL-1α, IL-1 ra and TNF-α

The concentration of IL-8 in the culture media without WISH cells was below the level of detection. Small amounts of IL-8 were detected in the supernatant of non-stimulated WISH cells after 24 hrs of incubation (Figure 1). When WISH cells were treated with various concen-
and U0126, we observed an increase in IL-8 production following IL-1α and TNF-α, C2-ceramide and fumonisin B1. WISH cells were treated with human IL-1α (1 nM), TNF-α (1 nM), C2-ceramide (10 µM) and fumonisin B1 (10 M). Data are expressed as the mean ± SD of triplicate samples from four separate representative experiments.

**Production of IL-8 following stimulation by IL-1α, TNF-α, C2-ceramide and Fumonisin B1**

When the WISH cells were treated with IL-1α and C2-ceramide, IL-8 production was greater than that by IL-1α treatment alone (Figure 2). IL-8 production in response to incubation with IL-1α and fumonisin B1 was not significantly decreased as compared with that by IL-1α treatment alone.

When the WISH cells were treated with TNF-α and C2-ceramide, IL-8 production was greater than that by TNF-α treatment alone (Figure 2). IL-8 production in response to incubation with TNF-α and fumonisin B1 was significantly decreased as compared with that by TNF-α treatment alone.

When WISH cells were treated with C2-ceramide alone, an accumulation of IL-8 was observed, but it was not significantly greater than that in the controls.

**Inhibition of IL-8 production following treatment with IL-1α, TNF-α, U0126 and SB203580**

When the WISH cells were treated with IL-1α, TNF-α and U0126, we observed an increase in IL-8 production following IL-1α and TNF-α treatment and a suppression of IL-8 production following U0126 treatment (Figure 3A). IL-8 production in response to incubation with IL-1α and U0126 was suppressed as compared with that by IL-1α treatment alone. IL-8 production in response to incubation with TNF-α and U0126 was also suppressed as compared with that by TNF-α treatment alone.

When the WISH cells were treated with IL-1α, TNF-α and SB203580, we observed an increase in IL-8 production following IL-1α and TNF-α treatment and a suppression of IL-8 production following SB203580 treatment (Figure 3B). IL-8 production in response to incubation with IL-1α and SB203580 was suppressed as compared with that by IL-1α treatment alone. IL-8 production in response to incubation with TNF-α and SB203580 was also suppressed as compared with that by TNF-α treatment alone.

**Discussion**

IL-8, a potent chemokine for neutrophils, has been shown to be involved in host response to microbial invasion of the amniotic cavity [5, 19]. Romero et al. [19] reported that the concentrations of this cytokine in amniotic fluid increased during preterm and term parturition. It has been demonstrated that concentrations of IL-8 in amniotic fluid were significantly higher in patients with histologic chorioamnionitis than in those without acute inflammatory lesions of the placenta [5]. In addition, the mean concentration of IL-8 in amniotic fluid obtained from women with positive cultures and in labor was 14-fold higher than that obtained from women at term and in labor [5]. IL-8 induces the activation and migration of cells from vessels into the surrounding uterine cervical tissue and stimulates the release of collagenase [20] and elastase [21] in the tissue, resulting in the digestion of collagen fibers during preterm and term labor.

IL-1 and TNF-α reportedly act by a mechanism that involves the sphingomyelin pathway [7] of signal transduction after binding to their specific receptors on the plasma membrane of the cell to form ligand-receptor complexes. This step initiates a complicated chain of signal transduction events within the cell, including activation of G-proteins and the formation of a variety of second-messenger molecules, including ceramide. This evidence suggests that the signal transduction of inflammatory cytokines within the cells occurs, in part, via ceramide as a second messenger [22].

Hydrolysis of sphingomyelin has been shown to mediate the actions of IL-1β in regard to the induction of prostaglandin H synthase (PGHS)-2 expression [23, 24] and IL-6 production [25] in fibroblasts. Indeed, we previously reported that ceramide enhanced the production of IL-1α-induced PGE2 or PGF2α production in amniotic cells [26] and endometrial stromal cells [24]. The concentration of ceramide in amniotic fluid is strikingly and consistently increased in premature labor in women with chorioamnionitis. In cases of preterm labor without symptomatic chorioamnionitis, the concent-
The regulation of ceramide has been shown to be higher than in cases of other term labor or no labor [8]. These findings suggest that the level of ceramide in the amniotic fluid may serve as an indicator of the imminence of preterm labor. However, the relationship between ceramide and the specific events leading to the onset of preterm labor is unclear. Keelan and Mitchell reported that the TNF-α-induced production of PGE2 was amplified by treatment with C2-ceramide [27]. It is suggested that IL-1 and TNF-α also act by a mechanism that involves the sphingomyelin pathway in amniotic cells. When we investigated the effect of treatment with C2-ceramide in addition to IL-1α or TNF-α, the accumulation of IL-8 was significantly increased. On the other hand, the increase of IL-8 was significantly decreased by treatment with TNF-α and fumonosin B1, but not by IL-1α and fumonisin B1. A differing affinity for the cells or in the effective concentrations of IL-1α or TNF-α may, perhaps, explain these differences. In our data, it is suggested that ceramide may contribute to increase the levels of IL-8 cooperated with IL-1α or TNF-α resulting to cause chorioamnionitis.

IL-1α and TNF-α have also been shown to induce the phosphorylation and activation of p38 MAP kinase in various cells [13, 28-31]. SB203580, a specific inhibitor of p38 MAP kinase activity, has been used to investigate the signal transduction pathway regulating IL-8 production in TNF-α-, and IL-1α-stimulated human pulmonary vascular endothelial cells [14, 32]. A class of pyridinyl imidazole compounds, which are inhibitors of p38 MAP kinase, block LPS-stimulated IL-1 and TNF-production at the translational level [32]. SB203580, which has recently been shown to be highly specific for kinase [14], completely inhibited p38 MAP kinase activity and IL-8 production in TNF-α- and IL-1α-stimulated human pulmonary vascular endothelial cells. These facts indicate that p38 MAP kinase plays an important role in the TNF-α- and IL-1α-activated signalling pathways which regulate IL-8 expression.

In the present study, treatment with U0126 and SB203580 markedly inhibited IL-1α- and TNF-α-induced IL-8 production. In a study by Song et al., ERK was activated by treatment with IL-1β and TNF-α, and this activation was suppressed by an MEK inhibitor in human airway epithelial cells [33]. Taken together, these results suggest that IL-8 production induced by IL-1α and TNF-α might be regulated by the mechanism involving the ERK pathway or p38 MAP kinase pathway from a point downstream of the IL-1 and TNF-α receptors in amniotic cells.
IL-1 activates a number of protein kinase pathways including those of the three types of MAPK (p42/p44, p54/JNK, and p38), and a β-casein kinase [30, 34-37]. These kinases may cause the activation of transcriptional factor resulting in increased expression of cytokines such as IL-8. In the present study, we judged that the inhibitory effects of SB203580 or U0126 at 0.1 M to 10 M were due to p38 MAPK inhibition. SB203580 inhibits the catalytic activity of p38 MAP kinase by binding to the ATP site and subsequently phosphorylating its substrate [38]. SB203580 might exert an inhibitory effect on the induced p38 MAP kinase activity and subsequent IL-8 production, but not on the basal levels of p38 MAP kinase activity and IL-8 production under our experimental conditions. These results may indicate that SB203580 could be used to control p38 MAP kinase-mediated IL-8 production in inflammatory disease.

In conclusion, the present study demonstrated that IL-8 production induced by TPA is regulated by MAP kinase and subsequent IL-8 production, but not on the basal levels of p38 MAP kinase activity and IL-8 production under our experimental conditions. 

Acknowledgement

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References


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The efficacy of paracetamol versus tenoxicam on postoperative pain and morphine consumption after abdominal hysterectomy: a placebo-controlled, randomized study

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Summary

Objective: The purpose of this study was to determine the analgesic efficacy and side-effects of paracetamol and tenoxicam in comparison with placebo in patients with postoperative pain after elective abdominal hysterectomy. Material and Methods: A total of 120 patients were randomly divided into three groups to receive either paracetamol 1 g, tenoxicam 20 mg or placebo intravenously at the end of surgery, and then morphine was administered by a patient-controlled analgesia device postoperatively. Results: Tenoxicam was associated with lower pain scores at the 2nd, 4th, 6th and 24th hour postoperatively. Total morphine consumption was 44.8 ± 17.4 mg, 64.6 ± 19.6 mg, 69.2 ± 22.1 mg (tenoxicam, paracetamol and placebo group, respectively) and there was a significant difference in the tenoxicam group compared with the other two groups (p < 0.05). Side-effects except for nausea were similar. Conclusion: A single dose of 20 mg tenoxicam provided effective analgesia and reduced total morphine consumption in comparison with paracetamol and placebo after abdominal hysterectomy.

Key words: Paracetamol; Tenoxicam; Morphine; Postoperative pain; Abdominal hysterectomy.

Introduction

Acute postoperative pain is a complex and multifactorial symptom that requires a thoughtful approach using a variety of treatment modalities to obtain an optimal outcome after surgery [1]. Single analgesics, such as opioid, nonsteroidal anti-inflammatory drugs (NSAIDs), are not able to provide effective pain relief without side-effects such as nausea, vomiting, sedation, or bleeding. Therefore, multimodal analgesia involving administration of a combination of opioid and nonopioid analgesics represents a popular approach to preventing postoperative pain [2, 3]. Morphine is often combined with NSAIDs or paracetamol as part of multimodal analgesia after surgery to decrease side-effects, and also to improve the quality of postoperative analgesia [3, 4]. Tenoxicam is a thienothiazide derivative of the oxicam class of NSAIDs, making it extremely suitable for postoperative analgesia [5, 6]. Studies have demonstrated the analgesic efficacy of 20 mg tenoxicam IV after third molar extraction [7], thoracic surgery [8] and cesarean section [9]. However, the use of NSAIDs are limited by contraindications and potential side-effects, such as gastrointestinal and perioperative bleeding, renal impairment, bronchospasm and homeostatic dysfunctions [10]. Paracetamol is a well established analgesic drug for the postoperative period, as an alternative to NSAIDs because it has a better record regarding adverse reactions [11]. Although it is widely used as a basis for pain treatment and after minor surgery [10, 12], some studies indicated that paracetamol might be effective in postoperative pain and opioid consumption after major gynecologic abdominal surgery [4, 13].

There are a lot of studies about multimodal analgesia with NSAIDs and/or paracetamol in the literature; however, no study has compared the analgesic efficacy of intravenous (IV) paracetamol and tenoxicam given intraoperatively, both compared with placebo after abdominal hysterectomy.

We aimed to assess postoperative morphine consumption, pain scores, and side-effects in patients who received 1 g paracetamol or 20 mg tenoxicam IV and to compare the results with the control patients who received placebo.

Materials and Methods

After ethics committee approval and having obtained written informed consent, 120 women, aged 44-65 years old, with the American Society of Anesthesiologist (ASA) physical status of class I or II, scheduled for elective abdominal hysterectomy were included in this randomized double-blind clinical trial. Patients with renal and hematopoietic disease, known gastric ulcer, asthma, hypersensitivity to NSAIDs and paracetamol, hepatic and cardiac dysfunction, bleeding disturbances, a history of drug or alcohol abuse, administration of an NSAID or opioid during 24 hours preceding surgery were excluded from the investigation.

By visiting the patients one day before the operation, related information and training was given about the anesthesia method to be applied, usage of the PCA (patient-controlled analgesia) device (Abbott Laboratories, North Chicago, IL, USA) and a visual analog scale (VAS). All patients were premedicated with 5 mg oral diazepam one night before surgery and two hours preoperatively. On arrival in the operating room, Lactated Ringer’s solution, with a rate of 10 ml/kg/h, was started through an 18-gauge (G) IV cannula and antacid prophylaxis consisted of 50 mg ranitidine and 10 mg metoclopramide IV. The women were then randomly allocated into one of three groups: 1 g of parac-
etamol (Perfalgan, 100 ml, IV, Bristol Myers Squibb, Group P), 20 mg of tenoxicam (Tilcotil, into 100 ml saline, Roche Laboratories, Group T) and placebo (100 ml saline, Group C), according to a computer-generated randomization table. All patients received standard general anesthesia which was induced with propofol 2 to 3 mg/kg, fentanyl 1 to 2 µg/kg and rocuronium 0.6 mg/kg and maintained with desflurane (3-6%) with N2O (66%) in oxygen (33%). The doses of the study drug in all groups were administered, IV infusion, for over 15 min at the end of surgery and then paracetamol 1g (Group P) or placebo (100 ml saline, Group T and Group C) were given every six hours for 24 hours. The study drugs as previously randomized were prepared by an anesthetic nurse who was not otherwise involved in the care of the patient and were administered by the same anesthetist not involved in the study follow-up.

At the end of the operation, the patients were transferred to the recovery room and all were given IV PCA with morphine (0.3 mg/ml morphine and a PCA device programmed for a 0.1 mg/kg loading dose, 0.02 mg/kg bolus with a 15-min lockout interval and no basal infusion). Postoperative pain was assessed using a 10 cm visual analog scale (VAS) with 0: no pain and 10: the worst imaginable pain. The patients were applied an additional 1 mg/kg of tramadol intramuscularly if VAS was ≥ 4.

Side-effects, such as nausea, vomiting, itching, respiratory depression, sedation and stomach irritation were recorded. Nausea and vomiting were treated with 8 mg ondansetron. The sedation levels of the patients were defined in accordance with the Ramsay sedation scale [14]. Postoperatively, all measurements were performed by another blinded and independent anesthetist.

The power analysis was based on a variation of morphine consumption from our pilot data. Sample size was calculated to detect a difference of 20% among groups in which morphine consumption was the lowest and the highest (±: 0.05, and β: 0.8), and 35 patients for each group were determined. Statistical analyses were performed with the SPSS (SPSS for Windows Release 13.0) Statistical Package. The results are presented as mean ± standard deviation (SD), median (range) or n (%) as appropriate. Age, weight, height, duration of anesthesia and surgery, intraoperative fentanyl requirement, morphine consumption, sedation and pain scores among the groups were compared using one-way analysis of variance. Side-effects and additional analgesic requirements were analyzed using chi-square and Fisher’s exact test. All post hoc comparisons were performed using Bonferroni correction. A p value < 0.05 was considered statistically significant.

Results

There were no differences among the groups in the demographic and baseline hemodynamic data, anesthesia and surgical time and intraoperative analgesic (fentanyl) requirement (Table 1).

VAS scores were lower in Group T at the 2nd, 4th, 6th and 24th hour than the other two groups (p < 0.05); there was no statistically significant difference between Group C and P (Figure 1).

Total morphine consumption at the 24th hour was 44.8 ± 17.4 mg, 64.6 ± 19.6 mg and 69.2 ± 22.1 mg, Group T; Group P and Group C, respectively. Additional analgesic requirement was also lower in Group T (Table 2). There was a statistically significant difference between Group T and the other two groups (p < 0.0001) (Figure 2).

Sedation scores were similar among groups except for the 30th min, at this time Group T was lower than Group C (p = 0.02). No patient suffered from oversedation at any time nor had respiratory depression. Although nausea was only statistically significant difference in Group T compared with Group C, vomiting and itching were similar among groups (Table 2).

Discussion

The results of this study demonstrated that 20 mg of tenoxicam provided effective analgesia with less additional analgesic requirements compared to paracetamol and placebo in the first 24 hours after abdominal hysterectomy. The incidence of side-effects was similar among groups except for nausea which was lower in the tenoxicam group than in the placebo group.

Single or multiple doses of intravenous paracetamol (1 g) generally provided significantly better analgesic efficacy than placebo treatment in adult patients who underwent dental, orthopedic or gynecological surgery [15, 16]. On the other hand, in some studies which compared paracetamol with NSAIDs, the analgesic efficacy of paracetamol in the postoperative period was found to be controversial since both effective [13] and ineffective [17, 18] results were reached. Varrassi et al. [13] indicated that propacetamol and ketorolac, combined with patient-controlled analgesia morphine, showed similar analgesic efficacy after gynecologic surgery. However, Munishankar et al. [17] demonstrated that patients given paracetamol were less satisfied than the patients given diclofenac alone and the combination of these after cesarean section. In this study, patients given a combination of diclofenac and paracetamol used 38% less mor-

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Table 1. — Patient, anesthesia, and surgical characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Duration of anesthesia (min)</th>
<th>Fentanyl requirement (µg)</th>
<th>VAS scores</th>
<th>Sedation scores</th>
<th>Nausea (%)</th>
<th>Vomiting (%)</th>
<th>Itching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td>40</td>
<td>48.3 ± 5</td>
<td>71.2 ± 9.6</td>
<td>162.2 ± 4.4</td>
<td>116.8 ± 20.3</td>
<td>72.1 ± 65</td>
<td>37.5 (27)</td>
<td>7.5 (9)</td>
<td>37.5 (15)</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
</tr>
<tr>
<td>Group P</td>
<td>40</td>
<td>47.8 ± 4.9</td>
<td>70.7 ± 9.7</td>
<td>160.7 ± 5.1</td>
<td>119.7 ± 8.3</td>
<td>71.6 ± 57</td>
<td>37.5 (27)</td>
<td>7.5 (9)</td>
<td>37.5 (15)</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
</tr>
<tr>
<td>Group T</td>
<td>40</td>
<td>49.8 ± 6.6</td>
<td>74.2 ± 12.2</td>
<td>163 ± 4.9</td>
<td>122.3 ± 17.3</td>
<td>89.6 ± 61</td>
<td>37.5 (27)</td>
<td>7.5 (9)</td>
<td>37.5 (15)</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
</tr>
</tbody>
</table>

p = 0.05 Group C versus Group T, Group T: tenoxicam group, Group P: paracetamol group, Group C: placebo group.

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Table 2. — Analgesic requirement and side-effects.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Fentanyl requirement (µg)</th>
<th>Nausea (%)</th>
<th>Vomiting (%)</th>
<th>Itching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td>40</td>
<td>72.1 ± 65</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
<td>7.5 (9)</td>
</tr>
<tr>
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<td>40</td>
<td>71.6 ± 57</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
<td>7.5 (9)</td>
</tr>
<tr>
<td>Group T</td>
<td>40</td>
<td>89.6 ± 61</td>
<td>37.5 (15)</td>
<td>22.5 (9)</td>
<td>7.5 (9)</td>
</tr>
</tbody>
</table>

Data are mean ± SD. SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, HR: heart rate, Group T: tenoxicam group, Group P: paracetamol group, Group C: placebo group.

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Data are mean ± SD, n, number of patient, *p < 0.05 Group T versus Group C and P. **p < 0.05 Group C versus Group T, Group T: tenoxicam group, Group P: paracetamol group, Group C: placebo group.

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phine compared to patients given paracetamol alone. Recently, a systematic review [19] reported that 24-hour morphine consumption decreased by 6.3 mg to 10.9 mg compared to placebo, when paracetamol, NSAID or COX-2 inhibitors were added to PCA morphine following major surgery. In the study, the authors concluded that NSAIDs seemed to be superior for postoperative pain management although there were differences in the efficacies of paracetamol and NSAIDs depending on the type of surgery performed. A qualitative review also determined that the efficacies of paracetamol and NSAIDs may depend on type of surgery [20]. The analgesic effect of paracetamol may be dependent on the rate and amount of active drug reaching the central nervous system, where its analgesic effect takes place [21]. Piquet et al. [22] indicated that intravenous paracetamol exerted a dose-dependent central antinociceptive effect in healthy subjects. A study by Juhl et al. [23] compared 1 g and 2 g paracetamol in dental surgery and found that the analgesic efficacy of a 2 g starting dose of IV paracetamol was superior over the recommended dose of 1 g in terms of magnitude and duration of analgesic effect for postoperative pain. Sinatra et al. [16] showed that the total morphine doses received over 24 h were 38.3 ± 35.1 mg for paracetamol 1 g and 57.4 ± 52.3 mg for placebo, corresponding decreases of 33% (19 mg) after major orthopedic surgery. However, we found that 24-hour morphine consumption decreased by 4.6 mg compared to placebo when paracetamol was added to PCA morphine in accordance with the literature [19]. Different results in studies may depend on the types of surgeries and different pain scores of the patients [19, 20].

Tenoxicam potentiates an opioid analgesic effect on the somatic and visceral types of pain to different extents [24]. Intravenous tenoxicam administration is preferable both in the immediate postoperative period to avoid any delay in absorption from either intramuscular or enteral routes. The drug is recommended at a once daily dosage of 20 mg [5]. A study showed that intraoperative injection of 20 mg tenoxicam decreased the demand ratio for PCA and 24-hour morphine consumption by approximately 30%. The authors suggested that intraoperative injection of 20 mg tenoxicam was sufficient to enhance intravenous PCA morphine on uterine cramping pain for the first 24 hours after cesarean section [24]. Munro et al. [5] reported that intraoperative intravenous administration of 40 mg tenoxicam during laparoscopic cholecystectomy, when compared with placebo, was associated with a significant reduction in consumption of morphine at six hours and 12 hours (p < 0.05) but not at 24 hours, when assessed by patient-controlled analgesia. There was no difference between the groups in pain scores, either at rest or on movement, nor in the incidence of nausea and vomiting. In contrast, Danou et al. [25] showed that the administration of 20 or 40 mg IV tenoxicam did not reduce fentanyl consumption via PCA compared with placebo after total abdominal hysterectomy. A difference between placebo and 40 mg tenoxicam groups was noted in VAS scores, both at rest and during coughing, at the 4th hour postoperatively but still failed to reach statistical significance. Furthermore, Merry et al. [8] performed a placebo-controlled trial using 20 mg tenoxicam in patients following thoracotomy and there was no significant difference between groups in terms of pain scores or side-effects. However, in other studies by the same authors, 20 mg and 40 mg of tenoxicam were superior to the control group regarding pain score and morphine consumption [26, 27]. There were no significant differences between study groups postoperatively in pain during coughing, opioid consumption, nausea, vomiting or sedation. The authors reported that these data support the inclusion of 20 mg tenoxicam IV in the management of pain, but do not show additional benefits for a higher dose.

Munishankar et al. [17] determined that morphine-related side-effects were similar in paracetamol, diclofenac, or the combination of diclofenac and paracetamol groups. Vandermeulen et al. [6] showed that morphine consumption in a control group was higher than in a 40 mg tenoxicam group at 24 h after hysterectomy. In the study, there were no important differences between placebo and tenoxicam groups in the incidence and severity of adverse events. In a study by Danou et al. [25], the incidence of nausea...
was similar in patients receiving 20 mg tenoxicam IV and 40 mg IV compared with placebo, but mild gastrointestinal symptoms were exhibited in tenoxicam groups. The authors reported that tenoxicam may increase intraoperative bleeding and gastrointestinal side-effects.

In our study, vomiting and itching were similar among groups although nausea was the only difference. Morphine sparing resulted in parallel reductions in opioid-related side-effects such as nausea in the tenoxicam group. Pain score and morphine consumption in placebo and paracetamol groups were higher at 6 h, probably because our patients were mobilized at the time. We believe that tenoxicam provided effective analgesia compared with the other two groups even on movement although VAS scores of the patients were evaluated only at rest.

One of our few limitations was that pain scores of the patients were only recorded at rest, not on movement and when coughing. Moreover, the amount of preoperative bleeding was not assessed but no patient was re-operated for bleeding or hematoma and received blood transfusions postoperatively.

In conclusion, tenoxicam provided reliable analgesia with comparable pain scores and reduced morphine consumption and nausea which is an opioid-related side effect after abdominal hysterectomy. Although paracetamol is claimed to be effective in postoperative pain after major abdominal surgery, we found no difference between paracetamol and placebo groups.

References


Malignant disease as a risk factor for surgical site infection

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Institute of Gynecology and Obstetrics, Medical School, University of Belgrade, Clinical Center of Serbia, Belgrade (Serbia)

Summary
Introduction and objective: Postoperative infections are a great constituent of surgical complications. The most common one is surgical site infection (SSI), as well as vaginal and/or urinary tract infections, infections affecting distant organs and systems and systemic circulation leading to sepsis and septic shock. Our aim was to emphasize the effect of malignant disease on postoperative infection and to establish malignant disease as a risk factor for SSI, per se. Material and Method: We designed a retrospective study in which 538 women who underwent surgery in the Gynecology and Obstetrics Clinical Center of Serbia during a six-month period in 2009 were analyzed. We collected relevant data regarding SSI incidence (CDC definitions), malignant disease (primary site, type and stage) and other potential risk factors for SSI. We used descriptive statistics, chi-square and Student’s t test for comparison of variables with statistical significance at \( p < 0.05 \). We also used univariate, multivariate logistic regression and ROC analysis. Results: Surgical site infection was present in 40 patients (7.5%). Univariate analysis revealed that the following factors were significantly related to SSI: age, malignant disease, stage of malignant disease, surgery longer than 120 min, postmenopause, diabetes mellitus, positive preoperative vaginal culture, ASA score and intraoperative blood loss. Multivariate analysis showed that the most important risk factors that contribute to SSI with RR of 4 and 5 are, respectively, FIGO II and FIGO III/IV stage of malignant disease (FIGO II \( p < 0.05 \) RR= 4.097; FIGO III/IV \( p < 0.01 \) RR = 5.061). Conclusion: In our study malignant disease erupted as the most important risk factor for SSI. This brings us to question the pathophysiological mechanisms and systemic effects associated with malignant disease. There are few studies discussing the issue of malignancy as an isolated risk factor that 4-5 fold increases the risk of SSI. It is of utmost interest to define protocols of antimicrobial prophylaxis for gynecological malignancy surgery as are suggested for some other malignancies.

Key words: Surgical site infection; Malignant disease.

Introduction

Postoperative infections are a great constituent of surgical complications. The most common one is surgical site infection (SSI), as well as vaginal and/or urinary tract infections, infections affecting distant organs and systems and systemic circulation leading to sepsis and septic shock. Postoperative infections result in higher morbidity and mortality, increased financial costs and prolonged hospital stay [1, 2]. In the 1990s, patients who developed SSI had longer and costlier hospitalizations than patients who did not develop such infections. They are twice as likely to die, 60% more likely to spend time in an ICU, and more than five times more likely to be readmitted to the hospital [3].

Our aim was to emphasize the effect of malignant disease on postoperative infection and to establish malignant disease as a risk factor for SSI, per se. In a wider sense, we investigated the incidence and other potential risk factors for SSI and tried to contribute to defining a relationship between different factors, especially malignancy, and SSI after surgical therapy of genital tract disease.

Material and Method

We designed retrospective study in which 538 women who underwent surgery in The Clinic for Gynecology and Obstetrics, Clinical Center of Serbia during six months period in 2009 were analyzed. Laparoscopy was performed in another specialized unit and was not included in the study. We used medical records to collect patient demographics and medical status. For statistical analyses we used descriptive statistics for distribution of variables. Incidence of SSI was compared among groups of patients depending on certain analyzed variables and potential risk factors using inferential statistics: categorical variables were compared using the chi-square test and continuous variables were compared using Student’s t-test; statistical significance was defined as \( p < 0.05 \). Predictors of SSI, overall morbidity, and mortality were analyzed by univariate and multivariate logistic regression with relative risk (RR) and confidence interval (CI) 95%. We also used ROC analysis for evaluation of surgery duration as a potential risk factor, defining cut-off value and calculating sensitivity and specificity.

Results

Average age of this group of 538 women patients was 50 years (SD = 14.01). Indications for surgery were cervical cancer in 9.5%, uterine corporal cancer 4.3%, vulvar cancer 2.6%, ovarian cancer 10.8%, uterine prolapse and other pelvic organ prolapse 12.2%, uterine leiomyoma 24%, benign adnexal tumors 27.7%, premalignant cervical lesions 7.8% and other organ malignancies 0.4%. Pathohistological analysis showed malignant disease FIGO Stage I or border-line in 84 (15.6%) patients, 18 (3.3%) FIGO Stage II, 40 (7.4%) FIGO III and three (0.6%) FIGO IV, while in 347 cases postoperative pathohistology analysis showed benign disease. Premalignant lesions of the cervix and vulva were found in 36 (6.7%), while for ten (1.9%) there were no available data of postoperative pathohistology diagnosis.
Surgical site infection was present in 40 patients (7.5% of total number of women). In abdominal procedures alone (409 cases), the incidence was 6.1%. Microorganisms as causative agents were isolated from 7.1% cultures of wounds. Most frequent was Enterococcus spp. in 2.2% of the total number of wounds. We also isolated methicillin-resistant Staphylococcus aureus in five cases and Acinetobacter (biggest challenge for antibiotic therapy in our hospital) in three patient cultures. Wound dehiscence was present in seven patients, and other complications found were enterocutaneous fistula, anterior abdominal wall hematoma and three patients were diagnosed with pulmonary embolus. Outcomes were such that out of 538 patients 530 were discharged home from hospital, six underwent repeated surgery in a short postoperative period, and two were diagnosed with pulmonary embolisms and transferred to a clinic for pulmonary disease for further treatment.

In the group of patients with malignant disease, 26 (17.8%) had diagnosed SSI compared to 14 (3.6%) in the group of patients with non-malignant disease - which presented a highly statistical significant difference ($x^2 = 30.407$, $p < 0.0001$). Distribution of incidence of SSI in benign and different stages of malignant disease of women’s genital tract is shown in Table 1. Besides the difference between groups of patients with malignant and non-malignant gynecological disease, a highly statistically significant difference was present among groups of patients in different stages of malignant disease, between the group who had benign/premalignant/FIGO I and those with FIGO II, FIGO III/IV ($x^2 = 75.870$, $p < 0.0001$). This was regardless of the organ with malignant alteration.

We also recognized a high statistical significance in distribution of SSI between groups of postmenopausal women and those that were not ($x^2 = 31.226$, $p < 0.0001$). In women who had diagnosed diabetes mellitus postoperative SSI was present in 26.3% and in those without diabetes mellitus in 6.9% which was also highly statistically significant ($x^2 = 9.985$, $p < 0.01$); there was also a statistically significant difference in those whose preoperative vaginal cultures were positive compared to the ones with no isolates ($x^2 = 4.012$, $p < 0.05$). Increasing ASA score showed a statistically significant increased occurrence of SSIs in ASA scores 1 through 5, respectively, four (2.4%), 12 (6.5%), seven (8.3%), ten (18.2%), six (28.6%) ($x^2 = 28.244$, $p < 0.0001$).

The average length of surgery in patients without SSI was 84.87 min, while in patients with SSI it was 133.21 min which was a high statistically significant difference ($t = 6.184$, $p < 0.0001$). Based on ROC analysis (area 0.745, SE = 0.06, $p < 0.0001$), the chosen cut-off value for the length of surgery was 120 min with sensitivity = 61.5%, specificity = 81.6%.

Results of univariate logistical regression analysis – which we used for determination of predictors of incidence of SSI – are shown in Table 2. The most important predictors were calculated using multivariate analysis including determined predictors of incidence of SSI (Table 2) are shown in Table 3.

### Table 1. — Distribution of surgical site infection (SSI) based on the FIGO stage of malignant disease.

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>No SSI</th>
<th>SSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>442</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>III/IV</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>481</td>
<td>39</td>
</tr>
</tbody>
</table>

### Table 2. — Determination of predictors of incidence of SSI.

<table>
<thead>
<tr>
<th>Variables</th>
<th>$p$</th>
<th>RR</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>1.035-1.088</td>
</tr>
<tr>
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<td>2.896-11.321</td>
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<tr>
<td>FIGO Stage II</td>
<td>&lt; 0.0001</td>
<td>9.444</td>
<td>3.093-29.353</td>
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<tr>
<td>FIGO Stage III/IV</td>
<td>&lt; 0.0001</td>
<td>15.111</td>
<td>6.920-32.997</td>
</tr>
<tr>
<td>Surgery length &gt; 120 min</td>
<td>&lt; 0.0001</td>
<td>7.442</td>
<td>3.763-14.720</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>&lt; 0.0001</td>
<td>7.909</td>
<td>3.428-18.244</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>&lt; 0.01</td>
<td>4.837</td>
<td>1.647-14.204</td>
</tr>
<tr>
<td>Positive preoperative vaginal culture</td>
<td>&lt; 0.05</td>
<td>2.231</td>
<td>0.999-4.980</td>
</tr>
<tr>
<td>ASA score</td>
<td>&lt; 0.0001</td>
<td>1.912</td>
<td>1.462-2.501</td>
</tr>
<tr>
<td>Intraoperative blood loss</td>
<td>&lt; 0.0001</td>
<td>3.140</td>
<td>1.634-6.036</td>
</tr>
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</table>

### Table 3. — Most important predictors of SSI occurrence.

<table>
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<tr>
<th>Risk factor</th>
<th>$p$</th>
<th>RR</th>
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<tbody>
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<td>FIGO Stage II</td>
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<td>4.097</td>
</tr>
<tr>
<td>FIGO Stage III/IV</td>
<td>&lt; 0.01</td>
<td>5.061</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>&lt; 0.05</td>
<td>3.168</td>
</tr>
<tr>
<td>Surgery length &gt; 120 min</td>
<td>&lt; 0.05</td>
<td>2.808</td>
</tr>
<tr>
<td>Positive preoperative vaginal culture</td>
<td>&lt; 0.05</td>
<td>2.521</td>
</tr>
</tbody>
</table>

### Discussion

Observed surgical site infection (7.5%) was higher than that reported in surveillance studies from developed countries of Western Europe, e.g., the United Kingdom (3.1%) and the Netherlands (4.3%) [5, 6]. Serbia is a country in transition with a significantly lower budget dedicated to public health. Also, our health system is in constant reform and adjustment with systems in developed countries and current medical protocols. The Clinic for Gynecology and Obstetrics, a tertiary level institution is the largest such hospital in Serbia. Nevertheless our data are not easy to compare, since for a period our clinic did not have uniform protocols for prophylactic antimicrobial therapy in accordance with different recommendations that have been used in developed countries for decades [7, 8]. Although, the incidence of SSI that our data show when it comes to gynecologic malignancy was similar to the one reported in the work of Latrakis et al. [9].

Univariate analysis revealed that the following factors were significantly related to SSI: age, malignant disease, stage of malignant disease, surgery longer than 120 min, postmenopause, diabetes mellitus, positive preoperative vaginal culture, ASA score, and intraoperative blood loss which are similar to those identified in different studies. Multivariate analysis showed that most important risk factors that contribute to SSI with RR of 4 and 5 were...
Malignant disease as a risk factor for surgical site infection

respectively, FIGO II and FIGO III/IV stage of malignant disease. This brings us to a question of immunologic abnormalities associated with malignancy, which have been widely observed through decades [10, 11]. In many instances, it is hard to ascertain whether the immune defect was an early event that led to failure of immune response, including tumor surveillance, or if it arose later, as a paraneoplastic process.

Diminished skin and in vitro T cell activity have been associated with decreased peripheral T lymphocyte counts which may be progressive with malignancy spread. Such observations were made in a variety of tumors originating in the head and neck, skin, breast, colon and pelvis [12, 13]. B cell numbers and antibody responses are relatively spared in most cancers [10].

Several other patient-related characteristics have consistently been identified as risk factors for SSI in well designed studies [14]. These risk factors include: diabetes, obesity, cigarette smoking, systemic corticosteroids or treatment with other immunosuppressive drugs, malnutrition, preoperative nasal carriage or colonization at other sites with S. aureus, the presence of a remote focus of infection, duration of preoperative hospitalization and preoperative severity of illness of the patient. There are few studies discussing the issue of malignancy as an isolated risk factor that 4-5 fold increases the risk of SSIs [15]. Many of these patient-related factors such as malignant disease and its stage cannot be altered preoperatively, therefore aiming to strengthen prevention and early detection of malignant tumors contributes to decreasing morbidity and mortality including surgical complications such as SSIs and others. This becomes much more than a standard medical doctrine phrase, keeping in mind the fact that Serbia is the country with the highest incidence of cervical cancer in Europe, with 27.2 newly diagnosed patients per 100,000 women per year in central Serbia, compared to 8.1 in the European Union and 15 globally.

The efficacy of antibiotic prophylaxis for reducing surgical SSI has been clearly established. Nevertheless, errors are common, such as shown in the study of 34,133 patients undergoing surgery in centers around the United States, where an antimicrobial was administered within one hour before incision to only 56% of patients, and antimicrobials were discontinued within 24 hours of surgery in only 41% of patients [16]. The 2009 American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin recommends antibiotic prophylaxis prior to the following gynecologic operations or procedures (7): hysterectomy, urogynecology procedures, including those involving mesh, hysterolaparoscopy or chromopertubation (only if the patient has a history of pelvic inflammatory disease or the procedure demonstrates dilated fallopian tubes), surgical abortion. Randomized trials have consistently demonstrated the efficacy of antibiotic prophylaxis for vaginal hysterectomy. There is a smaller, but significant, reduction in infectious complications for abdominal hysterectomy [17, 18].

In some recommendations for preparation for gynecologic surgery, malignancy is recognized as a risk factor per se and general recommendations are adjusted for patients with malignant disease. The risk of venous thromboembolism is particularly high in women undergoing surgery for gynecologic cancers, especially ovarian cancer. Combined mechanical and pharmacologic prophylaxis may be necessary for these patients [19, 20]. Our conclusion is that it is of utmost interest to define protocols of antimicrobial prophylaxis for gynecological malignancy surgery as suggested for some other malignancies [21]. Some randomized controlled studies need to be conducted in the future.

The following factors did not show any significant correlation: anemia, positive preoperative cervical culture, emergent surgery and length of preoperative hospital stay.

Limitations of this retrospective study are based on the fact that only data from medical records were available; therefore because of lack of definitions and data we could not analyze factors that in different studies are mentioned as risk factors for SSIs such as obesity [14], malnutrition, protocols of preoperative shaving [7] or class of surgical procedures. including those involving mesh, hysteroscopy or hysterectomy. For the same reason, further follow-up data were unavailable for the survey.

References


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The trend of VEGF-A and PI GF in pregnant patients: a perspective case-control study on 214 women

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Summary

Objective: The aim of this study was to measure plasmatic concentrations of vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PIGF) in pregnant women, and to evaluate their relationship with age, hormonal status, gestational age, and different diseases of pregnancy. Methods: We selected a control group of 163 patients (96 fertile and 67 in menopause) and a group of 214 pregnant patients during the whole gestational period. VEGF-A and PIGF were assayed by ELISA and EIA methods, respectively. Statistical analysis was performed using the Mann-Whitney test. Results: The control group showed mean VEGF-A and PIGF values of 89.87 pg/ml and 10.22 pg/ml, respectively; PIGF showed the highest values in menopausal patients. The group of pregnant patients showed VEGF-A values of 27.05 pg/ml and PIGF values of 231.36 pg/ml respectively, with lower (for the VEGF-A) and higher (for the PIGF) statistical significance. These values were not influenced by biological age, but were related to gestational age: VEGF-A showed a decrease and PIGF an increase particularly after the 20th gestational week. PIGF showed a statistically significant decrease compared to physiological gestation in spontaneous and threatened abortions (p < 0.0001) and in ectopic pregnancies (p < 0.0001), an increase in ultrasound and CTG alterations (p < 0.05), and threatened premature delivery and uterine hypercontractility (p < 0.01); on the other hand VEGF-A showed a statistically significant increase in ectopic pregnancies (p < 0.05). Conclusions: VEGF-A and PIGF may play a diagnostic and prognostic role in pregnancy. Further studies are required to better understand the meaning of variability of their values.

Key words: VEGF; PIGF; Pregnancy; Twin pregnancies; Preeclampsia; IUGR; Preterm delivery; Ectopic pregnancy; Abortion.

Introduction

Vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) belong to the family of vascular endothelial growth factors, which includes VEGF-A, -B, -C, -D, -E, PIGF, and endocrine gland-derived growth factor. All these growth factors carry out their activities by binding to specific receptors belonging to the family of tyrosine kinases [1-3].

VEGF-A is a dimerous glycoprotein which is coded by a gene located in the short arm of chromosome 6 – precisely at p21.3 according to some authors or at p12 according to other authors [1-3]. PIGF is a homodimerous glycoprotein coded by a gene located in the long arm of chromosome 14 (at q24.3).

The expression of gene coding for VEGF-A is minutely up-regulated by hypoxia through inducible transcription factors, such as hypoxia-inducible factor-1 (HIF-1) and hypoxia-inducible factor-2 (HIF-2), which bind to responsive elements located near the VEGF-A promoter [4, 5]. Additional mechanisms regulating VEGF-A transcription include several other growth factors, in particular epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-1), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) and a series of inflammatory cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, IL-10, IL-13 [6].

VEGF-A specifically binds to vascular endothelial growth factor receptor (VEGFR)-1, also called fms-like tyrosine-kinase (sFlt1), to VEGFR-2, also called fetal liver kinase (flk-1), and to neuropilins NRP-1 and NRP-2. VEGFR-1 and VEGFR-2 are both made up of seven extracellular domains similar to immunoglobulins, a transmembrane region, and an intracellular domain of a tyrosine kinase [7, 8].

PIGF binds exclusively to VEGFR-1 and Nrp-1, but it can also form a heterodimer with VEGF-A, which is capable of binding to VEGFR-2 and the heterodimerous VEGFR-I/VEGFR-2 as well [9, 10].

VEGF-1 is expressed by endothelial cells, osteoblasts, macrophages, trophoblasts, renal mesangial cells, and other hematopoietic cells. Its expression is increased by hypoxia and during angiogenesis. VEGFR-1 is first expressed by endothelial angioblasts during embryonic development and decreases at the end of it [11].

VEGF-2 is expressed by endothelial, neuronal and pancreatic ductal cells, osteoblasts, megacariocytes, and ascender Retinal cells [12].

VEGF-A strongly binds to VEGFR-1 and has a weaker

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affinity for VEGF-1648_29, even if the latter is the first receptor transmitting the VEGF signal to endothelial cells [13]. The VEGF/VEGFR-2 axis plays an important role both in physiological and pathological angiogenesis [14].

VEGF-A and PlGF are two pleiotropic growth factors that play distinct roles both in homoeostasis and development. Blood vessels are fundamental for normal development and growth because they supply oxygen and nourishment. Angiogenesis is a multistage process that depends on vascular growth factors.

The importance of VEGF-A is evidenced by the fact that its eventual decrease or damage, or damage to its receptors, causes an abnormal angiogenesis that could be lethal during embryo-fetal development [15]. On the other hand, PlGF plays a crucial role in pathological angiogenesis in adults, but an insufficient production of PlGF does not compromise the development of blood vessels, suggesting a less important role before birth [15].

VEGFR-1 and VEGFR-2 are also expressed by non-vascular tissues, which further suggests that they have other biological roles.

VEGF-A and its receptors are involved in the development and homoeostasis of many organs, such as the respiratory, skeletal, hematopoietic, nervous, renal, and reproductive systems, independently of their vascular role [16].

VEGF-A is produced by cytotrophoblastic cells and is poured in the syncytial lacunae, where it comes into contact with circulating maternal blood, in the same way as does human chorionic gonadotropin (hCG), whose detection in maternal blood is necessary for pregnancy tests.

Vasculogenesis and angiogenesis are essential for placental development and are both regulated by vascular growth factors [17]. Members of this family regulate angiogenesis, blood flow and vascular permeability, and coordinate various extracellular matrix bonds. VEGF-A and PlGF induce vasodilation by releasing nitric oxide (NO) and PGI-2 as a consequence of the stimulation of tyrosine-kinase receptors VEGFR-1 and VEGFR-2.

It has recently been discovered that PlGF is a powerful promoter of angiogenesis in vivo and is capable of stimulating the proliferation of microvascular endothelial cells in the human placenta at term [18].

The final configuration of the villous vascular beds is defined by the balance of VEGF-A, PlGF and their receptors. The predominance of VEGF-A promotes the formation of a rich arborization and of low-resistance capillary beds inside the mesenchymal and intermediate immature villi, which prevail in the first two trimesters of pregnancy. By contrast, the predominance of PlGF and its VEGFR-1 receptor in the last trimester is responsible for the absence of complex capillary beds, so that only poorly arborized capillary beds prevail [19].

Further studies are required to test the conflicting roles of VEGF-A, PlGF and their receptors in the genesis of villi [20, 21].

The balance between VEGF-A secretion and PlGF secretion can be regulated by partial oxygen pressure. VEGF-A and its receptors are up-regulated by conditions of reduced oxygenation both in the placenta and in vitro, while the opposite is true for PlGF.

As a consequence of these growth mechanisms, the villous vascular system differs from that of most human organs in two main respects. First of all, arteries and veins of this low-pressure system have a fairly thin middle tunic, and vasa vasorum are usually absent, except for some residual paravascular capillaries. Despite their intraluminal low pressure (10 and 20 mmHg, respectively), adequate compensation is obtained by the vascular walls because the surrounding intervillous space has a mean pO2 exceeding 40 mmHg.

The bed of peripheral capillaries in terminal villi is not represented by richly arborized capillary nets, but rather by a great number of stretched capillaries, coiled in lightly arborized spirals [22, 23].

The purpose of our perspective randomized study was to measure the plasma concentrations of VEGF-A and PlGF and to evaluate their relationship with age, hormonal status, gestational age, and different pregnancy-related diseases.

The main objective was to detect variations of plasma concentrations (either increasing or decreasing) in order to introduce the test of these two indicators as a tool for monitoring pregnancy and for early diagnosis of pregnancy complications.

Materials and Methods

This study, perspective case-control, evaluated a study group and a control group of pregnant women admitted to the Department of Gynaecology and Obstetrics of the Italian Universities of Padua, Parma and Sassari between September 2005 and May 2007. The study was approved by the Review Boards of all centres.

In the control group, which consisted of 163 subjects (96 fertile women and 67 menopausal women), we included 13 healthy volunteers and 150 patients hospitalized for different diseases (uterine prolapse, urinary incontinence, cystocele, ovarian cysts, pelvic colics).

The study group consisted of 214 pregnant women in the first, second and third trimester of pregnancy. These women were divided into ten sub-groups based on pregnancy complications: sub-group 1 (10 patients with spontaneous and internal abortions); sub-group 2 (19 patients with threatened abortion, bleeding and placenta previa); sub-group 3: (10 patients with ectopic pregnancies); sub-group 4: (12 patients with intrauterine growth restriction); sub-group 5 (20 patients with non-reassuring cardiotocography/umbilical artery Doppler pattern); sub-group 6: (6 patients with gestational diabetes); sub-group 7: (33 patients with preeclampsia); sub-group 8 (60 patients with threatened preterm delivery/premature rupture of membranes); sub-group 9 (7 patients with multiple pregnancies); and sub-group 10 (37 patients with uncomplicated pregnancies).

We obtained the patients’ informed consent to take peripheral venous blood samples and to determine the concentrations of VEGF-A and PlGF. Blood samples were introduced into two different Vacutainers: one with an ochre-yellow cap (capacity
The trend of VEGF-A and PlGF in pregnant patients: a perspective case-control study on 214 women

VEGF-A was tested using the ELISA method (Bender Med System). An anti-VEGF polyclonal coating antibody is adsorbed onto microwells. The VEGF-A present in the standard or sample binds to antibodies adsorbed in the microwells. A biotin-conjugated polyclonal VEGF-A antibody is added and binds to the VEGF-A captured by the receptors. Following incubation, unbound biotin-conjugated VEGF-A is removed during a wash step. Horseradish peroxidase (HRP)-conjugated streptavidin (streptavidin-HRP) is added and binds to the biotin-conjugated VEGF-A. Following incubation, unbound streptavidin-HRP is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of VEGF-A present in the sample. The reaction is terminated by the addition of acid and the absorbance is measured at 450 nm. For this test we prepared a standard curve from seven VEGF-A standard dilutions and then determined the VEGF-A concentration in the sample. The test is capable of determining values up to 0.26 pg/ml.

PlGF was tested using the EIA method (R & D Systems). This test employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PlGF is pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PlGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for PlGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of PlGF bound in the initial step. Colour development is stopped and the intensity of colour is measured. In this case, too, we prepared a standard curve from standard dilutions and analyzed the samples by spectrophotometry. The test is capable of determining values up to 0.26 pg/ml.

Results

The control group was composed of 163 women, with mean age 47.4 years (range 15-90 yrs). In this group, the mean VEGF-A concentration was 89.87 ± 100.31 pg/ml (range 0.26-538.8) and the mean PlGF concentration was 10.22 ± 6.21 pg/ml (range 0.26-31.26).

A total of 96 women were fertile and 67 were menopausal, with mean age of 35.2 and 65.6 years, respectively. Descriptive data for the two indicators concerning these two sub-groups are as follows: in the first sub-group (fertile women), mean VEGF-A values were 83.78 ± 92.50 pg/ml (range 0.26-474) and PlGF values were 8.71 ± 5.24 pg/ml (range 0.26 ± 27.30); in the second sub-group (menopausal women), mean VEGF-A and PlGF values were 94.2 ± 100.79 pg/ml (range 0.26-538.80) and 12.63 ± 12.7 pg/ml (range 0.26-31.20), respectively.

VEGF-A values were higher in the second sub-group than in the first one (92.78 vs 83.78 pg/ml), but they did not reach statistical significance. By contrast, mean PlGF values showed a statistical significant difference (p < 0.0001) with higher concentrations reported in menopausal women (12.63 pg/ml vs 8.71 pg/ml) (Figure 1).

We evaluated the presence of a correlation between the levels of the two indicators and the age of patients in the whole control group. VEGF-A levels showed no correlation with age, whereas PlGF levels showed a moderate but statistically significant increase with aging (r = 0.24; p = 0.0019).

We then carried out the same evaluation in the two sub-groups of the control group, but did not find any statistically significant correlation.

Descriptive data for the second group of pregnant patients are: mean age, 32 years (range 16-48); mean VEGF-A values, 27.05 ± 87.29 pg/ml (range 0.26-851.90); and mean PlGF values, 231.36 ± 275.78 pg/ml (range 3.50-1,000.00).
We compared mean PlGF and VEGF-A values between pregnant women and fertile women in the control group. Both indicators showed statistically significant differences ($p < 0.0001$), with mean PlGF values higher in pregnant patients (231.36 pg/ml vs 8.71 pg/ml) and mean VEGF-A values higher in fertile control subjects (27.05 pg/ml vs 83.78 pg/ml) (Figure 2).

No correlation was found between VEGF-A and PlGF levels and age of pregnant patients. On the other hand, a statistically significant correlation was found between VEGF-A/PlGF levels and gestational age ($r = -0.14$, $p < 0.05$ for VEGF-A; $r = 0.25$, $p = 0.0002$ for PlGF), showing an increasing trend for PlGF and a decreasing trend for VEGF-A (Figure 3).

For descriptive data purposes, the group of pregnant patients was divided into two sub-groups according to the gestational week: one sub-group with pregnant women before the 20th gestational week ($n = 46$), and another sub-group with women after the 20th gestational week ($n = 168$). Their descriptive data are as follows: pregnant patients before the 20th gestational week had mean plasma VEGF-A and PlGF concentrations of 47.99 ± 77.31 pg/ml (range 0.26-284.80) and 38.83 ± 59.76 pg/ml (range 4.90-226.60), respectively; pregnant patients after the 20th gestational week had mean plasma VEGF-A and PlGF concentrations of 21.31 ± 89.18 pg/ml (range 0.26-851.90) and 284.08 ± 288.16 pg/ml (range 3.50-1000.00), respectively. Differences in the indicator levels between the two sub-groups reached statistical significance, with a clear increase in PlGF after the 20th gestational week ($p < 0.0001$) and a decrease in VEGF-A ($p = 0.0006$).

Descriptive data distribution for the ten pregnant patient sub-groups was as follows: the sub-group with spontaneous and internal abortions ($n = 10$) had a mean PlGF value of 44.11 ± 98.55 pg/ml (range 5.7-324.00) and a mean VEGF-A value of 84.12 ± 179.73 pg/ml (range 0.26-387.10); the sub-group with threatened abortion and vaginal bleeding ($n = 19$) had a mean PlGF value of 167.14 ± 264.72 pg/ml (range 6.10-1,000.00) and a mean VEGF-A value of 25.64 ± 56.17 pg/ml (range 0.26-183.90); the sub-group with ectopic pregnancies ($n = 10$) had a mean PlGF value of 10.12 ± 5.19 pg/ml (range 4.90-22.30) and a mean VEGF-A value of 74.99 ± 103.84 pg/ml (range 0.26-284.80); the sub-group with intrauterine growth restriction ($n = 12$) had a mean PlGF value of 178.51 ± 184.28 pg/ml (range 14.30-611.00) and a mean VEGF-A value of 20.37 ± 47.56 pg/ml (range 0.26-138.60); the sub-group with cardiotochrome/umbilical artery Doppler alterations ($n = 20$) had a mean PlGF value of 206.51 ± 131.78 pg/ml (range 25.80-464.20) and a mean VEGF-A value of 14.18 ± 47.56 pg/ml (range 0.26-278.70); the sub-group with gestational diabetes ($n = 6$) had a mean PlGF value of 241.18 ± 182.42 pg/ml (range 60.70-589.30) and a mean VEGF-A value of 14.9 ± 35.86 pg/ml (range 0.26-88.10); the sub-group with toxemia of pregnancy and hypertension ($n = 33$) had a mean PlGF value of 188.78 ± 267.03 pg/ml (range 3.50-1000.00) and a mean VEGF-A value of 23.72 ± 56.22 pg/ml (range 0.26-214.40); the sub-group with threatened preterm delivery and uterine hypercontractility ($n = 60$) had a mean PlGF value of 374.11 ± 319.84 pg/ml (range 25.80-1000.00) and a mean VEGF-A value of 15.39 ± 50.76 pg/ml (range 0.26-312.30); the sub-group with spontaneous abortions and uterine bleeding ($n = 10$) had a mean PlGF value of 44.11 ± 98.55 pg/ml (range 5.7-324.00) and a mean VEGF-A value of 84.12 ± 179.73 pg/ml (range 0.26-387.10); the sub-group with uncomplicated pregnancies ($n = 37$) had a mean PlGF value of 201.88 ± 282.00 pg/ml (range 9.00-1000.00) and a mean VEGF-A value of 36.98 ± 143.69 pg/ml (range 0.26-851.90).

We compared the mean levels of the two indicators between the sub-group of women with uncomplicated pregnancies and the other nine sub-groups of women with complicated pregnancies. PlGF showed a statistically significant decrease in the sub-group with spontaneous and internal abortions ($p < 0.0001$) and in that with ectopic pregnancies ($p < 0.0001$), whereas it increased in the sub-group with ultrasound and cardiotochrome alterations ($p < 0.05$) and in that with threatened preterm delivery and uterine hypercontractility ($p < 0.001$). VEGF-A showed a statistically significant increase in the sub-group with ectopic pregnancies ($p < 0.05$).

Finally, we assessed the patterns of mean values of the two indicators among the ten sub-groups according to the gestational period (< 10, between 11 and 20, between 21 and 30, > 30 gestational weeks). Only PlGF showed sta-
The trend of VEGF-A and PIGF in pregnant patients: a perspective case-control study on 214 women

16 1260-31 - The trend of VEGF:1648_29 Incidence of multiple 21/02/12 12:56 Pagina 61

Figure 4. — PIGF pattern associated to different diagnoses, according to gestational week.

Table 4. — PIGF and VEGF-A values in patients with different diagnoses versus healthy volunteers.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PIGF (pg/ml)</th>
<th>VEGF-A (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>89.87 (68.00)</td>
<td>371.70 (10.1)</td>
</tr>
<tr>
<td>Patients</td>
<td>238.93 (10.2)</td>
<td>89.87 (68.00)</td>
</tr>
</tbody>
</table>

Discussion

Many studies in the last 15 years have examined the role of VEGF-A and PIGF both in uncomplicated and in complicated pregnancies. Nevertheless, this role is not completely clear yet. So far, no cut-off values have been proposed for diagnostic purposes, nor are such indicators commonly used in clinical practice, probably because of their high variability.

In our study, VEGF-A appeared to be more expressed than PIGF in the control group, with mean values of 89.87 pg/ml (median 68.00 pg/ml) and of 10.22 pg/ml (median 10.1 pg/ml), respectively. The control group was made up of patients hospitalized for gynecological (not oncological) problems or diseases, which according to the literature are unable to influence the values of the examined parameters. On the other hand, the PIGF and VEGF-A values observed in these patients did not substantially differ from those of a small group of healthy volunteers we were able to examine in our study.

The two sub-groups were not only largely different in size, but also had non-comparable mean ages (30.0 years for healthy volunteers vs 48.9 years for patients). Nevertheless, we observed that age did not influence the levels of the two indicators.

In fact, although a first evaluation of the control group demonstrated a weak but statistically significant correlation between age and PIGF (whereas no correlation was observed between age and VEGF-A), this fact can essentially be attributed to the subjects’ hormonal conditions. Consistently with the literature reports and physiology of embryogenesis, we observed an increase in PIGF and a decrease in VEGF-A in pregnant patients. This result has a great statistical significance if compared with the indicator values in fertile control subjects.

Confirming our findings in the control group, even in pregnant patients there was no statistically significant correlation between age and PIGF and VEGF-A, which were influenced by the gestational period: in particular, there was a positive statistically significant correlation between PIGF and increasing gestational weeks (p = 0.0002) and we observed that this factor started to increase clearly after the 20th gestational week. VEGF-A, too, was statistically correlated to the gestational period, though in a different way.

By dividing pregnant patients into two groups, before and after the 20th gestational week, we noted that in the latter group mean PIGF values were increased seven-fold and mean VEGF-A values were more than halved compared with the first group, with highly significant statistical differences (p < 0.0001 and p = 0.0006, respectively).

Furthermore, we examined the values of the two indicators in different pregnancy-related diseases, comparing them to those of uncomplicated pregnancy. This comparison was carried out by examining the values of the entire group of subjects in the different sub-groups and evaluating the pattern of data according to gestational weeks. However, this evaluation was not completely reliable due to the non-homogeneous distribution of our sub-groups in different classes of gestational weeks. Therefore, we observed some differences that had no statistical significance.

In the spontaneous and internal abortion sub-group, we found a statistically significant decrease in PIGF and in VEGF-A in the first 20 weeks of pregnancy, as reported in the literature [24]. Some studies have shown that an early decrease in NO and VEGF-A could be harmful to placental vascular growth and to endothelial regulation, leading to fetal death [25, 26].

A similar decrease in VEGF-A in the first ten weeks of pregnancy was also found in the threatened abortion sub-group.

In the ectopic pregnancy sub-group, too, there was a statistically significant decrease in PIGF in the first weeks of pregnancy, and an increase in VEGF-A, particularly between the 11th and the 20th gestational week.

The literature data concerning this condition show that due to the high sensitivity of serum hCG testing and to the increasing sensitivity of transvaginal ultrasound, the diagnosis of ectopic pregnancy has progressed dramatically in the last decade. The benefit of a single serum hCG measurement to confirm the absence of an ectopic pregnancy has been questioned, and serial measurements have been proposed [26, 27]. In fact, an abnormal pregnancy exists when serial serum hCG measurements decrease or do not increase in an appropriate way. Furthermore, serial hCG measurements are not easy to perform when the patient is in an emergency situation.

Serum VEGF-A levels are significantly increased in patients with ectopic pregnancies compared with controls (women with normal intrauterine pregnancies of match-
ing gestational age). The secretion and expression of VEGF-A could be induced by hypoxia, hormones, growth factors, and cytokines [28-30]. After 11 days from in vitro fertilization, VEGF-A levels higher than 700 pg/ml are strongly predictive of ectopic pregnancy [31]. Other authors have shown significantly increased VEGF-A levels in ectopic pregnancies, and have suggested the possibility of using a combination of VEGF-A and pregnancy-associated plasma protein A (PAPP-A) for early assessment of ectopic pregnancy [32]. This improvement could dramatically reduce the time required for diagnosis and the possibility of a tubal rupture and its sequelae.

Data for intrauterine growth restriction (IUGR) are extremely controversial in the literature. In our case series we did not find any statistically significant differences either for PlGF or for VEGF-A, but we observed that especially after the 20th gestational week, PlGF values tended to be lower than in uncomplicated pregnancy. Since reduced placental perfusion or a diminished density of villi is frequently observed in IUGR, studying placental vascular reactivity to VEGF-A and PlGF in IUGR is greatly useful [33]. The role of abnormal VEGF-A and PlGF expression in IUGR has been studied quite recently. When IUGR is caused by placental abnormalities or maternal diseases, growth retardation is usually a consequence of the inadequate presence of metabolic substrates and of reduced oxygen availability [34, 35]. Fetal ischemia is caused by placental failure: the foremost pathological factor associated with IUGR could be either the increase in vascular resistance or the upheaval of placental vascularization [36]. Data concerning VEGF-A and PlGF expression in IUGR are controversial. Several authors report both an increase and a decrease in VEGF-A and PlGF expression in the human placenta at the same stage of pregnancy [33]. In placental tissue, there are several potential sources of VEGF-A, but the contribution of mast cells to the VEGF-A pool could be significant, especially in pregnancies complicated by vascular abnormalities and hypoxia, like in IUGR [37]. It is believed that VEGFR-2 is more responsive to the vasodilating and hypotensive effects of VEGF-A. This greater reaction to VEGF-A rather than PlGF could be a consequence of the fact that the latter interacts only with VEGFR-1, while VEGF-A interacts both with VEGFR-1 and VEGFR-2 [21]. Relative deficiency of placental NO, or diminished sensitivity to VEGF-A and PlGF in pregnancies complicated by IUGR could strongly contribute to the development of a high impedance in fetal-placental circulation. By mainly interacting with VEGFR-2, VEGF-A is a powerful stimulus for increased vascular permeability. It may thus be inferred that abnormal placental transfer in IUGR could be related to pathological modifications of the placental vascular walls resulting from changes in the VEGF/VEGFR-2 system.

On the other hand, an increase in PlGF (statistically significant in the first group) and a concomitant decrease in VEGF-A (non-statistically significant) were observed in the sub-groups with ultrasound and cardiotocographic alterations and with gestational diabetes.

No statistically significant differences were found in the preeclampsia sub-group. However, the indicator values patterns according to gestational weeks showed an increase in VEGF-A in the first 20 weeks and a simultaneous decrease in PIGF, in agreement with the literature.

Recent studies have shown an increased placental expression and secretion of soluble fms-like tyrosine kinase-1 (sFlt-1), a VEGF-A antagonist normally circulating in patients with preeclampsia. In vitro studies indicate that an excessive placental production of sFlt-1 induces an angiogenetic status in the serum of women with preeclampsia, which can be opposed by exogenous VEGF-A and PIGF [38]. It has been confirmed that sFlt-1 increases both in the placenta and blood of women affected by preeclampsia [39]. Serum concentrations in women with uncomplicated pregnancies or with pregnancies complicated by preeclampsia suddenly decrease after delivery, as could be expected if the majority of circulating sFlt-1 during pregnancy originated from the placenta, and circulating concentrations of free PIGF were reduced in preeclampsia [40]. The highest concentration of sFlt-1 and the lowest concentration of PIGF were detected in blood samples taken from patients between the 21st and the 32nd gestational week before the development of preeclampsia, grouped by severity.

Studies concerning VEGF-A concentrations in preeclampsia have reported controversial results. In those where free VEGF-A was measured, levels were lower in patients with preeclampsia than in controls of matching gestational age [41]. In others, where total VEGF-A was measured, including VEGF-A bound to proteins, levels were mildly higher in preeclampsia patients [42]. However, since only free VEGF-A is biologically active and capable of interacting with receptors on the cellular surface, only the studies that measure free VEGF-A are relevant for discussion concerning its angiogenetic activity. As free VEGF-A levels in preeclampsia are close to the limit of detection using immunoadsorption, it is difficult to evaluate changes in these levels. Several studies have shown a great increase in circulating sFlt-1 starting about five weeks before onset of preeclampsia, along with a decrease in free circulating PIGF and VEGF. These findings support the hypothesis that circulating angiogenetic proteins could have a biological key role in preeclampsia [40]. Therapeutic strategies aimed at opposing endothelial dysfunction in preeclampsia with VEGF, P1GF and prostacyclins could be tested in patients with severe diseases, to evaluate if pregnancy can be continued safely.

In particular, these authors pointed out that women who tend to develop preeclampsia and hypertension have PIGF values lower than normotensive women, starting from the 10th to 11th gestational week; moreover, they tend to have lower VEGF-A levels in the last period of pregnancy, as we also found in our case series. In the sub-groups of patients with threatened premature delivery and hypercontractility, we found a statistically
significant increase in PI GF, both when we evaluated the whole sub-group and when we considered in particular the values of the last gestational period (> 30 weeks), compared with those in uncomplicated pregnancies.

Finally, although our case series was limited, in women with multiple pregnancies we found an increase in PI GF particularly marked in the last gestational period (> 30 weeks).

Conclusions

Our study led us to believe that the two analyzed growth factors could have a diagnostic and monitoring role during pregnancy, revealing deviations from a physiological pattern. They were indeed greatly different during pregnancy compared with control subjects, and were influenced by hormonal status and gestational age.

It is clear that further studies are needed to better assess the meaning of the variability of these values and to define cut-off values. Nonetheless, they seem to be particularly promising in the monitoring of some pregnancy-related conditions, such as ectopic pregnancy, threatened abortion or premature delivery, preeclampsia and IUGR, which are currently monitored only by ultrasound. The new opportunities offered by proteomics could also be beneficial to early diagnosis, leading to routine use of plasma assays for these two growth factors in all pregnancies.

We believe that it would be useful to further investigate these opportunities, by examining other pregnant patients and taking serial samples instead of single samples during pregnancy. By so doing, we could obtain more detailed information about the patterns of plasma concentrations in specific pregnancy-related diseases.

References


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Immunohistochemical changes of adenomyosis after heat therapy: comparison of radiofrequency myolysis and endoablation


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Summary

Purpose: To check the pathologic changes of focal adenomyosis after heat therapy using radiofrequency and to evaluate which approach – endometrial ablation or direct heat therapy – is better for adenomyosis. To evaluate whether the timing of the procedure and the menstrual cycle are related to pathologic outcomes after heat therapy.

Methods: This study included nine women who underwent total hysterectomy for adenomyosis (diameter, ≥ 6 cm). Six fresh uteri were excised in the midline and subjected to radiofrequency heat therapy at the center of the adenomyomas (direct heat therapy) and three uteri were subjected to endometrial ablation. Thereafter, 1 cm³ myometrial tissue was obtained at 1 cm, 2 cm, and 3 cm away from the endometrium. Tissue sections were stained with hematoxylin and eosin. Immunohistochemical analysis using antibodies against cytokeratin-19 (CK-19), actin, and estrogen receptor/progesterone receptor (ER/PR) was performed to evaluate CK-19 (endometrial epithelium marker), actin (myometrial marker) and ER/PR (checking the state of the menstrual cycle), respectively.

Results: After endometrial ablation, cauterized tissues were not noted 2 cm away from the endometrium. All tissues between the endometrium and center of adenomyosis were cauterized after direct heat therapy. During the uterine proliferative phase, unlike the secretory phase, subendometrial layers were cauterized 10 min after direct cauterization.

Conclusion: Direct heat therapy is more effective than endometrial ablation in adenomyosis, and heat is conducted effectively when the patients are in the proliferative phase.

Key words: Radiofrequency; Adenomyosis; Heat therapy.

Introduction

Adenomyosis uteri is a common gynecological disorder with unclear etiology leading to menorrhagia, dysmenorrhea, and infertility [1]. Classically, various surgical and medical treatments have been prescribed for managing uterine adenomyosis; however, the most effective treatment for symptomatic adenomyosis is still total hysterectomy [2].

To avoid total hysterectomy, less aggressive therapeutic options have been introduced. These include surgical excision of adenomyomas, uterine artery embolization, and heat therapy [3]. Radiofrequency myolysis, which is indicated as a topical heat therapy for uterine leiomyomas, results in 73% volume reduction and 90% symptom-improvement rates after an 18-month treatment regimen [4]. Heat therapy for adenomyosis is an endometrial ablation therapy because histopathologically, adenomyosis is caused by endometrial invasion into the myometrium [2, 5-7]. Although direct heat therapy for adenomyosis nodules has been applied using MRgFUS, this approach is still uncommon.

Although following radiofrequency myolysis, myomas show inflammatory reactions with hyaline degeneration [8], the histopathological changes following heat therapy in adenomyosis patients are still unknown. An adenomyosis nodule is composed of ectopic endometrium and hypertrophied myometrium [9]. Adenomyosis tissue reaction to heat may differ from leiomyoma reactions but the precise histopathological reactions have not been studied.

In this study, we used immunohistochemical analysis to study histopathological changes of fresh adenomyosis tissues after heat therapy. Heat-therapy-induced histopathological reactions differed with menstrual cycle, the depth of heat conduction, or the direction of heat application. Heat application was applied for complete endometrial ablation or for direct heat therapy into the adenomyotic lesions.

Materials and Methods

Patient selection

Nine patients, who underwent total hysterectomy due to previous focal uterine adenomyosis lesions of over 6 cm in diameter were enrolled in this study. Six patients were in the proliferative phase and three in the secretory phase (Figure 1). Fresh uteri were used for endometrial ablation and direct heat therapy after total hysterectomy. The study protocol was reviewed and approved by the Ethics and Research Committee of the Catholic University Medical College of Korea.

Methods

Endometrial ablation was done on three uteri in the proliferative phase (group A). Direct heat therapy on adenomyotic nodules was done on the remaining three uteri in the proliferative phase (group B) and three uteri in the secretory phase (group C). Endometrial ablation was done using a radiofrequency generator for 10 min at 80 W, a commonly used setting...
for radiofrequency endometrial ablation. Direct heat therapy on adenomyotic nodules was performed as follows: a needle was inserted parallel to but 3 cm away from the endometrium (at the center of the adenomyotic nodule) and heated for 10 min at 90 W, which is a usual setting for leiomyoma ablation (Figure 2). After heat therapy, the myometrium was biopsied using 1 cm³ of tissue sections taken at 1 cm (point 1, including endometrium), 2 cm (point 2), and 3 cm (point 3) away from the endometrium. Heat conduction direction was from point 1 to point 3 during endometrial ablation, and from point 3 to point 1 during direct heat therapy.

Staining

Hematoxylin-eosin staining and immunohistochemical analysis using cytokeratin-19 (CK-19) and actin antibodies were performed on the biopsied specimens.

Four-micrometer-thick paraffin sections were heated for 1 h at 58°C. For deparaffinization, tissue sections were treated with xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were treated with 0.01 M citric acid buffer (pH 6.0) (DakoCytomation, Carpinteria, CA, USA) and boiled in a microwave three times for 5 min. Sections were then treated with 3% hydrogen peroxide in methanol for 10 min at room temperature to quench endogenous peroxidase activity. After washing the excess solution off the slides, slides were incubated with the primary antibody for 1 h at room temperature and rinsed with the wash buffer. After 10 min, slides were incubated with Dako Envision/HRP-conjugated antibodies (secondary antibody and dextran polymerase) for 30 min and finally rinsed. The primary antibodies used were anti-mouse anti-SMA antibody against actin (Neomark) and RCK 108 (Biogenex, San Ramon, USA) against CK-19. For checking estrogen receptor/progesterone receptor (ER/PR), an immunohistochemistry kit (Abbott, Germany) was used. After incubation with 20% sheep serum for 15 min, sections were incubated with primary sheep monoclonal antibody (ERICA and PRICAm Abbott, Germany) at 4°C overnight. Sections were incubated with sheep anti-rat IgG for 2 h and washed in phosphate-buffered saline (PBS). To visualize immunohistochemical reactions, sections were incubated with the peroxidase-anti-peroxidase complex for 2 h and diaminobenzidine tetrahydrochloride and H₂O₂ were added and incubated for 12 min until color development. Counterstaining was in hematoxylin for 5 min.

The specimens from all patients were examined by two histologists who were blinded to the details of the study. The sections were evaluated for actin and CK-19 expression based on the percentage of stained cells: (−) trace (0–25% of cells stained), (+) weak positive (26–50% of cells stained), (++) moderate (51–75% of cells stained), and (+++) strong (76–100% of cells stained).

Results

Results of immunohistochemical analysis

After endometrial ablation in group A, ectopic endometrial tissue at point 1 (1 cm away from the endometrium) showed trace antibody reactivity to CK-19 and actin, whereas this reactivity increased from moderate to strong positive staining at points 2 and 3 (2 cm and 3 cm away from the endometrium, respectively). In group B, all ectopic endometrial tissues at points 1, 2, and 3 were weakly positive for CK-19 and actin after direct heat therapy. These results suggest that endometrial ablation could not effectively cauterize the adenomyotic lesions located deep in the myometrium 2 cm peripheral to the endometrium (Table 1).

To compare the results after direct heat therapy during the menstrual phase, group B and group C were examined.

Table 1. — Comparison of immunohistochemical findings for CK-19 and actin after endometrial ablation and direct heat therapy of adenomyosis.

<table>
<thead>
<tr>
<th>Distance from endometrium</th>
<th>Endometrial ablation</th>
<th>Adenomyosis direct heat therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cm</td>
<td>CK-19: ±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Actin: ±</td>
<td>+</td>
</tr>
<tr>
<td>2 cm</td>
<td>CK-19: ++</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Actin: +++</td>
<td>+</td>
</tr>
<tr>
<td>3 cm</td>
<td>CK-19: +++</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Actin: +++</td>
<td>+</td>
</tr>
</tbody>
</table>

*: distance from endometrium

±: trace, +: weakly positive, ++: moderately positive, +++: strongly positive.
Uteri in group B, which were in the proliferative phase, showed trace to weak CK-19 and actin immunoreactivity at points 1, 2, and 3. In group C, point 2 and 3 showed trace to weak CK-19 and actin immunoreactivity, but CK-19 and actin were moderately positive at point 1 (Figure 3). This finding suggests that cauterization after direct heat therapy was more effective in the proliferative phase.

**Discussion and Conclusions**

Adenomyosis is a common benign gynecological disorder characterized by presence of endometrial islets within the myometrium, typically situated at least 2.5 mm below the endometrium-myometrium (EM) junction [9]. The posterior uterine wall is more often affected than the anterior wall [10, 11].

Uterine extirpation had been the only therapy completely resolving uterine adenomyosis symptoms [2]. However, other options allowing uterine preservation in uterine adenomyosis patients comprise medical treatment, surgical resection, uterine artery embolization, and hysteroscopic procedures [3].

Cytoreductive surgery results in an increased sensitivity to hormonal treatment due to improved blood supply to the adenomyotic tissues and leads to improved immunity [12]. In contrast to leiomyoma, adenomyotic lesions are not well-demarcated or segregated from the adjacent myometrium. Removal of adjacent healthy myometrium may increase the risk of bleeding and negatively affect the uterine tensile strength during pregnancy and labor. Intra- and perioperative complications after adenomyomectomy such as pyrexia, infections, adhesions, and uterine rupture can develop after surgery [13, 14]. In leiomyoma patients, decreased ovarian function [15-17] and gestational complications [18] have been reported following uterine artery embolization while operational safety in adenomyosis patients was not guaranteed.

Endometrial ablation has been used for treatment of adenomyosis symptoms such as menorrhagia [2, 3]. It is believed that adenomyosis results from the abnormal
ingrowth and invagination of basal endometrium into subendometrial myometrium and an intact endometrium-myometrium interface may be important in preventing adenomyosis [19]. Wood et al. reported a symptom-relief rate of 55-67% after endometrial resection from 1993 to 1998 [2, 5, 6], but McCausland et al. reported no symptom improvement in seven adenomyosis patients treated by endometrial resection. The authors insisted that adenomyotic lesions located more than 2 mm from the endometrial layer have a poor chance of improvement after ablation therapy [20, 21].

In this report, adenomyotic lesions below 1 cm (point 1), 2 cm (point 2), and 3 cm (point 3) from the endometrium were examined. After endometrial ablation, ectopic endometrial tissues at points 2 and 3 were intact by immunohistochemical staining, indicating that these lesions were not cauterized and endometrial ablation was ineffective in managing uterine adenomyosis. Using direct radiofrequency heat therapy, the ablation needle was inserted up to 3 cm deeper than the endometrium. Immunohistochemical results in these lesions indicated that ectopic endometrial tissues at points 1, 2, and 3 were completely cauterized. Heat therapy is uncommonly used in adenomyosis. Rabinovici et al. reported successful gestation in patients with previous focal adenomyosis and treatment using heat therapy by MRgFUS [22]. Significant symptom improvements (26%) and 12.7% volume reduction were reported six months after heat therapy [23, 24].

The most important point in managing adenomyosis using heat therapy is the consideration that adenomyosis pathologically differs from leiomyoma. Adenomyotic nodules are a mixture of normal smooth muscle cells and ectopic glandular and stromal endometrial tissue, with hypertrophied smooth muscle cells around the ectopic endometrium. In these results, histopathology confirmed varying extent of tissue cauterization due to the menstrual cycle. In the proliferative phase, heat cauterized all the ectopic endometrial tissue from point 3 to point 1. However, in the secretory phase, point 1 was not cauterized. Heat conduction may have been affected due to prominent endometrial glandular secretions and prominent stromal vessels during the secretory phase. The conductive heat radiation was thought to be more effective in the living donors because of the blood flow. Therefore, much more time was required to cauterize adenomyotic lesions than leiomyomas. This possibility should be considered when performing heat therapy in uterine adenomyosis. The limitations of our study are the limited number of cases and conduct of the study in situ. Heat therapy could be a better therapeutic option considering preservation of the uterus in adenomyosis patients. Further heat-therapy studies are required to allow determination of the in vivo histopathological changes, patients’ symptom improvement, and pregnancy potential in adenomyosis patients.

References


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Risk factors and prevalence of urinary incontinence in postmenopausal women living in Turkey

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Summary

Purpose of investigation: To detect the prevalence, types and risk factors of urinary incontinence (UI) in postmenopausal women.

Methods: Three-hundred and thirty-three patients who were referred to our Menopause Clinic between August 2008 and May 2009 were included in the study according to the acceptance criteria. A detailed questionnaire was completed by the patients who were between 31-65 years of age. Results: The mean age was 52.5 and the mean age at which menopause symptoms started was 45.8. The prevalence of urinary incontinence was found to be 45.6%. The most frequently seen UI type was mixed urinary incontinence (68.4%). Advanced age, vaginal delivery, high BMI and no hormone replacement therapy were regarded as significant risk factors. Conclusion: UI is a common problem influencing the social experience of postmenopausal women. The prevalence of UI was detected as 45.6% in our study. The quality of life in postmenopausal women can be augmented by diagnosing the risk factors of UI and making an effort to improve the condition.

Key words: Menopause; Urinary incontinence; Vaginal delivery; Body mass index (BMI).

Introduction

Urinary incontinence (UI) is a medical and social problem which manifests with involuntary incontinence and has a high prevalence [1]. Continence occurs due to complex mechanisms and neurophysiologic functions of the bladder, urethra and pelvic floor [2]. Incontinence was detected in 70% of the women in the postmenopausal period. Previous studies regarding UI have shown wide variability about prevalence rates in women, with estimates ranging between 32% and 73% [3-5].

The most important results of UI concern the social and psychologic complications. It causes psychological problems varying from simple embarrassment to depression. Because of the decreasing self confidence, social activities diminish and eventually the patient’s quality of life becomes impaired [6]. UI is also an expensive health problem.

The risk factors for UI are age, parity, menopause, vaginal delivery, heavy lifting and hysterectomy [1, 2]. Age and obesity are well established risk factors for UI. Parity is another risk factor for moderate and severe stress and any UI [7, 8]. However, in relation to other risk factors, findings have been inconsistent with some studies showing significant associations between UI and mode of delivery [9-11], menopausal status [1], postmenopausal hormone use [12] and hysterectomy [13, 14] with other studies failing to show a significant association of UI with mode of delivery [15], menopausal status [16], postmenopausal hormone use [17] and hysterectomy [18]. We aimed to evaluate the prevalence, types and risk factors of urinary incontinence in postmenopausal women.

Material and Methods

Three-hundred and thirty-three postmenopausal patients whose ages ranged between 31 and 65 years participated in the study. The protocol for the research project was approved by our Institution’s Ethics Committee and conformed to the provisions of the Declaration of Helsinki. A signed consent form was submitted before obtaining demographic data. The patients were asked detailed questions about their UI. The questionnaire form consisted of two parts. The first part comprised questions on age, weight, height, birth pattern, onset of menopause age, menopause pattern (surgical or natural) and use or nonuse of hormone replacement therapy (HRT). Natural menopause was described as not having a menstrual cycle throughout one year. Undergoing bilateral oophorectomy accompanied or not accompanied by hysterectomy was described as surgical menopause. The second part consisted of three types of UI. UI types were described as follows: 1) Stress urinary incontinence (SUI): being incontinent while coughing, sneezing or laughing; 2) Urge urinary incontinence (UUI): being incontinent with urge symptoms; 3) Mixed urinary incontinence (MUI): having a combination of SUI and UUI.

Statistical Analysis and Graphics (NCSS 2007) and Power Analysis and Sample Size (PASS 2008) statistical software programs (Utah, USA) were used in this study. Student’s t-test, Mann-Whitney U test, chi-square test and logistic regression analysis were performed. Results were assessed with a 95% confidence interval and p < 0.05 significance level.

Results

Three-hundred and thirty-three postmenopausal women completed the study questionnaire. The mean age of the patients was 52.50 ± 7.44 years. Onset of menopausal age of the patients varied between 30 and 56 years and mean menopausal age was 45.80 ± 5.52. Body mass index (BMI) was between 18.73-48.44, mean BMI level was 30.02 ± 5.11. BMI was < 25 in 15% of the patients, while the remaining patients were within the >
25 BMI level. Birth numbers of the patients ranged from 0 to 13 and the mean birth number was 3.72 ± 2.05. Parity was < 3 in 25.5% of the women; the others were at level 3 or more parity. Vaginal birth rate was 99.1%. Menopause pattern was natural in 79% and surgical in 21% of the cases. Twenty-three percent of the patients were using HRT.

The patients were divided into two groups. The continent group consisted of 152 patients, while the control group (continent group) consisted of 181 patients. UI was seen in 45.6% of the patients. One hundred and four women (68.4%) were MUI, 32 cases (21.1%) were SUI and 16 (10.5%) were UUI.

The mean age of the continent women was higher than continent women and this value was found to be statistically significant (p < 0.01). There was no statistically significant difference between the two groups in relation to menopausal age. The BMI levels of the continent women were significantly higher than the continent group (p < 0.01). The parity of the continent women was also significantly higher than the continent group (p < 0.01) (Table 1).

Table 1. — Assessment of age, BMI and parity according to the groups.

<table>
<thead>
<tr>
<th>Incontinent (n = 152)</th>
<th>Continent (n = 181)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.20 ± 7.67</td>
<td>51.07 ± 6.95</td>
</tr>
<tr>
<td>Menopausal age</td>
<td>46.24 ± 5.54</td>
<td>45.43 ± 5.48</td>
</tr>
<tr>
<td>BMI</td>
<td>31.17 ± 5.32</td>
<td>29.06 ± 4.74</td>
</tr>
<tr>
<td>Parity</td>
<td>4.47 ± 2.16 (4)</td>
<td>3.07 ± 1.71 (3)</td>
</tr>
</tbody>
</table>

*p Student’s t-test; ** Mann-Whitney U test; *p < 0.05 (significant); **p < 0.01 (very significant).

Distribution of the risk factors according to the groups is demonstrated in Table 2. Menopausal age, BMI, parity, birth pattern, menopausal pattern, and use of HRT were compared according to the continent status.

When the risk factors influencing UI (BMI, parity, birth pattern, use of HRT) were assessed by logistic regression analysis, the model was found to be significant (p < 0.05), Nagelkerke R square value was detected to be 0.17 and expressiveness of the model quotient was good (68.3%) (Table 3). The effects of the parameters were found to be statistically significant (p < 0.05).

**Table 2. — Distribution of the risk factors according to the groups (chi square test).**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Incontinent (n = 152)</th>
<th>Continent (n = 181)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopausal age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>98 (64.5%)</td>
<td>137 (75.7%)</td>
<td>0.583</td>
<td></td>
</tr>
<tr>
<td>&gt; 50</td>
<td>54 (35.5%)</td>
<td>44 (24.3%)</td>
<td>0.36-0.94</td>
<td>0.02**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>136 (89.5%)</td>
<td>147 (81.2%)</td>
<td>1.966</td>
<td></td>
</tr>
<tr>
<td>&gt; 25</td>
<td>16 (10.5%)</td>
<td>34 (18.8%)</td>
<td>1.03-3.72</td>
<td>0.03*</td>
</tr>
<tr>
<td>Parity (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>23 (15.1%)</td>
<td>62 (34.3%)</td>
<td>1.70-5.01</td>
<td>0.01**</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>129 (84.9%)</td>
<td>119 (65.7%)</td>
<td>2.922</td>
<td></td>
</tr>
<tr>
<td>Birth pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>147 (96.7%)</td>
<td>153 (84.5%)</td>
<td>5.380</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>5 (3.3%)</td>
<td>28 (15.5%)</td>
<td>2.02-14.30</td>
<td>0.01**</td>
</tr>
<tr>
<td>Menopausal age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>121 (79.6%)</td>
<td>142 (78.5%)</td>
<td>0.54-1.58</td>
<td>0.79</td>
</tr>
<tr>
<td>Surgical</td>
<td>31 (20.4%)</td>
<td>39 (21.5%)</td>
<td>0.933</td>
<td></td>
</tr>
<tr>
<td>Vaginal Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>133 (87.5%)</td>
<td>122 (67.4%)</td>
<td>3.385</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>19 (12.5%)</td>
<td>59 (32.6%)</td>
<td>1.91-6.00</td>
<td>0.01**</td>
</tr>
</tbody>
</table>

*p < 0.05 (significant); **p < 0.01 (very significant).

**Table 3. — Logistic regression analysis results of UI cases.**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>B</th>
<th>S.E.</th>
<th>Sig</th>
<th>Exp(B)</th>
<th>95.0% CI for EXP (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &gt; 25</td>
<td>0.798</td>
<td>0.343</td>
<td>0.02*</td>
<td>2.220</td>
<td>1.13-4.35</td>
</tr>
<tr>
<td>Parity &gt; 3</td>
<td>0.759</td>
<td>0.309</td>
<td>0.02*</td>
<td>2.135</td>
<td>1.16-3.91</td>
</tr>
<tr>
<td>Vaginal Delivery</td>
<td>1.354</td>
<td>0.544</td>
<td>0.02*</td>
<td>3.873</td>
<td>1.33-11.24</td>
</tr>
<tr>
<td>Not to use HRT</td>
<td>1.279</td>
<td>0.301</td>
<td>0.01**</td>
<td>3.593</td>
<td>1.99-6.48</td>
</tr>
</tbody>
</table>

*p < 0.05 (significant); **p < 0.01 (very significant).

**Discussion**

Our study was performed in postmenopausal patients only and UI prevalence was detected as 45.6% during this period. Sakondhavat et al. [1] reported the prevalence of UI in women aged 45-50 years to be 38.86%, whereas Hsieh et al. [19] reported a prevalence estimate of 29.8% for women aged 60 years or older.

When the incontinence types were evaluated the proportions of MUI, SUI and UUI were found to be 68.4%, 21.1% and 10.5%, respectively. Many studies have shown similar results [1, 2]. Different results in previous studies may be due to the distinct methodology and different classifications. The precise pathogenesis of urge incontinence among elderly women is not understood. It may involve anomalies of neuroendocrine control, an element of obstruction or premature activation of the micturition reflex [1, 2, 5].

BMI, parity, vaginal delivery, advanced age and non-use of HRT were detected as significant risk factors in our study.

Our study confirms that a high BMI is a risk factor for UI. The likelihood of UI was almost two times greater for obese women than for women with a BMI under 25 kg/m². We found a statistically significant relationship between the rate level and UI. Having a parity ≥ 3 increased the UI risk 2.92 times.

Cesarean section was more protective than vaginal delivery in relation to the pelvic floor in previous studies with large patient numbers [9, 15]. We also found vaginal delivery to be a significant risk factor for UI. Herrmann and colleagues stated that parity of more than three was an especially significant risk factor for UI [10].

Some [20], but not all [13, 14] studies indicate that hysterectomy is a risk factor for UI. It is biologically plausible that nerve and urethral support structure damage associated with hysterectomy could result in a mixed pattern of symptoms. Alternatively, hysterectomy may be associated with either stress or urge or both, but in our study the association may have achieved statistical significance in the mixed UI group only because of statistical power, as the mixed UI group had the largest number of women. We found that hysterectomy was not a significant risk factor for UI.

Estrogen receptors were commonly seen in the lower genitourinary tract and hypoestrogenic situation cases of UI.
Although the role of estrogen replacement therapy is controversial, using estrogen for urogenital atrophy was reported to be beneficial [22]. Using HRT improved the incidence of UI in postmenopausal patients. The findings in our study supported these data. The proportion of UI was significantly high in cases that did not use HRT in comparison with the cases using HRT. Not using HRT increased the risk of UI 3.38 times.

There are many studies which have been carried out in Turkey. Kocak and colleagues investigated female urinary incontinence in Western Turkey [4]. Onur et al. researched prevalence and risk factors of female urinary incontinence in Eastern Turkey [5]. Both studies indicated the same results as our study.

The data were obtained from detailed conversation with postmenopausal patients, although this study was descriptive. The women in this study could ask about the questions put to them. Thus, a detailed answer rate was obtained for each question. However, the limitation of this study was making the classification of UI, not using any physical examination or validated urodynamic investigation, assessment being made only according to the clinical symptoms of the patients and assessing the answers to the questions asked.

Consequently, UI is a common problem which influences the social life of the menopausal patients. Our results highlight the fact that being obese and parous were positively associated with the likelihood of having UI. The quality of life can be improved by diagnosing and preventing the relevant risk factors in postmenopausal UI patients.

References

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Comparison of propofol/ketamine versus propofol/alfentanil for dilatation and curettage

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Summary
Background and Objectives: The use of propofol with an analgesic agent is probably the principal technique for the induction of anesthesia for dilatation and curettage (D&C) at the present time. We designed a randomized, double-blind study to compare the clinical efficacy of ketamine and alfentanil when combined with propofol for short-lasting anesthesia during D&C. Methods: The study included 60 patients scheduled for D&C. Either alfentanil 10 µg/kg⁻¹ IV (Group A) or ketamine 0.5 mg/kg⁻¹ IV (Group K) were given to each patient with propofol 0.7 mg/kg⁻¹ IV for anesthesia induction. Surgeon and patient satisfaction, Aldrete score, Verbal Pain Scale rating, total propofol dose, orientation time, and adverse events such as bradycardia, hypotension, nausea, and vomiting were evaluated. Results: In Group A orientation time was significantly shorter and was significantly lower than in Group K. Conclusions: Both alfentanil/propofol and ketamine/propofol combinations provide reliable and effective hypnosis and analgesia; however, the ketamine/propofol combination leads to higher consumption of propofol and results in a longer orientation time than the alfentanil/propofol combination.

Key words: Alfentanil; Ketamine; Propofol; Dilatation curettage; Analgesia; Orientation time.

Introduction
Dilatation and curettage (D&C) is a short-lasting procedure that generally causes considerable pain due to cervical dilatation performed usually by Hegar dilators and tissue extraction. Prevention of movement responses to pain during D&C is important. The use of propofol with an analgesic agent is probably the principal technique for induction of anesthesia for D&C at the present time. Alfentanil is a synthetic opioid with a rapid onset and short elimination half-life used for short-time procedures [1, 2]. Theoretically, alfentanil may increase the incidence and duration of apnea due to respiratory depressant effects, and may enhance the depressant effects of propofol on blood pressure and heart rate [3].

Ketamine has intrinsic analgesic and amnestic properties, and thus may be a suitable choice for short-lasting procedures [4-6]. However, it has the potential for undesirable side-effects that include unpleasant emergence hallucinations and emesis [7, 8]. To our knowledge, no anesthesia studies have been done in which alfentanil or ketamine are added to propofol for D&C. We designed a randomized, double-blind study to compare the clinical efficacy of ketamine versus alfentanil when combined with propofol for short-lasting anesthesia during D&C.

Patients and Method
The study was approved by the ethics committee of our institution, and each patient included provided informed written consent. The study included 60 patients between the ages of 18 and 60 who were scheduled for D&C procedures for evaluation of abnormal uterine bleeding. Their physical status, as rated by the American Society of Anesthesiologists (ASA) criteria, ranged from I to II. Patients with pulmonary, hepatorenal, neuromuscular, and neuropsychiatric disease, body mass index over 30 kg/m², regular use of sedative medication or substance abuse, and patients undergoing emergency curettage for massive bleeding or hemodynamic instability were excluded from the study. Patients unable or refusing to give informed consent were also excluded.

Before anesthetic induction, standard monitoring was applied (electrocardiogram, pulse oximetry, and noninvasive blood pressure monitoring) to all patients in the operating room. Lactated Ringer’s solution was infused at a rate of 5 ml/kg. Each patient included was then randomly assigned to receive either a combination of propofol/alfentanil (Group A) or propofol/ketamine (Group K) for anesthesia. Patients were preoxygenated with 100% oxygen for 3 min, just before anesthesia induction.

Alfentanil (Rapifen, Janssen-Cilag, Germany) 10 µg/kg⁻¹ IV was given to each patient in Group A, and ketamine (Ketalar 500 mg flc, Phizer, Luleburgaz, Turkey) 0.5 mg/kg⁻¹ IV to each patient in Group K, followed 60 sec later in both groups for anesthesia induction applied with propofol (propofol 1% Fresenius, Fresenius Kabi, Australia) 0.7 mg/kg IV. If the eyelid reflex failed to disappear with this medication, an additional half of the induction dose of propofol was administered. After loss of consciousness, ventilation was assisted manually via a face mask as necessary with a fresh gas flow of 6 l/min (3 l/min N₂0, 3 l/min oxygen). N₂0 was discontinued when the gynecologist declared the D&C procedure completed.

During the D&C, an additional propofol bolus of half the induction dose was given if any of the following signs were detected: heart rate (HR) > 15% above preoperative baseline or > 90 beats/min; systolic arterial pressure (SAP) > 15% above preoperative baseline; extremity or body movement. Administration was repeated after 1 min if necessary. Blood pressure, HR, and oxygen saturation were recorded at 2 min intervals.
Adverse events such as hypotension (mean arterial pressure < 30% pre-induction baseline value, SAP < 80 mmHg), or bradycardia (HR < 50 beats/min^-1) were also registered and were treated with IV ephedrine 5-10 mg or atropine 0.5 mg, respectively.

A modified Aldrete scoring system was used to evaluate recovery of patients [9] and a verbal pain scale (VPS) used to evaluate pain intensity, with scores of 0-3 (0: no pain; 1: light pain; 2: moderate pain; 3: severe pain) at 5 and 10 min postoperatively. Tramadol 1 mg/kg IV was administered to patients with a score > 1. Two hours later, all patients were questioned about the occurrence of nausea or vomiting.

The total dose of propofol was recorded, as well as duration of surgical procedure, duration of anesthesia (from first propofol injection to open eyes), and orientation time (from N2O discontinuation until able to recall name and date of birth). After the operation, surgeons were questioned about their subjective evaluation of surgical working conditions during the D&C, and patients were questioned at discharge about their anesthetic experience (0: not satisfied; 1: satisfied; 2: extremely satisfied). Surgeon and patient satisfaction scores, Aldrete scores, VPS, nausea, and vomiting were recorded by independent anesthesiologists or nurse anesthetists blinded to the study groups. The primary endpoint was defined as orientation time, and the secondary endpoint was defined as adverse events such as hypotension, bradycardia, nausea, and vomiting.

**Statistical analysis**

After the power analysis (priority analysis) according to orientation time, we found the total sample size to be 58, power 0.95, and effect size 0.9 (alpha = 0.05, actual power = 0.95, delta = 3.3). Results are expressed as the median (range), mean ± SD, and patient number. A normalization test was done using the Kolmogorov-Smirnov Z-test for parametric data. The independent Student’s t-test was used to compare parametric variables and the Mann-Whitney U test for non-parametric variables; a p value < 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS 10.0 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, USA).

**Results**

Data regarding demographics, duration of surgery, and anesthesia of patients in the two groups are summarized in Table 1. There were no statistically significant demographic differences between the two groups. Duration of surgery and duration of anesthesia were also similar between the two groups (Table 1).

Postoperative evaluation data are shown in Table 2. Orientation time was significantly shorter in Group A than Group K (4.6 ± 1.2 min vs 7.1 ± 1.1 min [p < 0.001], respectively). In addition, propofol consumption was significantly lower in Group A than Group K (86.7 ± 36.5 mg vs 142.6 ± 42.4 mg [p < 0.001], respectively).

There were no statistically significant differences between groups regarding surgeon satisfaction, patient satisfaction, VPS, and Aldrete score. Similarly, no statistically significant differences were found between the groups in terms of adverse events (hypotension, bradycardia, nausea, and vomiting) (Table 3).

**Discussion**

This study shows that both anesthesia protocols were effective and reliable for D&C. There were significant differences only in propofol consumption and orientation time between groups. Propofol consumption was greater in the ketamine group than in the alfentanil group, depending on the vital signs and movement of patients in this study. It may be that use of a low ketamine dose caused patients in the ketamine group to feel more pain compared with the alfentanil group [10]. In fact, ketamine is known to have both analgesic and anesthetic properties. The analgesic effect of ketamine is explained by its non-competitive antagonism at the N-methyl-D-aspartate (NMDA) receptor, which plays a significant role in the pathogenesis of pain perception [11, 12]. Ketamine administered intravenously or epidurally in a low-dose manner has been shown to decrease pain scores and reduce postoperative analgesic consumption by 35-40% [13-16]. According to the results of this study, alfentanil was a more effective analgesic than ketamine during the intraoperative period. We believe that the propofol/alfentanil combination provided more effective analgesia and anesthesia than the propofol/ketamine combination. Perhaps the need for propofol is associated with the synergistic effect between these two drugs and propofol.

Both the propofol/ketamine combination [17, 18] and the propofol/alfentanil combination [19-22] interact additively to produce hypnosis and immobility and suppress...
responses to both noxious and non-noxious stimulation. These reports show that the synergistic effect of the drug combinations is more important than their individual effects. We propose that the alfentanil/propofol combination provides more effective hypnosis and immobility than the ketamine/propofol combination.

In this study, although there was no statistically significant difference between groups in terms of Aldrete recovery score, the orientation time was longer in the ketamine group than in the alfentanil group. This may seem contradictory, but we suspect that the extended orientation time is associated with the stronger amnestic effect of ketamine and the use of higher doses of propofol in the ketamine group. St. Pierre et al. [23] reported an extended recovery time for propofol/ketamine compared with a propofol/alfentanil combination.

Ketamine produces sympathetic stimulation which leads to increased SAP and HR [24]. When administered with propofol to induce anesthesia, even in subanesthetic doses, it may produce hemodynamic stability by neutralizing the sympatholytic activity of propofol [24-26]. This study expected the finding that a ketamine/propofol combination would provide a stable hemodynamic course because of previous studies [21, 24, 27, 28]. Due to the cardio-depressant activity of both propofol and alfentanil, their combination caused more hemodynamic instability than the ketamine/propofol combination. Similar results were reported by Fruya et al. [25], and Salihoglu et al. [29] who showed that SAP and HR were significantly lower in an alfentanil group compared with a ketamine group. With the alfentanil/propofol combination, decreased SAP and HR do occur, but there was no statistical difference between groups in bradycardia and hypotension as defined in this study. In addition, alfentanil was used in a low dose with propofol and the propofol was administered gradually according to need. We consider that this method of alfentanil use provided a reliable hemodynamic effect.

Bege et al. [30] and Chiaretti et al. [31] investigated these two drug combinations and found both protocols effective to obtain good sedation and analgesia. However, alfentanil caused respiratory depression in both studies. These two studies used different methods and twice the alfentanil dose compared with our study. We can infer that both the type and dose of agent used is important for safe anesthesia and analgesia during short procedures.

Besides the depressive cardiac and respiratory effects of opioids, the most frequently mentioned adverse effect related to ketamine is emergent delirium or hallucinations. This occurs more commonly if ketamine is used as the sole agent for sedation. In the present study, no patients reported emergent delirium or hallucinations. The combination of propofol/ketamine has been known to eliminate the side-effects of ketamine [24]. The combination of either alfentanil [32] or ketamine [26] with propofol reduces the levels of both the hypnotic and anesthetic dose of propofol.

Patient satisfaction, surgeon satisfaction and Aldrete recovery scores indicated that comfortable and reliable anesthesia was achieved in both groups. The VPS showed that both study drugs provided effective and equal postoperative analgesia.

We note several limitations of this study. First, we compared the hemodynamic data between groups only in terms of bradycardia and hypotension. Second, N2O is an agent with analgesic properties, and we did not take its use into account when evaluating data for either group. Although the present study has clinical importance, our findings could be considered preliminary data and our results, especially the lower frequency of adverse effects, should be confirmed by larger studies with more adequate power. In addition, further studies should be designed to determine the optimal drug and dose for D&C.

In conclusion, the results of this study suggest that both alfentanil and ketamine in combination with propofol provide reliable and effective hypnosis and analgesia, but that the ketamine/propofol combination results in higher consumption of propofol and longer orientation time than alfentanil/propofol.

References


Comparison of propofol/ketamine versus propofol/alfentanil for dilatation and curettage


Removal of uterine fibroids during cesarean section: a difficult therapeutic decision

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²Dr. Zekai Tahir Burak Training and Research Hospital, Ankara (Turkey)

Summary

Purpose of investigation: Myoma excision during cesarean delivery has traditionally been discouraged, however controversy persists among studies of myomectomy being performed during cesarean section. In this study, medical records of patients who underwent cesarean section our institution were evaluated retrospectively. Methods: A total of 70 cases of cesarean myomectomy done during this period were included (group 1) and compared with the patients who underwent cesarean section alone (group 2). Results: Mean surgical time of the myomectomy group was 58.1 ± 23 minutes which was significantly increased (p < 0.01). Mean postoperative hemoglobin value was 9.6 ± 1.5 in the myomectomy group and 10.8 ± 1.01 in controls (p = 0.01). Length of hospital stay was significantly longer in the myomectomy group (p < 0.05). Conclusion: This study shows that myomectomy during cesarean section is a feasible procedure without any serious complications. The procedure is related with increased blood loss that does not require blood transfusion.

Key words: Leiomyoma; Pregnancy; Cesarean section; Hemorrhage; Pregnancy complications.

Introduction

Leiomyomas or fibroids are the most common pelvic tumors in women, with a reported incidence of 20-25% [1]. Fibroids affect mainly women in reproductive age and are mostly asymptomatic. The estimated prevalence of fibroids in pregnancy is 1-4% [2, 3]. Myomectomy at the time of cesarean section has traditionally been strongly discouraged. The most common reasons for this practice are uncontrollable hemorrhage associated with myomectomy which may require hysterectomy and increased postoperative morbidity [2, 3]. The experience of myomectomy at cesarean section is still limited and not used as a routine procedure, however several recent reports suggest myomectomy during pregnancy and cesarean section in experienced hands is possible and safe [1, 4, 5].

The aim of the study was to determine whether myomectomy at the time of cesarean section leads to increase incidence of intrapartum and postpartum complications in a retrospective controlled study design.

Material and Methods

This study was conducted at Dr. Zekai Tahir Burak Training and Research Hospital in a period of six years from 1st January 2004 to 31st December 2009. The study protocol was approved by the local ethical committee of the institution. The study was conducted in accordance with the basic principles of the Declaration of Helsinki. Seventy patients with uterine myomas who were treated by cesarean myomectomy were compared retrospectively with 70 women without uterine fibroids who had routine cesarean section during the same period. In all, 140 women were enrolled in the study. The inclusion criteria were: (1) presence of leiomyoma located anterior of the uterine body documented by antepartum ultrasound or by intraoperative findings; (2) no evidence of antenatal bleeding; (3) no other surgical procedures at the time of cesarean section besides myomectomy; (4) no co-morbid conditions with evidence of coagulopathy. All operations were performed under regional (either spinal or epidural) anesthesia. The number and sizes of uterine fibroids removed were documented in the operation notes. When there was more than one myoma the biggest myoma diameter was measured. Myomectomy for all types of myoma was performed at cesarean section after the delivery of the fetus. Sixty-eight patients were delivered by lower uterine segment incision while two patients were delivered through a classical uterine incision because of the fibroid in the cerviciodinal junction making the lower uterine segment inaccessible. Irrespective of the location of the fibroid, a conventional method of incision over the myoma followed by earectation was employed. The dead space was obliterated in layers by interrupted sutures with 1-0 vicryl. The serosa was sutured using a continuous absorbable suture (2-0 or 3-0 vicryl). Uterine incision for cesarean section was closed in two layers with 1-0 vicryl and the abdomen was closed after ensuring hemostasis. A standard uterotonic treatment was used in all patients. Twenty units (2 ml) of oxytocin per liter of infusate was added and this solution was administered after delivery of the placenta at a rate of 10 ml/min for 12 hours.

The cases and controls were compared for age of the patient, parity, hemoglobin levels before and 12 hours after the operation, change in hemoglobin values, incidence of blood transfusion, length of operation, frequency of postoperative fever and hemorrhage, and duration of postoperative hospital stay. Length of operation was calculated from skin incision to skin closure. Hemorrhage was defined as decrease in hematocrit of ten points from the preoperative value to the postoperative value or the need for intraoperative transfusion. Postoperative fever was defined as temperature greater than or equal to 38°C. Statistical analysis was performed with the SPSS/PC 15.0 package. The chi square test and Student’s t-test were used for statistical analysis; p < 0.05 was considered statistically significant.
Results

Obstetric characteristics of the groups in terms of age, parity, previous abortions and gestational age were similar (Table 1). A total of 73 fibroids were removed. Ninety-seven percent of the patients had only one fibroid removed. Table 2 shows the localization, number and type of myomas. Intramural fibroids were the most common form (45.2%) which were mostly located on the uterine fundus (36.9%). Thirty-one sub-serous myomas were removed, of which ten were pedunculated with a short stalk. In ten women the myomas were located to the lower uterine segment (14%). Two of these patients were delivered through a classical uterine incision. Most of the myomas were between 4-8 cm in size (57.5%). Seventy-one percent of the patients required two uterine incisions. Mean pre- and postoperative hemoglobin value, duration of operation, incidence of blood transfusion and incidence of postpartum fever are shown in Table 3. The length of mean operating time for cesarean myomectomy (58.1 min) was significantly longer than for the control group (32.1 min) \((p < 0.01)\). Mean postoperative hemoglobin values significantly differed between the groups \((p = 0.001)\). Mean change in hemoglobin values was significantly higher in the myomectomy group than the controls \((p < 0.01)\). Mean duration of hospital stay was also significantly longer in the myomectomy group \((p = 0.02)\). Emergency cesarean rates, incidence of postpartum atony, need for blood transfusion during the early postoperative period and frequency of postoperative fever and hemorrhage were not significantly different between the groups. Intraoperative blood transfusion, peripartum hysterectomy, hypogastric artery ligation or other procedures to control bleeding were not necessary in any case.

Discussion

Resection of myomas during pregnancy is generally contraindicated unless the myoma is pedunculated, however the management of fibroids encountered during cesarean section remains a therapeutic dilemma [2, 3, 6, 7]. The main concern associated with cesarean myomectomy is excessive hemorrhage which may require an emergency hysterectomy due to increase in blood supply of the uterus throughout pregnancy. On the other hand, it may be time to reconsider this topic. Fibroids complicating pregnancy occur more frequently now than in the past because many women are delaying childbearing to their late thirties, which is the time for greatest risk of myoma growth. Several studies performed over the last decade have clearly shown that myomectomy at the time of cesarean section is a safe and feasible procedure in experienced hands [1, 4, 8-12]. Kaymak et al. reported 40 cases of myomectomy at cesarean section [1]. In this study the incidence of hemorrhage in the myomectomy group was 12.5% as compared with 11.3% in the isolated cesarean group and the difference was not statistically significant. Ehigiegbga et al. assessed the intraoperative and postoperative complications of cesarean myomectomy in 25 pregnant women [12]. Five required blood transfusions and none required a hysterectomy. They concluded that with adequate experience and the use of high dose oxytocin infusion (intra- and postoperatively), myomectomy at cesarean section is not as hazardous as many believe. Bhatla et al. Li et al. and Hassiakos et al. have also reported similar results with a good surgical outcome of cesarean myomectomy cases [4, 10]. In this present study, there was no difference in the incidence of postoperative fever and hemorrhage between the myomectomy group and the controls. The difference in hemoglobin levels before and 12 hours after the operation was statistically significant compared with patients who underwent cesarean section without myomectomy \((p < 0.05)\). However ten patients who underwent cesarean myomectomy and five patients in the control group needed blood transfusions but the difference was not significant. Of the 70 cases in the study group, none had severe hemorrhage necessitating emergency hysterectomy or any further surgical interventions. Although estimated blood loss, based

<table>
<thead>
<tr>
<th>Table 1. — Patient characteristics.</th>
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<tr>
<td></td>
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<tr>
<td>Maternal age (years)</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>Previous abortions</td>
</tr>
<tr>
<td>Gestational age</td>
</tr>
</tbody>
</table>

NS: not statistically significant.

<table>
<thead>
<tr>
<th>Table 2. — Size, location and type of the removed fibroids.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
</tr>
<tr>
<td>Type</td>
</tr>
<tr>
<td>Intramural</td>
</tr>
<tr>
<td>Subserous</td>
</tr>
<tr>
<td>Submucosal</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

| Size        |
| < 4 cm      | 12 (16.5%) |
| 4-8 cm      | 42 (57.5%) |
| > 8 cm      | 19 (26%)   |

| Location    |
| Fundus      | 27 (36.9%) |
| Corpus      | 25 (34.1%) |
| Cervicoisthmic | 10 (14%)  |
| Multiple    | 11 (15%)   |

<table>
<thead>
<tr>
<th>Table 3. — Surgical outcomes of myomectomy and control groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Mean operative time (min)</td>
</tr>
<tr>
<td>Cesarean myomectomy</td>
</tr>
<tr>
<td>Cesarean alone</td>
</tr>
</tbody>
</table>

| Preoperative hemoglobin values (g/dl)                        |
| 12.7 ± 1.06        | 12 ± 1.1    | NS       |

| Postoperative hemoglobin values (g/dl)                        |
| 9.6 ± 1.5          | 10.8 ± 1.01 | 0.01     |

| Mean difference in hemoglobin change (g/dl)                   |
| 3.09 ± 1.2        | 1.25 ± 0.77 | < 0.01   |

| Emergency cesarean rate                                     |
| 20 (31)           | 31          | NS       |

| Incidence of atony                                          |
| 7 (10%)           | 4 (7.14%)   | NS       |

| Frequency of hemorrhage                                     |
| 12 (17.14%)       | 5 (7.14%)   | NS       |

| Frequency of blood transfusion                              |
| 10 (14.28%)       | 5 (7.14%)   | NS       |

| Frequency of postoperative fever                            |
| 15 (21.4%)        | 8 (11.4%)   | NS       |

| Length of hospital stay (days)                              |
| 3.02 ± 1.58       | 2.40 ± 1.09 | 0.02     |

NS: not statistically significant.
on hemoglobin decrease, was higher in cases of cesarean myomectomy than average blood loss after an uncomplicated cesarean section, the present study shows that myomectomy during cesarean delivery may not be as dangerous as most obstetricians are trained to believe. Our results are in agreement with findings of other researchers. We recommend the use of standard dose oxytocin to obtain a sustained uterine contraction during the myomectomy and for 12-24 h after surgery as was used in this series [13].

In our set-up cesarean myomectomy added a mean of 26 min to the duration of surgery which is longer than the findings in previous reports [1, 4]. The duration of hospital stay in the myomectomy group was also significantly longer which ranged between two and 11 days with a mean of three days, although early postoperative morbidities did not differ from the patients who underwent an isolated cesarean section. Previous studies have yielded conflicting results regarding the effects of cesarean myomectomy on the mean hospital stay and mean operating time. Roman and Tabsh in a retrospective study involving 111 women with myomectomy at cesarean section and 257 women undergoing cesarean section alone noted no significant difference in operative time and length of hospital stay [11]. Previous studies have reported similar results. On the other hand, conflicting results of significantly longer operating time and longer hospital stay in cesarean myomectomy patients than controls have also been reported [1, 14].

Consequently, this study has clearly shown, like several other studies, that myomectomy during cesarean section is not always a hazardous procedure. The advantages include the fact that interval myomectomy is avoided including the risk of a second surgery and anesthesia, and is also cost saving. With the use of standard dose oxytocin infusion during the intra- and postoperative period, the procedure can be performed without any serious bleeding by experienced obstetricians.

References

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The impact of HPV diagnosis on women’s sexual and mental health: preliminary findings

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Summary

Purpose of investigation: The objective of the present study was to demonstrate the impact of HPV diagnosis on sexual function and mental health of Greek women. Methods: The study population included 51 patients who proceeded to the gynecological outpatient clinic of “Aretaieion” Hospital, Athens, during 2008-2009. The participants were asked to complete a questionnaire regarding demographic characteristics, knowledge on HPV, gynecological and sexual history, as well as questions regarding their mental and sexual health after the diagnosis. Results: Mean age of the participants was 36 years and mean age of their first Pap smear test was 24.4 years. Mean age of HPV diagnosis was 34 years and mean number of sexual partners was four. Regarding mental health, the majority experienced anxiety after the HPV diagnosis as well as fear regarding their health in the future. Nearly half of the women experienced guilt and anger and some of them distress, shame, diminution of self-esteem and stigmatization. Diminution in the level of sexual interest and desire and decrease in sexual intercourse frequency were quite often reported. Conclusion: Except for the important physical impact of HPV infection, its diagnosis seems to trigger several negative feelings and reduce sexual desire.

Key words: HPV; Infection; Psychosexual consequences; Psychological/Psychosocial/Interpersonal impact; Psychological aspects of HPV; Reactions to HPV diagnosis.

Introduction

HPV infection is very common among sexually active women. Previous studies have demonstrated that a HPV diagnosis may have an important impact not only on women’s physical health, but also on their sexual and emotional well being. Although there is limited literature on psychosocial reactions to HPV diagnosis, research among women who have received abnormal cervical smear results indicates that they often experience psychological consequences, including anxiety, fears about cancer, sexual difficulties, changes in body image, and concerns about the loss of reproductive functions [1-4]. In addition to the distress caused by these psychological side-effects, fears about gynecologic investigations and treatments have been shown to decrease adherence to follow-up recommendations among women with abnormal Pap tests [3], suggesting that patient counselling to reduce such side-effects has the potential to both enhance psychological well being and improve follow-up and clinical outcomes [5].

Several studies over the last few years have demonstrated negative effects of HPV diagnosis on women’s mental health, including hypochondriac fears, anger, depression, isolation, fear of rejection, shame, guilt, anxiety, stigma, confusion and distress, as well as personal stigmatizing with a significant adverse impact on self esteem and significant relationships [6-8]. Moreover, the receipt of a positive HPV test result often calls sexual fidelity into question, while emotions related primarily to stigma, fear, self-blame, powerlessness, and anger may raise additional concerns for women regarding disclosure of the disease to significant others, usually to a sexual partner [9-11]. Finally, the presence of anogenital warts can cause feelings of anxiety, guilt, anger, loss of self esteem and create concerns about future fertility and cancer risk [12].

Women who tested positive for HPV stated that this diagnosis made them feel ‘less attractive’, ‘tarnished’, ‘let down by their bodies’, ‘defiled’, ‘contaminated’ and ‘dirty’ [13].

HPV seems to also have negative influence on sexual life, affecting sexual enjoyment and activity. Impairments in sleep and sexual interest, as well as a high percentage of sexual impairments after therapy and worsening of the emotional relationship with the partner were noted, aggravating existing sexual problems [1, 14-16]. Additionally, as noted before, testing positive for HPV raised concerns about women’s attractiveness and sexual relationships in terms of trust, fidelity, blame, and protection, particularly for women in long-term monogamous relationships, affecting directly or indirectly sexual life and satisfaction [9-11, 16].

Materials and Methods

The study population included 51 patients who proceeded to the gynecological outpatient clinic of “Aretaieion” Hospital, Athens, during 2008-2009. The initial number of women who were asked to participate in the study was 80, but their final participation depended on their time availability, as well as comprehension of the Greek language (some of the patients were
foremost), therefore the final sample included 51 women. Patients had already been diagnosed with HPV during a previous visit and particularly the HPV subtypes that are responsible for cervical cancer. Therefore they were advised to regularly perform a colposcopy, usually every six months. None of the women participating in the study had a history of another sexually transmitted disease besides HPV.

Participants were asked to complete a questionnaire that included demographic and medical data, as well as questions regarding their sexual and mental health. The completion of the questionnaire took place after the gynaecologic exam (usually colposcopy); it was anonymous and was distributed by a member of the research team (usually a physician other than their gynecologist).

Other than the demographics, the participants were asked to give some information regarding their knowledge of HPV and sexually transmitted diseases (STDs), as well as information regarding diagnosis of HPV (age and duration of diagnosis, how it was diagnosed, the presence of genital warts or not, disclosure to partner), their gynecological history (age of first Pap smear, frequency of Pap smear testing, time since last Pap smear) and their sexual history (age of first sexual intercourse, number of sexual partners, and method of contraception they use).

In the second part of the questionnaire, the participants were asked to state their feelings after HPV diagnosis, in order to investigate the impact of the diagnosis on women’s mental health. Specifically, participants were asked to complete a questionnaire that included 12 different items regarding feelings and reactions after HPV diagnosis. A five-graded scale ranging from “not at all” to “very much” followed the statement: “After HPV diagnosis did you experience: a) physical distress, b) anxiety, c) guilt, d) anger, e) shame, f) negative effect in your self-confidence, g) stigma, h) fear – anxiety regarding your health in the future, i) diminution of sexual intercourse frequency, and k) less sexual life satisfaction”. Answers within the range “quite” to “very much” were considered as positive and therefore the specific feeling or reaction was regarded as present. Participants were also asked to answer the question: “Did HPV diagnosis have a negative effect on your sexual relationship?”. Answering alternatives were “not at all, a little, quite, much, very much”. Similarly, answers ranging from “quite” to “very much” were considered positive answers. Additionally, patients were asked to report the level of worry they experience regarding their HPV infection. Choosing from a six-graded scale: “not at all, not much, a little, quite, much, very much, extremely”. In the third part, the participants were asked to complete the Symptom Checklist of Sexual Function, a short four-item self-report checklist regarding men’s and women’s perception of and satisfaction with sexual function (two versions available, one for each gender), developed by the 2nd International Consultation on Sexual Medicine [17]. The brief symptom checklist is suitable for use in primary care settings and addresses the level of satisfaction with sexual function, using a single question: “Are you satisfied with your sexual function? (Yes/No). In case of a negative response, three additional questions exist, assessing duration that subjects are dissatisfied with their sexual function, the type/s of sexual problems experienced, as well as the willingness of the person to discuss the problem with a health care provider. Its administration time is less than five minutes [17]. Finally, women were asked to report their level of worry regarding their sexual problem, as well as the feelings they would experience if they had to live with it for all their life.

Results

Study population - demographics

The main characteristics of the subjects are shown in Table 1. Mean age of the participants was 36 (min 21, max 68) years and 86.3% of them lived in Athens. The majority (62.8%) were highly educated (college or university) and married or in a stable relationship (60.8%).

Knowledge of HPV and STDs

Most of the participants reported that they knew about HPV (76%) and cervical cancer (70%), as well as the fact that HPV is the main causal factor of cervical cancer (82.2%). Furthermore, they believed that HPV is frequent or very frequent (82%) among sexually active women and that condom use can prevent HPV transmission (72%) as well as the development of cervical cancer (62%). The vast majority of women were also aware of the vaccine against HPV (78%). Regarding other STDs, the majority (> 76%) were aware that HIV, syphilis, Chlamydia, hepatitis and genital herpes are sexually transmitted, while half of them (50%) knew about the sexual transmission of gonorrhea. Finally, the major source of information for the patients regarding STDs was medical staff (80%), followed by magazines (50%), friends, TV and the Internet (32-38%).

Diagnosis of HPV

Mean age of HPV diagnosis was 34 years. Most women found out about HPV through Pap smear testing (72%) and the rest by the presence of genital warts (18%), colposcopy (8%) or biopsy (2%). One third of the participants (32%) had genital warts at some point during their infection. Interestingly enough, nearly all women (89.8%) told their sexual partner about the HPV diagnosis.

Gynecological & sexual history

Mean age of first Pap smear testing was 24.4 (min 17, max 40) years and mean frequency of testing for the women in our sample was every 9.4 months. The majority of participants had had sexual activity during the previous year (88.2%) and 60.8% of them during the prior month. The most common contraceptive method they used was the condom (37.3%) and 23.5% preferred withdrawal during ejaculation of the partner, while 37.3% of our sample did not answer regarding the contraceptive method they used. Interestingly enough, none of the women currently use the pill, although 41.2% have taken it at some point in their life mainly for gynecological reasons (e.g. menstrual cycle regulation). Mean number of sexual partners during the lifespan was four (min 1, max 10), except for five participants who stated to have had more than ten sexual partners in their life. These data points were not taken into account and were excluded as outliers since they had a strong influence on the mean number of sexual partners. Finally, mean age of first sexual intercourse was 18.8 (min 15, max 32) years.
The impact of HPV diagnosis on women’s sexual and mental health: preliminary findings

Is there an impact of HPV on women’s mental health?

HPV diagnosis was a stressful life event for most of the participants, since 76.5% of them experienced anxiety (grades “quite-very much”) as well as fear and anxiety regarding their health in the future (82.4%). Nearly half of them (41.1%) experienced guilt (grades “quite-very much”) and anger (43.1%). Overall, 17.6% of the subjects experienced physical distress, 21.5% shame, 21.6% a diminution of their self-esteem and 15.7% felt stigmatized by the diagnosis (grades “quite-very much”). Finally, 80% of the participants stated that they were still worried (ranging from “quite” to “extremely”) regarding their health problem related to HPV (Table 2).

Is there an impact of HPV on women’s sexual health?

Nearly half of the participants (41.2%) reported that they experienced a diminution of sexual desire after HPV diagnosis, as well as a diminution in the frequency of sexual intercourse (43.1%). One third (33.3%) felt “less sexual” after the diagnosis and 21.5% reported that they experienced less satisfaction from their sex life. Nevertheless, not many women (11.7%) believed that HPV diagnosis did negatively affect their relationship with their partner. Regarding the participants’ answers on the SCSF, 74.5% reported satisfaction with their sexual function, while the most prevalent sexual problem was little or no interest in sex (35.3%), followed by pain during sex (23.5%), orgasmic problems (21.6%), problems with reduced genital sensation (17.6%), problems with reduced vaginal lubrication (13.7%) and other problems (7.8%) [Table 3].

Women who are married or in a stable relationship compared to single women

Chi-square analysis was performed to compare married women (or women who were in a long-term stable relationship) to single ones, regarding their mental and sexual health after the HPV diagnosis. No significant differences were found on any of the variables of mental health, except for the item “shame”, while married women seemed to experience shame more often than singles (p < 0.05). Similarly, no statistically significant differences were found regarding sexual health, except for the problem “little or no interest in sex”, where married women reported it more often than single ones (p < 0.05).

Discussion

The primary objective of the present study was to assess the degree to which HPV diagnosis affects women’s sexual and mental health. The sample included well-educated women, highly informed regarding HPV as well as other sexually transmitted diseases. Probably, seeking information regarding HPV follows a HPV diagnosis while Greek women do not get official education on STDs or sexual health during high school. Additionally, based on the current results, a quite long period of time (6 years)

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Table 1. — Demographic and social characteristics.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29 years old</td>
<td>19 (37.3%)</td>
</tr>
<tr>
<td>30-39 years old</td>
<td>16 (31.4%)</td>
</tr>
<tr>
<td>40-49 years old</td>
<td>7 (13.7%)</td>
</tr>
<tr>
<td>&gt; 50 years old</td>
<td>9 (17.9%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relationship status</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>17 (33.3%)</td>
</tr>
<tr>
<td>Divorced</td>
<td>8 (15.7%)</td>
</tr>
<tr>
<td>Single/In occasional relationships</td>
<td>12 (23.5%)</td>
</tr>
<tr>
<td>In a long-term relationship</td>
<td>14 (27.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High school</td>
<td>19 (37.2%)</td>
</tr>
<tr>
<td>College or University graduate</td>
<td>32 (62.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Employment status</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Public employee</td>
<td>14 (27.5%)</td>
</tr>
<tr>
<td>Private employee</td>
<td>10 (19.6%)</td>
</tr>
<tr>
<td>Self-employed</td>
<td>5 (9.8%)</td>
</tr>
<tr>
<td>Student</td>
<td>7 (13.7%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>7 (13.7%)</td>
</tr>
<tr>
<td>Housewife</td>
<td>4 (7.8%)</td>
</tr>
<tr>
<td>Pensioner</td>
<td>4 (7.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athens</td>
<td>44 (86.3%)</td>
</tr>
<tr>
<td>Rural areas</td>
<td>7 (13.7%)</td>
</tr>
</tbody>
</table>

Table 2. — Mental health and psychological reactions after HPV diagnosis.

| After HPV diagnosis, did you experience: | Number of women (%)| |
|----------------------------------------|--------------------|
| Distress                               | 17.6%              |
| Anxiety                                | 76.5%              |
| Guilt                                  | 41.1%              |
| Anger                                  | 43.1%              |
| Shame                                  | 21.5%              |
| Diminution of self-esteem              | 21.6%              |
| Stigmatization                         | 15.7%              |
| Fear and anxiety regarding your health in the future | 82.4% |

| How much do you worry about your problem (HPV infection)? | Number of women (%)| |
|---------------------------------------------------------|--------------------|
| Extremely                                               | 11.2%              |
| Very much                                               | 28.9%              |
| Quite                                                   | 40%                |
| A little                                                | 6.7%               |
| Not much                                                | 11.1%              |
| Not at all                                              | 2.2%               |

Table 3. — Sexual health and sexual function after HPV diagnosis.

| After HPV diagnosis: | Number of women (%)| |
|---------------------|--------------------|
| 'I felt less “sexual”' | 33.3%             |
| 'The level of my sexual desire decreased' | 41.2% |
| 'The frequency I had sexual intercourse decreased' | 43.1% |
| 'I was dissatisfied with my sexual life' | 21.5% |
| 'My relationship was negatively affected' | 11.7% |

| Symptom Checklist of Sexual Function - Women (SCSF) | Number of women (%)| |
|-----------------------------------------------------|--------------------|
| Are you satisfied with your sexual function?        | 74.5%              |
| Do you have problems with little or no interest in sex? | 35.3% |
| Do you have problems with reduced genital sensation? | 17.6% |
| Do you have problems with reduced or loss of vaginal lubrication? | 13.7% |
| Do you have orgasmic disorders?                     | 21.6%              |
| Do you have pain during intercourse?                | 23.5%              |
| Do you have other sexual problems?                  | 7.8%               |

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Discussion

The primary objective of the present study was to assess the degree to which HPV diagnosis affects women’s sexual and mental health. The sample included well-educated women, highly informed regarding HPV as well as other sexually transmitted diseases. Probably, seeking information regarding HPV follows a HPV diagnosis while Greek women do not get official education on STDs or sexual health during high school. Additionally, based on the current results, a quite long period of time (6 years)
elapsed between first intercourse and first gynecological check up, demonstrating that women should be educated and motivated to seek preventive care earlier in their life.

Except for the important physical impact of HPV infection, its diagnosis seems to trigger several negative feelings to a woman such as anxiety, guilt, anger and fear of negative consequences on their general health in the near future. These findings are consistent with previous studies [1-4, 6-8,12], and they all demonstrate the importance of emotional well being for these women, since it may increase adherence to follow-up recommendations and improve clinical outcomes. HPV diagnosis is a stressful life event that raises fears and worries regarding physical health, reproduction and sexuality, which are quite important issues for women in their mid-thirties.

Additionally, several sexual dysfunctions may arise following HPV diagnosis, with the most prevalent being reduced sexual desire disorder and pain during coitus. Supported also by other studies [1, 14-16], testing positive for HPV raises concerns about a woman’s attractiveness and self-esteem, trust in the relationship with the partner and blame for the infection, affecting both sexual life and pleasure.

Limitations of the present research include the likelihood of some degree of sample self-selection since participants volunteered to participate, depending on their time availability and their academic abilities to answer the questions. A second limitation is the fact that the results were based on a small, non-randomized, specific sample. Nonetheless, since the present study presents preliminary findings and is still underway, further research based on larger sample sizes is essential to investigate the extent that HPV diagnosis influences women’s emotional and sexual well being.

Conclusion

Although the results of the present study are not representative of the general population, HPV diagnosis seems to have an adverse impact on self-esteem and significant relationships. Since evidence on the psychosexual impact of HPV is scarce, further research is important to determine patients’ needs and further improve their management.

References

Magnetic resonance hysterosalpingography in the evaluation of tubal patency in infertile women: an observational study

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¹Department of Radiological Sciences, ²Department of Gynecology and Obstetrics “Sapienza” University of Rome, Rome (Italy)

Summary

The purpose of this study was to evaluate the ability of magnetic resonance hysterosalpingography (MR-HSG) to demonstrate fallopian tube patency in infertile women and to improve the MR-HSG technique. Sixteen consecutive infertile women were recruited for this trial. All subjects underwent clinically indicated MR-HSG: 10-15 ml of 1:10 solution of gadolinium and normal sterile saline (0.9%) was gently hand-injected intracervically through a 7 French balloon catheter while seven consecutive flash-3D dynamic (FL 3D DY) T1-weighted MR sequences were acquired. Two readers independently assessed image quality as well as anatomic and pathologic correlations. Patient comfort was evaluated using a specific score questionnaire. MR-HSG was successfully completed in all patients. In 14/16 (87.4%) patients, MR-HSG showed bilateral tubal patency with symmetric contrast agent diffusion and a regular tubo-ovarian relationship. One patient (6.3%) with monolateral hydrosalpinx presented no contrast agent diffusion in the affected side (monolateral tubal occlusion); in another patient (6.3%) the fallopian tube was displaced upward causing loss of the tubo-ovarian anatomical relationship resulting in asymmetric and inadequate contrast agent diffusion. Eight women (50%) were found to have abnormalities on MR imaging; these abnormalities included multi follicular ovaries (5 cases 31.1%), endometrioma (1 case, 6.3%), leiomyoma (1 case / 6.3%) and endometrial polyp (1 case / 6.3%). The average time required for the study was 25-30 minutes. Analysis of the questionnaires administered to the patients showed that 15/16 patients (93.7%) were fully satisfied with the procedure. All examinations were judged to be of high diagnostic quality and the two readers made similar diagnoses. In conclusion, MR-HSG is a feasible, useful and well tolerated tool for the assessment of the uterus, fallopian tubes, ovaries and extra-uterine structures. MR-HSG is a new promising imaging approach to female infertility.

Key words: Female infertility; Tubal factor; Magnetic resonance hysterosalpingography.

Introduction

The current trend to defer childbirth to a later age has resulted in an increasing number of couples presenting with infertility problems. Approximately 15% of couples of reproductive age are confronted with infertility, which is defined as the failure to conceive after one year of unprotected intercourse [1-2]. In about half of these cases infertility is caused by female factors, and abnormalities of the reproductive tract are commonly detected. The etiology of female infertility can be found in several factors (tubal, ovulatory, cervical, uterine, endometriosis, age and lifestyle) that may be isolated or combined [3-6].

Tubal pathology is one of the main causes of infertility. It is estimated to account for 12%-33% of cases [7-9]. This figure is probably underestimated, as most aspects of tubal dysfunction escape observation.

Tubal pathology is usually associated with peritubal adhesions and tubal occlusion. In routine fertility work-up, the ability to evaluate tubal function is limited and the degree of tubal damage is judged mainly by tubal patency and the extent of peritubal adhesions [10].

Hysterosalpingography (HSG) is a radiographic method used in routine infertility evaluation. It is used in many infertility centers as a preliminary investigation tool and it is considered the gold standard for assessing fallopian tube patency. HSG is a reproducible examination, easily and rapidly performed at a reasonable cost [10-15]. This procedure allows diagnosis of congenital uterine abnormalities, acquired alterations of the uterine cavity and stenosis or occlusion of the fallopian tubes [11]. HSG shows a good diagnostic accuracy (sensitivity 81.8%, specificity 77.1%) in the evaluation of fallopian tube patency while sensitivity and specificity are low in the evaluation of abnormalities of the uterine cavity. HSG is considered mainly a diagnostic tool, but there may also be a possible therapeutic benefit in connection with this procedure due to the flushing effect [12-15]. However, conventional HSG exposes the reproductive organs of young potentially fertile woman to ionizing radiation, and moreover, the procedure can be uncomfortable or painful [16-18].

For these reasons, the scientific community’s attention has in recent years been focused on alternative methods such as hysterosalpingo-contrast-sonography (HyCoSy) and magnetic resonance imaging (MRI) which would allow a full assessment of the potential causes of infertility in women without administration of ionizing radiation [15, 19-23]. MRI is gradually gaining importance in the investigation of the female reproductive tract [19-23] for its ability to display soft tissue contrast. Gadolinium-based con-
The aim of the present study was to evaluate the feasibility of MR-HSG in the demonstration of fallopian tube patency in infertile women and to optimize the MR-HSG technique.

Materials and Methods

This prospective observational study was approved by the local ethics committee and written informed consent was obtained from all patients. The sample was built up from December 2010 to March 2011 at the Department of Radiological Sciences, University of Rome “Sapienza” among women with primary or secondary infertility. Absence of pregnancy after 12 months of unprotected intercourse, exclusion of male infertility and hormonal disorders provided the indication for MR-HSG.

Exclusion criteria were refusal or inability to sign the informed consent form, age < 18 years, clinical history of intolerance all patients received oral administration of 300 ml of Lumirem (Ferumoxsil 175 ml/l, Laboratoire Guebert, France).

MR examination was performed on a 1.5T MRI unit (Avanto, Siemens Medical Solutions, Germany) using a four-element phased-array surface coil (Body Matrix Coil; Siemens Medical Systems; Germany).

For the catheterization a 7 French balloon catheter (PBN Medicals Denmark A/S, Stenlose, Denmark) was used. Catheterization was performed on a non-magnetic stretcher in the medical center next to the MRI room under sterile conditions and adequate privacy. The patients were placed in a gynecological position, the vagina was dilated by a gynecological dilator and the external orifice of the uterus was catheterized. A balloon catheter was inflated with 2-3 ml of normal sterile saline and its stability was verified by gentle traction in the caudal direction.

The speculum was removed and circa 5-10 ml of sterile saline (0.9%) was injected into the uterine cavity through the catheter. The patients were transported to the MRI room on a non-magnetic stretcher and placed in the supine position on the mobile MRI bed.

Before the procedure, the correct position of the balloon catheter was verified using a sagittal T2-weighted half-Fourier acquired single-shot turbo spin-echo (HASTE) sequence. Subsequent standard imaging of the pelvis consisted of axial and coronal T2-weighted HASTE sequences performed in order to
Table 2. — Analysis of the questionnaires administered to the patients 7 days after MR-HSG examination.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Score (mean values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain during catheterization</td>
<td>2.97</td>
</tr>
<tr>
<td>Execution time</td>
<td>2.73</td>
</tr>
<tr>
<td>Pain during contrast injection</td>
<td>2.74</td>
</tr>
<tr>
<td>Lumirem palatability</td>
<td>2.15</td>
</tr>
<tr>
<td>Total score</td>
<td>10.59</td>
</tr>
</tbody>
</table>

study the morphology of the uterine cavity which was hyperintense due to injection of saline and the field of view (FoV) for the subsequent dynamic sequences was set. FoV was oriented parallel to the major axis of the uterus and was large enough to also cover the ovaries.

Subsequently, approximately 10-15 ml of a 1:10 mixture of Dotarem (Acidum gadoteras, 0.5 mmol/ml; Laboratoire Guerbert, France) diluted with normal sterile saline (0.9%) was gently hand-injected through the catheter into the uterine cavity when the acquisition of dynamic sequences was started. All patients were told to inform the radiologist if pain occurred during the injection.

For the dynamic MR-HSG, seven consecutive fat-saturated T1-weighted gradient echo FLASH 3D DYNAMIC (FL 3D DYN) sequences were acquired during intrathecal injection of contrast solution.

One of the following two findings led to the diagnosis of tubal patency: 1) contrast enhancement in the periovian region and 2) evidence of contrast agent in the pouch of Douglas.

Following an axial T1-weighted turbo spin-echo (TSE) sequence with fat saturation (FS), an axial T2- weighted TSE sequence was performed to visualize the distribution of contrast solution in the peritoneal cavity. The MR-HSG protocol is reported in Table 1. All patients were kept under observation for about half an hour after the examination and they were given a telephone number to contact in case of complications.

Seven days later when the patients came to collect the MRI report, a questionnaire was administered to assess the degree of satisfaction with the examination. The parameters were: pain during catheterization (mild, moderate, severe); pain during injection of contrast medium (mild, moderate, severe); execution time (short, acceptable, long); palatability of Lumirem (good, acceptable, poor).

Each parameter was assigned a score from 1 to 3. The score (range 4-12) was evaluated as follows: 4-6 = examination is not satisfactory; 7-9 = examination is satisfactory; > 10 = examination is considered very satisfactory. In the same questionnaire the patients were asked to state possible onset of bleeding, vaginal discharge, vaginal itching/burning and pelvic pain in the days following the examination.

A set of dynamic subtraction images was reconstructed and evaluated similar to the MRI angiographic procedure. Both the subtracted images and the anatomic images were reviewed independently on a PACS system (Infinitt Healthcare Ltd, Korea) by two expert readers. The readers evaluated image quality, the different parts of the pelvic anatomy, unilateral or bilateral fallopian tubes as well as the uterine cavity with regard to congenital anomalies or pathologies such as fibroids, adenomyotic lesions and uterine polyps. The subtracted images were viewed by manually scrolling through the series on the PACS workstation and were not viewed in cine mode. Assessment of functional aspects included fallopian tube patency in general, localization and extent of filling defects as well as spillage and distribution of contrast agent into the peritoneal cavity.

Results

A total of 16 infertile women (mean age 35.5; range 28-44 years) were recruited for this study. Indications were primary infertility in 12 patients (75%) and secondary infertility in four patients (25%).

MR-HSG was successfully completed in all patients: positioning of the catheter was feasible in all patients and no complication occurred during cervical cannulation. In one (6.3%) patient with moderate stenosis of the external orifice of the uterus, dilation was performed using a Hegar dilator size 4; another patient (6.3%) whose cervical canal was bent consented to caudal traction of the cervix using Collin’s uterine forceps in order to straighten the cervical canal and thereby facilitate the introduction of the catheter. In all cases T2-weighted HASTE sequences showed correct positioning of the catheter (Figure 1) using the scans in the sagittal plane.

Manual injection of 5-10 ml sterile saline (0.9%) made the uterine cavity hyperintense and T2 HASTE sequences in the coronal plane showed the regularity of the profile in 15/16 (93.7%) cases and a slightly arched uterine fundus in the remaining cases (6.3%).

Analysis of dynamic sequences, subtraction images and maximum intensity projection (MIP) reconstructions confirmed tubal patency in 14/16 (87.4%) cases. The images showed sequential opacification of the uterus and intramural portion of the tubes as well as bilateral spillage of contrast agent into the peritoneal cavity permitting also evaluation of diffusion time and symmetry (Figure 2 a). In eight cases (50%) the ampullary portion of the tube was depicted (Figure 2 b), while it was possible to identify the intramural portion in only two cases (12.5%) (Figure 2 c).

In 14/16 (87.4%) cases contrast agent diffusion was symmetrical and comparison of base-line and dynamic images using dedicated software confirmed the regularity
of the tubo-ovarian relationship (Figure 2 d/e). In one case (6.3%) hydrosalpinx of the left tube with unilateral tubal occlusion were detected. In another case (6.3%), the right fallopian tube was cranially displaced resulting in loss of the anatomical relationship with the ipsilateral ovary and inadequate contrast agent diffusion.

The endometrium, myometrium, ovaries and pelvic ectopic structures were studied using T2-weighted HASTE, TSE and T1-weighted TSE FS sequences.

Eight patients (50%) were found to have abnormalities on MRI including multifollicular ovaries: five patients (31.1%) had multiple small follicular cysts surrounded by thickened and luteinized theca suggestive of polycystic ovarian syndrome [29-32]; one had endometrioma (6.3%), one had small intramural myoma (6.3%) and one had an endometrial polyp (6.3%) that appeared as a small filling defect in the dynamic sequences. Average time required to perform the examination was 25-30 min: balloon catheter positioning 5-10 min and MRI data acquisition 20-25 min. Analysis of the questionnaires administered to the patients one week after the procedure showed that 15/16 patients (93.7%) were satisfied with the procedure resulting in an average score of 10.59 (Table 2). Catheter implantation and MRI examination were generally well tolerated and only 1/16 patients (6.3%) indicated “severe pain”. Lumirem palatability was considered poor by most of the patients (68.7%) and one patient had adverse reactions such as nausea and diarrhea. This event did not affect the outcome of the examination but it significantly reduced the score assigned by the patient in question (score 8). There were no cases of bleeding, vaginal discharge, symptoms related to bacterial vaginosis or pelvic pain in the days following the procedure. All examinations were judged to be of high diagnostic quality, and the two readers provided similar diagnoses.

Discussion and Conclusions

To our knowledge, this is the first Italian experience with MR-HSG in infertile women. The purpose of the present study was to evaluate the ability of MR-HSG to depict tubal patency and to optimize the technique in order to reduce complications and failure rate. At present HSG is the method of choice for the evaluation of tubal patency in infertile women. However, the most important

Figure 2. — FLASH 3D DYN T1-weighted images after intrauterine contrast injection (MIP). Accumulation of contrast solution in the uterine cavity appears regular and there is initial filling of the intramural portion of the tubes with bilateral and simultaneous spilling of contrast medium into the peritoneal cavity: bilateral tubal patency (a). Initial non subtracted FLASH 3D DYN T1-weighted images from series of 7 sequences after intrauterine contrast injection reveal the ampullary portion of the left tube (arrowhead) (b) and the intramural portion of the right tube (arrowhead) (c). Comparative analysis of final non subtracted FLASH 3D DYN T1-weighted images from the same series (d) and baseline T2-weighted TRUE FISP (e) shows the symmetrical spillage of contrast agent in the periovarian region on the left and in the periuterine region on the right (arrows).
limitations of HSG are the insufficient evaluation of other causes of infertility, such as myometrial abnormalities and extraterine diseases, and the exposure to ionizing radiation [5-7]. Ovarian doses range from 3.1 mGy in analog systems to 0.5 mGy in digital systems [16-18] and the procedure usually involves at least two radiographic exposures in addition to fluoroscopic exposure. Furthermore, although HSG is a relatively quick outpatient procedure, it is uncomfortable and often painful to the patient due to osmotic irritation of the endometrial and peritoneal tissue caused by iodinated contrast agents [9, 10].

Over the years, MRI has become an increasingly used diagnostic tool [19-25] and this method has the potential to become useful also for evaluating pelvic disorders associated with female infertility. Women affected by infertility are often referred to MRI for the diagnosis of possible uterine and extrauterine abnormalities, and simultaneous assessment of tubal patency would therefore be beneficial, as this would permit the patient to avoid administration of ionizing radiation [19, 20, 30-32]. The MR-HSG procedure performed in this study clearly demonstrated the anatomy of the reproductive organs including the myometrium and ovaries allowing at the same time assessment of tubal patency and the study of the extrauterine pelvic structures. MR-HSG may thus provide a more accurate diagnosis of all the possible causes of female infertility.

Assessment of diffusion time and symmetry as well as spillage of contrast agent into the pelvic cavity makes it possible to evaluate the functional aspects of the tubal factor assuming that delayed and/or asymmetrical contrast agent diffusion is suggestive of functional abnormalities of the fallopian tubes. The tubal factor includes also loss of a tubo-ovarian anatomical relationship mainly due to peritubal adhesions caused by inflammation and/or endometriosis [8]. This aspect was studied using dedicated software which allowed a combined analysis and comparison of baseline and dynamic sequences in order to establish the relationship between the uterine tubes and ovaries. MR-HSG thus allows an overall assessment of the tubal factor by studying both morphological and functional aspects.

The most important disadvantages of MR-HSG are the high costs and the duration. However, these disadvantages are largely weighed up by the advantage of obtaining more information about all the organs of the pelvis and especially by the fact that exposure to ionizing radiation is avoided [28]. All patients enrolled in this study stated that they were satisfied with the duration of the examination.

In the present study the attention was focused on certain aspects of the procedure and the attempt to improve the technique in order to avoid the difficulties reported in the literature. MR-HSG was performed in the early follicular phase of the menstrual cycle after exclusion of infections. The endometrium is thin during this proliferative phase and this facilitates a better image interpretation and should also ensure that the patient is not pregnant. Later in the cycle, focal contour irregularities of the endometrium may be mistaken for small polyps or focal areas of endometrial hyperplasia and may cause a false-positive diagnosis of corneal occlusion [14]. The absence of post-procedure infectious complications in the present study population may be related to the microbiological screening and antibiotic prophylaxis administered before the examination.

Winter et al. [28] studied 37 patients and reported an incidence of malpositioning of the catheter in 5.4% of the patients and displacement during injection of contrast medium in 8.1%; moreover, in 5% of cases the examination was not concluded due to strong pain during contrast injection. Sadowski et al. [29] performed MR-HSG in 17 patients and reported a failure rate of 5.4% caused by excessive patient movement due to strong pain felt during the automatic injection of contrast agent.

To reduce the incidence of such events, the anatomy of the external orifice of the uterus and the cervical canal was studied in each patient to detect possible anatomical abnormalities which might prevent positioning and/or stability of the catheter during injection of contrast medium.

The absence of complications related to catheterization (malpositioning or dislocation of the catheter) seems to confirm the importance of preliminary assessment to determine the possible need for procedures in addition to the standard protocol such as dilation of the cervical canal using a Hegar dilator and/or traction using Collin’s uterine forceps. Ultrasound determination of the position of the uterus performed by the same radiologist, who carried out MR-HSG, led to the decision to perform the examination with a full or empty bladder in order to reduce the flexion of the cervical canal and thereby facilitate the introduction of the catheter. To avoid pain during injection of contrast agent, muscle relaxant medication was orally administered two hours before the procedure, and injection of contrast agent was carried out manually. Instead of intravenous medication as administered by Winter et al. [28] oral medication was preferred as it is more easily accepted and better tolerated by the patients. In our opinion, the use of an automatic injector device is not beneficial because the inability to control and modulate the quantity, pressure, and timing of injection of contrast agent increases the likelihood that the patient feels pain. Moreover, a fast and continued distention of the uterine cavity may cause a reflex spasm of the intramural portion of the tube leading to a false-positive diagnosis of proximal occlusion.

The ability to adjust injection speed in order to avoid pain is essential to the good outcome of the examination, as the response of the myometrium during the introduction of contrast agent is highly subjective and influenced by pain. Also the fact that only a small quantity of contrast agent is injected (10-15 ml) has a positive effect on the patient’s perception of pain. Finally, manual injection may reduce the risk of dislocation of the catheter in the vagina.

Lumirem is a negative, superparamagnetic contrast agent that has so far mainly been orally administered for...
bowel contrast in MRI intestinal exploration. The ration-
ale for its use in this study lies in the ability to reduce sig-
als coming from the intestinal contents and to prevent artifac-
to MR-HSG images. In all cases the images were of excellent diagnosti-
uc quality, but due to the poor palatability of Lumirem, the real utility of this product and its use in future examinations will be subject to eval-
uation.

In this study preliminary assessment of the cervix and position of the uterus, oral intake of muscle relaxant med-
ication and manual injection of contrast agent produced ex-
cellent results. Also the fact that all examinations were completed, unlike other studies [28, 29], allows us to assert that the above procedures are useful and can be re-
commended.

In conclusion, this study has provided proof of the util-
ity of dynamic MR-HSG assessment of the uterus, extra-
uterine pelvic structures and tubal factor in a “one-stop shop” session without exposure to ionizing radiation. MR-HSG was furthermore well tolerated by the patients, but further research is required to determine if this tech-
ique can become an alternative to conventional HSG.

References

[9] Cheong Y.C., Li T.C.: “Evidence-based management of tubal dis-
Effects of methylene blue, pentoxyphylline and enoxaparin on postoperative adhesion formation and markers of angiogenesis in a rat uterine horn model

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Summary

Objective: Postoperative adhesions still remain as a common and serious problem leading to morbidity, mortality and economic loss. Adhesions are the major cause of postoperative intestinal obstruction, infertility, and chronic pelvic pain. In this study, we aimed to compare adhesion prevention effects of pentoxyphylline, enoxaparin and methylene blue and to investigate the effects of these agents on angiogenesis, which is suggested as an important step in wound healing, in rat uterine horn model. Material and Methods: Forty female Wistar albino rats were randomized into four subgroups and underwent laparotomy. Adhesions developed following cauterization at the anti-mesenteric surfaces of both uterine horns. After 14 days, adhesions were investigated by using macroscopic, histopathological and immunohistochemical [vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF-β), platelet-derived growth factor (PDGF)] methods. Results: We found that enoxaparin significantly reduced adhesion formation. Pentoxyphylline had no significant effect on adhesion formation, whereas methylene blue had a significant decreasing effect on histopathologically determined adhesion markers and it may affect angiogenesis through PDGF. Conclusion: Among three agents, which were intraperitoneally given by a single dose manner in order to prevent postoperative adhesions, methylene blue and enoxaparin exhibited a positive effect, while no such effect was shown with pentoxyphylline.

Key words: Rat; Uterus; Adhesion; Methylene blue; Pentoxyphylline; Enoxaparin; Angiogenesis.

Introduction

Adhesions are the structures which contribute to revascularization of tissues with impaired blood supply, limit infection and prevent leakage from anastomosis during the healing period of traumatized tissues [1, 2]. Pathological adhesions are the primary cause of postoperative intestinal obstruction, infertility and chronic pelvic pain [3].

By microscopic evaluation, it was shown that there are very thin vascular structures, namely angiogenesis, within adhesions [4]. Angiogenesis is a complex phenomenon involving migration, proliferation, maturation and organization of endothelial cells within capillary tubes by angiogenic stimulation. Angiogenic stimulation occurs via release of pro-angiogenic cytokines and growth factors from inflammatory cells, pericytes and tumor cells, mainly from leukocytes, macrophages and mast cells. Some of these factors induce proliferation and migration of endothelial cells by directly binding to surface receptors, whereas others stimulate local stromal and inflammatory cells to induce angiogenesis [5, 6]. Some of these are vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF).

The role of VEGF, through effects on fibroblast function, has been demonstrated in the restoration process of tissues, such as early inflammatory response, wound repair and remodeling [7]. Basic fibroblast growth factor (later called bFGF), also termed as FGF-2, is a well-documented angiogenic growth factor and induces endothelial cell replication, migration and extracellular proteolysis [8-10]. PDGF-BB is involved in neo-vessel stabilization and functionalization by inducing anastomoses and recruiting pericytes. Vessel stabilization has been shown to be dependent on expression of PDGF β-receptors, which are expressed by fibroblasts, endothelial cells and smooth muscle cells [11, 12]. PDGF-BB also stimulates production of extracellular matrix proteins from pericytes, including fibronectin, collagen and proteoglycans which are necessary for the basal membrane of capillaries. In addition, PDGF-BB increases expression levels of VEGF in mural cells and stimulates fibroblasts to produce and secrete collagenases, which are key factors for cell migration in the angiogenesis [13]. TGF-β may play a role in the formation and maintenance of fibrous adhesions following intraperitoneal injury [14].

Production of oxygen radicals, inhibition of nitric oxide synthase (NOS) and K channels can be specified among pharmacological effects of methylene blue (MB) [15]. It was shown that 1% concentration of MB was effective in the prevention of adhesion formation [16]. However, it was mentioned that both higher (5-7%) and lower (0.1-0.5%) concentrations were ineffective [17].

Pentoxyphylline is a non-selective phosphodiesterase inhibitor, which has significant immunoregulatory and anti-inflammatory effects. Pentoxyphylline inhibits activation and adhesion of peripheral blood T lymphocytes in...
vitro [18]. It was reported that it reduced formation of intraperitoneal adhesions in the anastomosis area in rats after intesinal resection [19].

Enoxaparin is a low molecular weight heparin (LMWH). LMWHs are fragments of heparin, which can no longer be fragmented, and produced by controlled enzymatic and chemical depolymerization [20]. It was observed that heparins inhibited capillary tube formation by human endothelial cells from a macrovascular bed, which is stimulated by proangiogenic factors such as FGF-2 and VGEF; it was also suggested that this inhibitory capacity of heparin is dependent on its molecular weight [21].

In this study, it was aimed to evaluate the effects of methylene blue, pentoxyphylline and enoxaparin on adhesions in a uterine horn model by using macroscopic, histopathological and immunohistochemical methods.

Materials and Method

Forty female Wistar albino rats (10-12 weeks old; 200-220 g weight) were used. They were housed five animals per cage with the appropriate diet and water ad libitum. All rats were observed for several days to ascertain health before operations. All procedures were approved by and performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University.

Each rat was anesthetized by using 40 mg/kg intravenous ketamine hydrochloride before the surgery, the abdomen was shaved and prepared with povidone iodine solution. Using a sterile technique, a 3 cm midline vertical incision was made and both uterine horns were exposed; then 2 cm segments of each uterine horn were traumatized at ten spots on the anti-mesenteric surface using unipolar cautery (Elman Surgitron, Leo-farma, Istanbul, Turkey). Care was taken to avoid gross bleeding from injured sites. Handling of other tissues was minimized.

Rats were randomly assigned into four groups each consisting of ten rats. Treatment groups were as follows: (C) control group, 2 ml saline solution only; (E) enoxaparin group; (MB) methylene blue group; (P) pentoxyphylline group.

Enoxaparin (Clexane, Sanofi Aventis, Istanbul, Turkey) solution was obtained by diluting 50 anti-Xa IU/ml of enoxaparin. Methylene blue (Methylene blue, Sigma, USA) was diluted to obtain 1% solution and pentoxyphylline (Trental ampul, 100 mg/5 ml, Aventis Pharma, Istanbul, Turkey) solution was obtained by diluting 50 anti-Xa IU/ml of enoxaparin. Methylene blue (Methylene blue, Sigma, USA) was diluted to obtain 1% solution and pentoxyphylline (Trental, 100 mg/5 ml, Sanofi Aventis, Istanbul, Turkey) solution was diluted to obtain a solution of 5 mg/ml. Before abdominal sterilization, all therapeutic agents (2 ml) and saline (2 ml) were instilled onto uterine horns. The incision was closed in a single layer, excluding the peritoneum with a running 4-0 monofilament delayed absorbable suture. The total operative time was less than 10; a 2-week recovery period was allowed.

On postoperative day 14, animals were sacrificed by cervical dislocation. A transverse sub-costal incision was made above the cephalad extent of the midline laparotomy site, and the abdominal cavity was inspected for the presence of adhesions. The extent and severity of adhesions in the operation site for each uterine horn were evaluated and recorded by an investigator blinded to the treatment group according to criteria proposed by Linsky et al. [22]. The extent of adhesions was graded as follows: 0, no adhesion; 1, 25% of traumatized area; 2, 50% of traumatized area; 3, total involvement. The severity of adhesions was graded as follows: 0, no resistance to separation; 0.5, some resistance (moderate force required); 1, sharp dissection needed. Total adhesion score (TAS) was recorded as arithmetic sum of severity and extent of adhesions [23].

Biopsy materials of all four groups were fixed by using 10% formaldehyde solution to perform histological evaluation and histochemical and immunohistochemical staining. Hematoxylin-eosin stained slides obtained from paraffin blocks

Table 1. — Histological adhesion score.

<table>
<thead>
<tr>
<th>Grade</th>
<th>None</th>
<th>Mild</th>
<th>None</th>
<th>Mild</th>
<th>None</th>
<th>Mild</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; vs control group.

Table 2. — Macroscopic median adhesion scores of all groups, (minimum-maximum).

<table>
<thead>
<tr>
<th>Adhesion</th>
<th>Control (n = 10)</th>
<th>Enoxaparin (n = 10)</th>
<th>Methylene blue (n = 10)</th>
<th>Pentoxyphylline (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
</tr>
<tr>
<td>Severity</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
</tr>
</tbody>
</table>

* p < 0.05; vs control group.

Table 3. — Histopathological and histochemical median findings of all groups (minimum-maximum).

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Control (n = 10)</th>
<th>Enoxaparin (n = 10)</th>
<th>Methylene blue (n = 10)</th>
<th>Pentoxyphylline (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
</tr>
<tr>
<td>Activity</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
<td>2 (0-4)</td>
</tr>
<tr>
<td>Reaction</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
</tr>
<tr>
<td>Collagen</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
<td>4 (2-3)</td>
</tr>
</tbody>
</table>

* p < 0.05; vs control group.

Table 4. — Immunohistochemical adhesion scores of all groups.

<table>
<thead>
<tr>
<th>VEGF Score</th>
<th>Control (n = 10)</th>
<th>Enoxaparin (n = 10)</th>
<th>Methylene blue (n = 10)</th>
<th>Pentoxyphylline (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>0.5 (0-4)</td>
<td>0.5 (0-4)</td>
<td>0.5 (0-4)</td>
<td>0.5 (0-4)</td>
</tr>
<tr>
<td>PDGF Score</td>
<td>2.5 (1-4)</td>
<td>2.5 (1-4)</td>
<td>2.5 (1-4)</td>
<td>2.5 (1-4)</td>
</tr>
</tbody>
</table>

* p < 0.05; vs control group.
which were prepared by routine tissue management, were assessed in blind manner to macroscopic adhesion scores. The method proposed by Kanbour-Shakir et al. [23] was used to semi-quantitatively grade (grade 0 to 4) inflammation on the serosal surface, fibroblastic activity, foreign body reaction, collagen formation and severity of vascular proliferation (Table 1). Moreover, Masson’s trichrome histochemical staining was performed to make severity of collagenization more pronounced in all preparations. Results were scored as 0, 1+, 2+ and 3+; 4 µm thick sections were obtained to interpret angiogenesis, a step in the formation of adhesions. VEGF Ab-7 (mouse monoclonal antibody, Cat # MS-1467-R7, Neomarkers, USA, 2007), bFGF (mouse monoclonal antibody, Cat # AM 359-5M, Biogenex, USA, 2007), PDGF (rat monoclonal antibody clone, Cat # RB-9257-R7, Neomarkers, USA, 2007) and TGF-β (rabbit monoclonal antibody, Cat # RB-9262-R7, Neomarkers, USA, 2007) markers were used in the immunohistochemical evaluation. Results were scored as 0, 1+, 2+, 3+ and 4+.

Statistical Analysis

Data are presented as median (min-max). Kruskall Wallis ANOVA and Tukey post hoc test were used to compare scores of adhesion extent, adhesion severity, total adhesion, inflammation, fibroblastic activity, foreign body reaction, collagen formation, vascular proliferation, MT, VGEF, PDGF, TGF and aFGF between groups. A p value < 0.05 was considered as significant.

Results

There was no mortality in the study group. Forty female rats recovered without incident after operation and resumed preoperative physical activity and feeding patterns postoperatively. All animals appeared healthy; they were evaluated and there were no signs of impaired wound healing or bleeding complications.

Table 2 presents macroscopic adhesion scores of all groups (extent, severity, and total score of adhesions). The extent scores of adhesions in the control, enoxaparin control, methylene blue and pentoxyphylline groups were observed as 3 (2-3), 1.5 (1-3), 3 (1-3) and 3 (2-3), respectively. The severity scores of adhesions in the control, enoxaparin control, methylene blue and pentoxyphylline groups were observed as 1 (0.5-1), 0.5 (0-0.5), 1 (0-1), and 1 (1-1), respectively. As shown in Table 2, the adhesion extent score of the enoxaparin group was found to be significantly lower than the score of the pentoxyphylline group (p < 0.05). When adhesion severity scores of the control, enoxaparin, methylene blue and pentoxyphylline groups were compared, the score of the control group was found to be significantly higher than the score of the enoxaparin group (p < 0.05). When total adhesion scores of the control, enoxaparin, methylene blue and pentoxyphylline groups were compared, only the score of the enoxaparin group was found to be significantly lower than those of the control, methylene blue and pentoxyphylline groups (p < 0.05) (Figures 1 and 2).

Table 3 shows histopathological (inflammation, fibroblastic activity, foreign body reaction, collagen formation, and vascular proliferation) and histochemical (Masson’s trichrome scores) findings of all groups. When inflammation scores of the control, enoxaparin, methylene blue and pentoxyphylline groups were compared, only total adhesion score of the enoxaparin group was found to be significantly higher than scores of the control, methylene blue and pentoxyphylline groups (p < 0.05). The fibroblastic activity score of the methylene blue group was found to be significantly lower than scores of the control, methylene blue and pentoxyphylline groups (p < 0.05). The fibroblastic activity score of the methylene blue group was found to be significantly lower than scores of the control, methylene blue and pentoxyphylline groups (p < 0.05). The vascular proliferation score of the methylene blue group was found to be significantly lower than scores of the control, methylene blue and pentoxyphylline groups (p < 0.05). The Masson’s trichrome (MT)
scores of the control, enoxaparin, methylene blue and pentoxyphylline groups were compared and only the MT score of the methylene blue group was found to be significantly lower than scores of the control, enoxaparin and pentoxyphylline groups ($p < 0.05$).

Table 4 presents immunohistochemical adhesion scores (VEGF, PDGF, TGF and aFGF) of all groups. When VEGF, TGF and aFGF scores of the control, enoxaparin, methylene blue and pentoxyphylline groups were compared, no significant difference was found between the groups, whereas only PDGF score of the methylene blue (MB) group was found to be significantly lower than scores of the control, enoxaparin and pentoxyphylline groups ($p < 0.05$) (Figure 4).

Discussion

There is no in vivo study that has investigated the effect of MB on angiogenesis in the rat adhesion model in the literature. In our study, we found no significant reduction in total adhesion score in rats, of which 1% MB was administered intraoperatively; however, there was a significant reduction in adhesion severity score compared to controls. When fibroblastic activation, collagen formation, collagenization and vascular proliferation scores were compared, a significant reduction was observed in the MB group. However, it was shown that MB had no effect on cytokines linked to angiogenesis, such as VGEF, bFGF and TGF, although there was a significant reduction in PDGF compared to other groups. We think that MB has a reducing effect on adhesions and may be related to reduction in fibroblastic activation, collagen formation, and vascular proliferation that occurs through angiogenesis. In the literature it was shown that MB had an anti-angiogenic effect in chicken chorioallantoic membranes [24]. However histological examination of the lungs showed that VEGF-positive cells were decreased in rats treated with MB, in which hepatopulmonary syndrome developed due to common bile duct ligation and the authors concluded that MB treatment decreased the proliferation of alveolar capillary vessels and angiogenesis [25].
The administration of intraoperative single dose MB leads to fragmentation of fibrinous proto-adhesions by up-regulating the fibrinolytic system during the consequent 24 hours [26]. Also it was reported that MB stimulated NADPH production via pentose phosphate and an increase occurred in (tissue plasminogen activator) (tPA) secretion after day 2 or 4 [27]. This can also explain the adhesion prevention effect of MB by fibrinolysis. In addi-
tion, it was reported that methylene blue, as an inhibitor of superoxide generation by xanthine oxidase [28], was very effective in preventing formation of peritoneal adhesions. Its activity is probably through inhibition of free-radical generation [29]. In addition to all this information, our results and other studies [24, 25] showing an angiogenesis inhibition effect of MB suggest that adhesion preventive effect of MB can occur through inhibition of angiogenesis.

Gude et al. [30] found that pentoxiphylline inhibited angiogenesis induced by tumoral activity. This effect was probably through a decrease in endothelial cell proliferation and levels of urokinase type PA, which are released from these cells. In a study by Barros et al. [31] phosphodiesterase inhibitors, cilostazol and pentoxiphylline were administered over seven days after operation by gavage, and they showed that both agents decreased angiogenesis in sponge-induced intraperitoneal adhesions in mice; however, it was also shown that there was a more significant decrease in the levels of VEGF and TGF-β within adhesion tissue at high doses (500 mg/kg for pentoxiphylline). In our study, it was shown that single dose intraoperative administration had no significant effect on adhesion formation and markers of angiogenesis. Pentoxiphylline increases blood flow by reducing blood viscosity and increasing fibrinolytic activity of plasma. It was reported that intravenous and intraperitoneal administrations of pentoxiphylline decreased adhesions induced by cecal abrasion with gauze by altering fibrinolytic activity [32]. Kaleli et al. [33] found that single dose intraperitoneal pentoxiphylline decreased intraabdominal adhesions in a rat horn model, which was developed by denuding the serosa on the proximal antimesometric area with a scalpel until macroscopic punctate bleeding was observed over most of the uterine horn surface. In addition, the peritoneum was stripped from the lateral abdominal wall over an area of 1 x 1 cm and adhesion was achieved by suturing after bleeding. The results of the last two studies seemed to be inconsistent with our findings; we believe that this inconsistency might be caused by the difference of the adhesion formation models (we used a cauterization method which causes ischemia in tissues instead of bleeding in our adhesion model). In rats, it was shown that pentoxiphylline reduced intraperitoneal adhesion formation at the anastomosis site after intestinal resection, but single dose intraperitoneal and 50 mg/kg intramuscular administration had no effect on the non-anastomotic regions [19]. Parra-Membrives et al. showed that there was a positive effect on healing by increasing fibrosis on adhesion tissue in the model of ischemic colon anastomosis [34]. In addition, Steinleitner et al. [35] showed that pentoxiphylline reduced adhesion development after adhesiolysis by administering 2.5 mg/kg intravenous doses six times with 12-hour intervals in a rabbit uterine horn model. In light of these data, we think that the adhesion model should be considered when assessing the efficiency of pentoxiphylline on preventing adhesion formation, and also drug dose and administration route are important factors that can affect results.

Intraperitoneal heparin administration was found to be effective in animal models [36, 37]. Arikan et al. [38] found that enoxaparin decreased intraabdominal adhesions without compromising wound healing. However, Diamond et al. [39] reported that heparin, administered by intraperitoneal lavage, intravenous injection or the intraabdominal route was ineffective in preventing adhesion formation in a rabbit uterine horn model. Similarly, it was reported that administration with Interceed was effective, but not with carboxymethyl cellulose or dextran 70 [39]. It was detected that 500 and 1000 USP units of heparin, which were administered to horns of each rabbit, had significant efficiency in preventing adhesions [40]. Both unfractionated heparin and LMWH alter the bioavailability and activity of growth factors. LMWH affects fibrin structure and inhibits angiogenesis in vitro [41]. It was observed that heparins inhibited capillary tube formation by human endothelial cells from a macrovascular bed, which is stimulated by proangiogenic factors such as FGF-2 and VGEF; it has also been suggested that this inhibitory capacity of heparin is dependent on its molecular weight [21]. When total adhesion scores were considered, it was seen that the enoxaparin group had the lowest score among groups. However, it was detected that it decreased inflammation scores among adhesion markers, which were evaluated in a histopathological manner, but had no significant effect on fibroblastic activity score, foreign body reaction score, collagen formation score, vascular proliferation score and MB (collagenization) score. In our study, it was shown that single dose intraperitoneal enoxaparin administration had no significant effect on growth factors related to angiogenesis, although it decreased adhesions. Although a number of studies have sought to identify a broad spectrum of the biological effects of heparin, many challenging questions remain unanswered [42].

In conclusion, our results demonstrated that MB had a positive effect on prevention of postoperative adhesions and this effect could be through inhibition of angiogenesis via PDGF. Intraperitoneal administration of single dose pentoxiphylline had no significant effect on the formation of adhesions and enoxaparin had no significant effect on growth factors related to angiogenesis, although it led to a decrease in adhesions. Prevention of postoperative adhesions has complex and multifactorial characteristics. We believe that combination treatment or multifactorial effect treatment is more successful than the treatments related to one of these factors. At present, many more specific and original studies are necessary for the purpose of improving effective and inexpensive treatment methods.

References

Effects of methylene blue, pentoxiphylline and enoxaparin on postoperative adhesion formation and markers of angiogenesis in etc.


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Molecular diagnosis of CMV infection in fetal aborted tissues in the region of Thrace

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Summary

Purpose: To detect the incidence of CMV infection in spontaneous abortion in Thrace. Methods: Genetic material from 143 fetuses aged from 11 to 39 weeks was examined. The material originated from various regions of Thrace. All fetuses and the respective placentas underwent routine histopathology. DNA was isolated from sections of paraffinized tissues. Detection of CMV in the DNA genomic samples was performed using a commercial PCR-based detection kit. Results: From the 143 fetuses that were examined, two were found to be CMV positive. Pathological findings related to inflammatory corruptions were observed in the placentas of 97 embryos, including the CMV infected ones. Conclusions: This study indicates CMV-DNA infection in 1.4% of aborted fetuses. CMV infection incidence in aborted fetuses is similar to this reported in other European regions. The molecular technique of PCR applied on paraffin-embedded biopsy material is proven to be an accurate, valid and fast method for investigating the CMV infection in aborted fetuses.

Key words: Spontaneous abortion; Fetus; Cytomegalovirus - CMV; PCR.

Introduction

Cytomegalovirus (CMV) (originating from the Greek cyto "cell" and megalο "large") was isolated from Weller and his collaborators in 1956 in liver biopsies and in the urine of different patients [1]. It is the most common cause of congenital infection in humans worldwide with an incidence of 0.5-2.5% of live births [2, 3].

Microscopically, formation of big cells (cytomegalia) and inclusion bodies (owl’s eye) in the nucleus and protoplasm of cells in vitro and in vivo are the two major characteristics of CMV. The virus is developed only in cultures of human fibroblasts manifesting cytopathological corruption after one to six weeks [4].

In terms of clinical manifestations of the infection, CMV causes a wide spectrum of symptomatology ranging from minor illness up to heavy mental retardation and other critical damage including petechiae, hepatosplenomegaly, jaundice, microcephaly, chorioretinitis, and typical lymphocytosis with a mortality rate ranging from 10-30% [4, 5].

This virus can be transmitted in utero or perinatally, person-to-person via close non-sexual contact, through sexual activity, organ transplantation, blood transfusions and breastfeeding [6, 7]. Furthermore, recent molecular epidemiological studies prove that a risk factor for CMV transmission is close interaction with young children that have been infected because they confect high concentrations of the virus in urine and salivary secretions [8, 9].

The virus can be transmitted in the fetus after primary infection of the mother (0.7-4.1% of pregnancies with 40% average rate of transmission) or due to the activation of latent infection during gestation (0.15-3%) [3, 4].

Indisputably, CMV constitutes the most common cause of congenital infection, as proven by several reports, over against controversial studies regarding the correlation of CMV and intrauterine fetal death [2, 3, 10]. The association of spontaneous abortion and CMV has not yet been clarified, whereas, CMV infection is an important agent of adverse outcome in infants and moreover, the nucleic acid and viral agent are often detected in aborted material [10].

In this study we examined human fetuses of spontaneous abortions for CMV infection, using the accurate and sensitive molecular method of polymerase chain reaction (PCR) in order to investigate whether CMV could provide the basis and reason behind these abortions.

Materials and Methods

Tissue specimens

In the present study 143 fetuses were examined in the Laboratory of Histology and Embryology of Democritus University of Thrace, deriving from spontaneous abortions in the Department of Obstetrics and Gynecology of University General Hospital of Alexandroupolis. The fetuses’ age ranged from the 11th to 39th gestational week. The gestational week was estimated using developmental anatomical criteria.

The experimental procedure included the preparation of the tissue and its fixation in paraffin, standard histopathological examination, isolation of DNA, PCR procedure and electrophoresis of the respective PCR reaction products.
Sections from fetal liver, placenta and membranes of each embryo were employed. Each tissue was dehydrated and fixed in paraffin. Two μm sections were cut from paraffin blocks of the placenta and membranes and stained with hematoxylin and eosin for histopathological evaluation and 20 μm tissue sections of placenta and fetal liver were cut for the PCR procedure, respectively.

The tissue used for PCR amplification in the majority of fetuses was the placenta, except for multiple gestations where the examined tissue was the fetal liver of each fetus.

Extraction of DNA was performed using the Macherey-Nagel nucleospin tissue kit (GmbH & Co. KG, Germany), according to the manufacturer's protocol for DNA extraction from paraffin-embedded tissues. A positive control was included in each assay using 10 μl of control cDNA supplied in the PCR kit.

PCR was performed using CMV major immediate early gene, primer set kit (Maxim Biotech Inc, San Francisco, USA). The primers used to amplify the sequence of CMV according to the manufacturer were: 5' Oligo: CCAAGCGGCCTCTGATAAAGAAGCC, 3' Oligo: CAGCACAATCTACTTTCCCTTCCTGTG. (alignment on database M21295) that were available as pre-mixed primers. PCR amplification of DNA was performed in a 50 μl total volume reaction using 1U of Taq polymerase, 10 μl of DNA and 40 μl of optimized buffer (including chemicals, enhancer, stabilizer, dNTPs). The amplification protocol was as follows: 96°C (1 min) for one cycle, 94°C (1 min), 58°C (1 min), 72°C (1 min) for 40 cycles, followed by a 10 min extension at 72°C. Presence of PCR products in the specimens was visualized on a 2% agarose gel stained with ethidium bromide. A negative control containing water instead of DNA was included in all assays. The positive control was also included using 10 μl of control cDNA supplied in the kit.

Data of serological examination from maternal sera of some cases were given by obstetricians.

Results

Molecular detection of CMV infection revealed two positive fetuses out of 143, as indicated by the appearance of a 435 bp DNA band, as a PCR product deriving from CMV DNA amplification of placenta tissues (Figure 1). Both positive fetuses were male aged 20 and 23 weeks, while the mothers' ages were 21 and 41 years, respectively (Table 1).

A male fetus positive to specific CMV antibodies indicated by serological examinations, proved negative for the presence of CMV genome by PCR (line 4 - Figure 1).

No other pathological findings related to congenital CMV infection were found by gross examination in the CMV positive fetuses (Table 1).

Histological examination of the placental samples revealed acute chorioamnionitis (Figure 2) in 86 of the 143 investigated fetuses (including the two positive for CMV) and acute placentalitis (Figure 3) in 11 of these fetuses (Table 1), which may correlate to fetal death (Table 2).
Table 1. — Summary of the candidate gene variants and genotyping methodology.

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Gender</th>
<th>Gestational age</th>
<th>Mother’s age</th>
<th>Clinical features/pathological findings</th>
<th>Chorioamnionitis (+, ++, ++++)</th>
<th>Placentitis (+, ++, ++++)</th>
<th>CMV (+, –)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♀</td>
<td>18 w</td>
<td>30 y</td>
<td>Cystic hygroma</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>21 w</td>
<td>34 y</td>
<td>Dropsy</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>♂</td>
<td>15 w</td>
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<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>♀</td>
<td>19 w</td>
<td>24 y</td>
<td>Cheiloschisis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>♀</td>
<td>32 w</td>
<td></td>
<td>Cyclopia</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>♀</td>
<td>14 w</td>
<td>28 y</td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>♂</td>
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<td>24 y</td>
<td>Dropsy</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>♂</td>
<td>14 w</td>
<td>32 y</td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>Congenital abnormalities</td>
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<td>–</td>
</tr>
<tr>
<td>11</td>
<td>♀</td>
<td>27 w</td>
<td>21 y</td>
<td>Recessive development</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
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<td>12</td>
<td>♀</td>
<td>25 w</td>
<td>36 y</td>
<td>Recessive development, oligohydramnios</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>♀</td>
<td>24 w</td>
<td>30 y</td>
<td>Gastrochisis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>♀, ♀</td>
<td>28 w</td>
<td>38 y</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>♀</td>
<td>39 w</td>
<td>23 y</td>
<td>Lung abnormalities, parencephalism</td>
<td>hemmorrhage</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>♀</td>
<td>20 w</td>
<td>21 y</td>
<td>Congenital abnormalities</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>♀</td>
<td>23 w</td>
<td>27 y</td>
<td>Congenital abnormalities</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>♀</td>
<td>36 w</td>
<td>36 y</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>♀</td>
<td>27 w</td>
<td>21 y</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>♀</td>
<td>19 w</td>
<td>19 y</td>
<td>Congenital abnormalities</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>♂</td>
<td>16 w</td>
<td>16 y</td>
<td>Gastrochisis, cheiloschisis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>♀</td>
<td>28 w</td>
<td>28 y</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>23</td>
<td>♀</td>
<td>22 w</td>
<td>20 y</td>
<td>Heart abnormalities</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>24</td>
<td>♀</td>
<td>22 w</td>
<td>26 y</td>
<td>Anencephaly, meningocele</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>25</td>
<td>♀, ♀</td>
<td>23 w</td>
<td>26 y</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>26</td>
<td>♀</td>
<td>17 w</td>
<td>24 y</td>
<td>Anencephaly, omphalocele</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>27</td>
<td>♀</td>
<td>20 w</td>
<td>22 y</td>
<td>Anencephaly</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>28</td>
<td>♀</td>
<td>17 w</td>
<td>31 y</td>
<td>Down’s syndrome</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>29</td>
<td>♀</td>
<td>21 w</td>
<td>43 y</td>
<td>Encephalocele, meningocele</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>♀</td>
<td>23 w</td>
<td>27 y</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>31</td>
<td>♀</td>
<td>20 w</td>
<td>17 y</td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>32</td>
<td>♀</td>
<td>17 w</td>
<td>24 y</td>
<td>Anencephaly</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>33</td>
<td>♀</td>
<td>18 w</td>
<td>21 y</td>
<td>Anencephaly, spina binida aperta</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>34</td>
<td>♀</td>
<td>34 w</td>
<td>28 y</td>
<td>Dropsy</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>35</td>
<td>♀</td>
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<td>24 y</td>
<td>Gastrochisis</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>36</td>
<td>♂</td>
<td>16 w</td>
<td>35 y</td>
<td>Down’s syndrome</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>37</td>
<td>♂</td>
<td>23 w</td>
<td>24 y</td>
<td>Heart and lung abnormalities</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>38</td>
<td>♂</td>
<td>22 w</td>
<td>25 y</td>
<td>Procephaly</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>39</td>
<td>♂</td>
<td>17 w</td>
<td>24 y</td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>40</td>
<td>♀</td>
<td>21 w</td>
<td>40 y</td>
<td>Down’s syndrome</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>41</td>
<td>♂</td>
<td>18 w</td>
<td>25 y</td>
<td>Nuchal cord</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>42</td>
<td>♂, ♂, ♂</td>
<td>22 w</td>
<td>35 y</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>43</td>
<td>♂</td>
<td>12 w +3d</td>
<td>26 y</td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44</td>
<td>♂</td>
<td>23 w</td>
<td>33 y</td>
<td>Immature malignant sacrococcygeal teratoma</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>45</td>
<td>♂</td>
<td>18 w</td>
<td>22 y</td>
<td>Umbilical cord ischemic necrosis</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>46</td>
<td>♂</td>
<td>24 w</td>
<td>33 y</td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>47</td>
<td>♂</td>
<td>19 w</td>
<td>29 y</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>48</td>
<td>♂</td>
<td>23 w</td>
<td>39 y</td>
<td>Umbilical cord ishaemic necrosis</td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>49</td>
<td>♂</td>
<td>22 w</td>
<td>19 y</td>
<td>Anencephaly</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>50</td>
<td>♂</td>
<td>23 w</td>
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<td>24 w</td>
<td>32 y</td>
<td>Edward’s syndrome</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>52</td>
<td>♀, ♂</td>
<td>32 w</td>
<td>27 y</td>
<td>Hyaline membrane syndrome, atelectasis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>53</td>
<td>♂</td>
<td>32 w 3d</td>
<td>29 y</td>
<td>Hyaline membrane syndrome</td>
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<td>–</td>
<td>–</td>
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<td>♂</td>
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<td>32 y</td>
<td>Fallot tertalogy, kidney abnormalities</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>♂</td>
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<td>21 y</td>
<td>Kidney abnormalities</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>56</td>
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<td>20 w</td>
<td>41 y</td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>57</td>
<td>♂</td>
<td>20 w</td>
<td>40 y</td>
<td>Anencephaly, meningoymelocele</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>58</td>
<td>♂</td>
<td>14 w</td>
<td>31 y</td>
<td>Head edema</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>59</td>
<td>♂</td>
<td>12 w</td>
<td></td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>60</td>
<td>♂</td>
<td>25 w</td>
<td>32 y</td>
<td>Heart abnormalities</td>
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Table 1. — Summary of the candidate gene variants and genotyping methodology.

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Gender</th>
<th>Gestational age</th>
<th>Mother’s age</th>
<th>Clinical features/pathological findings</th>
<th>Chorioamnionitis (+, ++, ++++)</th>
<th>Placenta (+, ++, ++++)</th>
<th>CMV (+, –)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>♀</td>
<td>26 w 28 y</td>
<td></td>
<td>Heart abnormalities</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>62</td>
<td>♂</td>
<td>17 w 30 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>63</td>
<td>♂</td>
<td>20 w 16 y</td>
<td></td>
<td>Total edema</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>64</td>
<td>♂</td>
<td>15 w 30 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>65</td>
<td>♀</td>
<td>17 w 29 y</td>
<td></td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
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<td>66</td>
<td>♂</td>
<td>20 w 30 y</td>
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<td>Syndrome XXY</td>
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<td>67</td>
<td>♂</td>
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<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>68</td>
<td>♂</td>
<td>16 w 36 y</td>
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<td>Procephaly</td>
<td>+</td>
<td>–</td>
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<td>69</td>
<td>♂</td>
<td>16 w 19 y</td>
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<td>–</td>
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<tr>
<td>70</td>
<td>♂</td>
<td>20 w 30 y</td>
<td></td>
<td>Aspiration of amniotic fluid</td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>71</td>
<td>♂</td>
<td>18 w 16 y</td>
<td></td>
<td>Devisceration, sull dissection</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>72</td>
<td>♂</td>
<td>14 w 30 y</td>
<td></td>
<td>Organ autolytic corruptions</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>73</td>
<td>♂</td>
<td>21 w 39 y</td>
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<td>Down’s syndrome</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>74</td>
<td>♂</td>
<td>20 w 33 y</td>
<td></td>
<td></td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>75</td>
<td>♂</td>
<td>23 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>76</td>
<td>♂</td>
<td>11 w 31 y</td>
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<td>Heart abnormalities</td>
<td>+</td>
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</tr>
<tr>
<td>77</td>
<td>♂</td>
<td>20 w 28 y</td>
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<td></td>
<td>++</td>
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</tr>
<tr>
<td>78</td>
<td>♂</td>
<td>18 w 29 y</td>
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<td>Dropy</td>
<td>++</td>
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</tr>
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<td>79</td>
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<td>20 w 19 y</td>
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<td>++</td>
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</tr>
<tr>
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<td>♂</td>
<td>23 w 31 y</td>
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<td>++</td>
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</tr>
<tr>
<td>81</td>
<td>♂</td>
<td>14 w 28 y</td>
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<td>Organ autolytic corruptions</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>82</td>
<td>♂</td>
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<td>+++</td>
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<td>83</td>
<td>♂</td>
<td>25 w 30 y</td>
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<td></td>
<td>+++</td>
<td>+++</td>
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<td>84</td>
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<tr>
<td>85</td>
<td>♂</td>
<td>23 w 20 y</td>
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<td>–</td>
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</tr>
<tr>
<td>86</td>
<td>♂</td>
<td>20 w 31 y</td>
<td></td>
<td>Cysts of lung and kidneys</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>87</td>
<td>♂</td>
<td>22 w 34 y</td>
<td></td>
<td></td>
<td>+++</td>
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<td>♂</td>
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<td>++</td>
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</tr>
<tr>
<td>90</td>
<td>♂</td>
<td>13 w 37 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>91</td>
<td>♂</td>
<td>19 w 28 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
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</tr>
<tr>
<td>92</td>
<td>♂</td>
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<td>–</td>
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</tr>
<tr>
<td>93</td>
<td>♂</td>
<td>19 w 32 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>94</td>
<td>♂</td>
<td>11 w 31 y</td>
<td></td>
<td></td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>95</td>
<td>♂</td>
<td>12 w 24 y</td>
<td></td>
<td>Procephaly, organ autolytic corruptions, umbilical cord ischemic necrosis</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>96</td>
<td>♂</td>
<td>21 w 28 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>97</td>
<td>♂</td>
<td>13 w 26 y</td>
<td></td>
<td>Dropsy, heart abnormalities</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>98</td>
<td>♂</td>
<td>21 w 25 y</td>
<td></td>
<td>Dropsy, heart abnormalities</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>99</td>
<td>♂</td>
<td>31 w 28 y</td>
<td></td>
<td>Lung abnormalities</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>100</td>
<td>♂</td>
<td>26 w 24 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>101</td>
<td>♂</td>
<td>18 w 38 y</td>
<td></td>
<td>Meningomyelocele</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>102</td>
<td>♂</td>
<td>19 w 41 y</td>
<td></td>
<td>Down’s syndrome</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>103</td>
<td>♂</td>
<td>17 w 34 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>104</td>
<td>♂</td>
<td>19 w 17 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>105</td>
<td>♂</td>
<td>25 w 17 y</td>
<td></td>
<td>Clubfoot, talipes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>106</td>
<td>♂</td>
<td>17 w 34 y</td>
<td></td>
<td>Down’s syndrome</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>107</td>
<td>♂</td>
<td>20 w 27 y</td>
<td></td>
<td>Anencephaly, neck failure</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>108</td>
<td>♂</td>
<td>13 w 37 y</td>
<td></td>
<td></td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>109</td>
<td>♂</td>
<td>16 w 34 y</td>
<td></td>
<td>Organ autolytic corruptions</td>
<td>++</td>
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<td>–</td>
</tr>
<tr>
<td>110</td>
<td>♂</td>
<td>27 w 35 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>111</td>
<td>♂</td>
<td>19 w 28 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>112</td>
<td>♂</td>
<td>22 w 21 y</td>
<td></td>
<td></td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>113</td>
<td>♂</td>
<td>18 w 41 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>114</td>
<td>♂</td>
<td>19 w 28 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>115</td>
<td>♂</td>
<td>27 w 37 y</td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>116</td>
<td>♂</td>
<td>27 w 30 y</td>
<td></td>
<td>Hemosiderine deposition</td>
<td>++</td>
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</tr>
<tr>
<td>117</td>
<td>♂</td>
<td>18 w 22 y</td>
<td></td>
<td>Encephalomeningocele</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>118</td>
<td>♂</td>
<td>12 w 24 y</td>
<td></td>
<td>Encephalomeningocele</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>119</td>
<td>♂</td>
<td>23 w 32 y</td>
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<td></td>
<td>–</td>
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Discussion

In this study we examined the incidence of CMV in intrauterine fetal death in the region of Thrace by localizing the genetic material of the virus in aborted tissues using the PCR technique. Of 143 samples of aborted fetuses that were examined, two were found positive for the presence of CMV genome. Furthermore, the placentas of these two fetuses were positive for acute chorioamnionitis diagnosed with Hm-E staining, while no other pathological findings related to congenital CMV infection were observed.

For the majority of the cases no sufficient data about the maternal sera were provided to us, except for certain ones. For that reason we could not investigate whether the presence of specific antibodies against the virus, in correlation with PCR, would provide more composite results.

In order to ensure accurate results, PCR amplification of CMV genomic DNA was the method of choice. Published data have proven that experimental methods based on isolation of DNA from paraffin-embedded tissues and amplification of virus DNA with PCR have been successful [11, 12].

Alternative methods that can be used for CMV diagnosis consist of microscopic examination, immunohistochemistry, the culture of cells (CC and SV) [13, 14], the CMV pp65 antigenemia test (AGC)\(^{3}\), the determination of CMV-specific IgG and IgM and others [10]. It is however reported that the culture of cells (CC and SV) and CMV pp65 antigen test have failed to provide the effectiveness of PCR [13, 14]. Niubo J. et al. presented data comparing results on peripheral blood samples of patients with heart transplantation for prognosis of CMV disease, and the reliability documented for each of the methods employed was: 89.9% reliability for PCR, 33.4% for CC (tube culture) culture, 42.6% for SV (shell vial culture) and 68.1% for pp65 antigen test. The numerous variants of PCR are applied internationally in the laboratories of molecular diagnostics for the detection of CMV [15].

Considering previous studies that proved the focal distribution of CMV [10, 16] and that the virus may not present in all different tissues of the same case, as well as the fact that placentas of CMV infected incidents show various pathological findings from absence of abnormalities up to variant inflammatory corruptions [10, 17], we performed the experimental procedure in different aborted tissues (placenta, membranes, liver) in order to have more reliable results.

The idea of using the placenta as an accessory tissue for DNA extraction is based on the knowledge that CMV or other pathological agents are transmitted by mother to the fetus, transplacentally [18-21]. Further studies have established that the CMV genome is localized on the villi
of the placenta, including the mesenchyme, trophoblasts and decidual cells [16].

Regardless of the above-confirmed facts, the cases of multiple gestations examined in the present study were treated with special caution because the type of placenta cannot determine which of the fetuses the virus may infect. Furthermore, it has not even been proven whether transmission by one fetus to the other is effected [22]. For that reason, and in order to insure reliability of results for such cases, we chose to use the fetal liver from each fetus body rather than the placentas. Alternatively, similar studies have employed biological fluids such as amniotic fluid [23, 24], peripheral blood [25, 26] or urine [27].

It is of great value to note that in our study the examination of a male fetus aged 12 weeks by PCR was proven negative for CMV infection, while it was expected to be positive, as the mother had appeared positive following serological examinations. This phenomenon is possible due to the fact that the presence of CMV-specific IgM is not always indicative of primary infection because of its production in low levels in reactivated CMV infection. Furthermore, the sensitivity of serological CMV IgM assay is 70% [28]. This has also been observed by other research [26] including a recent study in Turkey describing the opposite case where serological analysis was negative for CMV IgM and the CMV-DNA were found to be positive by PCR for two newborns [28].

CMV is the most common agent of congenital infection in humans [2] and the incidence of its specific antibodies in the adult population is high ranging – based on several studies conducted in Greece and worldwide – from 40-90% [2, 10]. It is also known that the incidence of congenital CMV infection depends on socioeconomic conditions of the population. Consequently in our study we extrapolated that a part of the examined fetuses derived from the minority population of Thrace.

After the successful application of PCR and the electrophoresis of products in agarose gel, we estimated that frequency of appearance of the virus in the examined aborted fetuses was 1.4% (2 out of 143). This percentage appears to be in line with the percentage of CMV-infected samples that were spontaneously aborted in other respective studies where the percentage ranged from 3-16% [29, 30]. Furthermore, the high rate of chorioamnionitis that was seen is obviously basal but it is not clear whether CMV caused it in the two positive fetuses.

It is important to note that although several relevant studies have been conducted in various regions of Europe [8, 29] to estimate the relation to CMV infection with intrauterine fetal death, the exact percentage for incidence of the infection has not been defined.

Conclusion

In this study the molecular technique of PMR was employed in an attempt to determine whether CMV infection has a potential involvement in fetal death. Even though the fact that two out of 143 aborted fetuses that were examined and found positive in CMV gives an abtional role to CMV in Thrace with a percentage of 1.4%, we propose that this field of study involving the role and effect of CMV in embryology should be further investigated, as other studies – in accordance with ours – point out the incidence of CMV infection and its relation to congenital abnormalities and fetal death.

Acknowledgments

The authors would like to thank the midwives of the Department of Obstetrics and Gynecology, Democritus University of Thrace, for supplying the placentas and embryos. The skillful technical assistance of Irene Apostolou is gratefully acknowledged.

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Examination of the effect of melatonin use in Pomeroy method of tubal ligation on ovarian histology in rats

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2Department of Obstetrics and Gynecology, Adana Numune Research Hospital, Adana
3Department of Pathology, Inonu University School of Medicine, Malatya (Turkey)

Summary

Objective: To examine the effects of melatonin use in the unilateral Pomeroy method of tubal ligation 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 abdomen was opened and closed. G2 (n: 10): The group where the abdomen was opened, and the Pomeroy method of tubal ligation was performed. G3 (n: 10): The group where the abdomen was opened, and Pomeroy method of tubal ligation was performed 15 min after 10 mg/kg/ip melatonin administration. Abdomens of all rats were opened six months later and left oophorectomy was performed. Samples of the left ovary were fixed in formaldehyde. The preparations were stained with hematoxylin eosin, and prior to statistical analysis of data; p < 0.05 were considered significant.

Results:

An ordinal scale was formed for the regression of angiogenesis within the corpus luteum and presence of fibrosis (none: 0p, present: 1p, markedly present: 2). Follicle cysts in the ovary were counted. Kruskal Wallis variance analysis was used in the statistical analysis of data; p < 0.05 were considered significant. Results: The comparison between G1 and G3 showed that all values were similar (p > 0.05, Kruskal Wallis variance analysis). When G2 was compared with G1 and G3, regression of angiogenesis in the corpus luteum was found to be significantly lower (p < 0.05, Mann Whitney U test), while atretic follicle count and fibrosis were significantly higher in G2 (p < 0.05, Mann Whitney U test). Conclusion: The Pomeroy method of tubal ligation reduces regression of angiogenesis in the corpus luteum, and increases atretic follicles and fibrosis development. Melatonin use restores these harmful effects. Melatonin can be used to refrain from this negative effect of the Pomeroy method of tubal ligation on the ovary.

Key words: Melatonin; Ovarian histology; Pomeroy tubal ligation; Rat.

Introduction

Tubal sterilization is the most commonly used contraceptive method among women over 30 years of age in the United States. In both humans and rats, ovarian blood flow is impaired when the fallopian tube is destroyed, and this may damage ovarian tissue [1-4].

Zackrisson et al. [5] established in their rat study that ovarian artery ligation (OL) or uterine artery ligation (UL) had negative effects on ovarian blood flow and functions. A procedure conducted on the fallopian tube (tubal lig., salpingectomy, etc.) may theoretically 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 in the mesosalpinx, and are necessary for the feeding of the ovary [6].

Ischemia and/or reperfusion injury leads 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 [7]. Consequently, cell membrane, lysosome membrane, and membranes of such cell organelles as endoplasmic reticulum, etc. are destroyed, cells break down, and necrosis results [8]. This event is called lipid peroxidation. Sugino [9] reported that oxygen radicals and the antioxidant system in the ovary have 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 the reactive oxygen species (ROS) inhibited oocyte development, and increased degenerated oocyte count and apoptosis [9]. However, lipid peroxidation stimulates collagen gene transcription in cell culture [10, 11].

Melatonin, a pineal secretory product, modulates ovarian function and reproduction in mammals [12]. Melatonin is present in human pre-ovulatory follicular fluid concentrations 3-fold higher than in peripheral serum [13].

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 [14]. Ishizuka et al. found that melatonin supported fertilization and early embryo development after in vitro fertilization because melatonin is a ROS scavenger [15].

Takasaki et al. [16] used oral melatonin in infertile
women with poor quality oocytes and found a high amount of intrafollicular melatonin and a low amount of lipid peroxide. Melatonin use reduced degenerated oocyte count and increased fertilized oocyte count. Melatonin is effective on hydroxyl radicals, singlet oxygen, peroxyl radicals and superoxide anion, among oxygen radicals. It protects nucleus DNA, membrane lipids, and cytosolic proteins against oxidative stress [17]. Moreover, it supports SOD, GSH-Px, glutathione reductase, and glyoxyl-6-phosphate dehydrogenase of the antioxidant system [18]. It has an inhibitor effect on nitric oxide synthetase [19]. In addition, 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 and penetrates against oxidative stress. Furthermore, it has a protective effect on mitochondrion, which is a cell organelle [20].

Our Pub Med scan (melatonin, Pomeroy tubal ligation, rat) did not show any experimental study on this topic. We attempted to examine the effects of melatonin use in the unilateral Pomeroy method of tubal ligation on ovarian histology in rats.

Material and Method

This study was conducted in the experimental Animals Laboratory of Fırat University Medical School. Thirty 12-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 and 12-hour dark photo period, 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. Oral feeding was stopped 18 hours before the experiment, and only water was allowed. The rats, which 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 and the abdomen was opened with a midline incision. Anesthesia was induced, and the abdomen was opened, and the left ovary was taken out. The left Pomeroy method of tubal ligation was performed 15 min after 10 mg/kg/IP melatonin (1 g flacon, N-acetyl-5-methoxytriptamine; Sigma Chemicals Co.) administration. Layers of the abdomen and skin were closed with 3/0 silk. The rats were monitored throughout the study with blood pressure, heartbeats and body temperature measurements. They were kept in different cages in groups of five. On the postoperative 180th day, the animals were anesthetized in the same way. The abdomens were opened, and the left ovary was taken out. Ovarian tissue was fixed in 10% formaldehyde for histological examination, and placed into paraffin blocks from which 4 µm cross sections were prepared. The cross sections were stained with hematoxylin cosin. 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 [21]. 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 the corpus luteum was examined. Presence of fibrosis on the ovarian stroma was also 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. Kurskal Wallis variance analysis was employed in the statistical analysis of continuous and ordinal data. The Bonferroni correction Mann-Whitney U test was carried out for parameters; level of significance was set at $p < 0.05$.

Results

The experiment was successful in all rats. All values were similar in the comparison between G1 and G3, (Kruskal Wallis variance analysis). The comparison of G2 with G1 and G3 showed that regression of angiogenesis in the corpus luteum was significantly lower in G2 ($p < 0.05$, Mann-Whitney U test). Atretic follicle count and fibrosis were significantly higher ($p < 0.05$, Mann-Whitney U test). Ovarian follicle reserve was high in G1 and G3, but there was no statistically significant difference (Kruskal Wallis variance analysis).

All examined parameters are presented in Table 1.

<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>9.2 ± 3</td>
<td>9.2 ± 4.7</td>
<td>9.5 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Primary follicle (no.)</td>
<td>8.9 ± 3</td>
<td>7.7 ± 4.7</td>
<td>9 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary follicle (no.)</td>
<td>0.4 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>0.5 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Tertiary follicle (no.)</td>
<td>3.5 ± 0.7</td>
<td>3.7 ± 2.5</td>
<td>3.3 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian follicle reserve (no.)</td>
<td>22 ± 4.9</td>
<td>21 ± 10</td>
<td>22.5 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Corpus luteum (no.)</td>
<td>6.8 ± 1.3</td>
<td>6.7 ± 1.2</td>
<td>7 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Corpus albicans (no.)</td>
<td>0.3 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.4 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>7.1 ± 1.2</td>
<td>7 ± 1.4</td>
<td>7.3 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Atretic follicle (no.)</td>
<td>0.2 ± 0.2</td>
<td>4.4 ± 1.81</td>
<td>0.01 ± 0.32</td>
<td>*</td>
</tr>
<tr>
<td>CL angiogenesis (points)</td>
<td>2 ± 0.01</td>
<td>1 ± 0.02</td>
<td>2 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Stromal fibrosis (points)</td>
<td>0 ± 0.02</td>
<td>1 ± 0.01</td>
<td>0 ± 0.02</td>
<td>*</td>
</tr>
<tr>
<td>Cystic follicle (microscopic)</td>
<td>0.1 ± 0.3</td>
<td>0.1 ± 0.3</td>
<td>0 ± 0</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = non significant.

$* = p < 0.05$ (Kruskal Wallis variance analysis).

Means are placed in descending order in the numbering process.

Discussion

The left Pomeroy method of tubal ligation performed by laparotomy in rats reduces regression of angiogenesis in the corpus luteum, and increases atretic follicles and fibrosis development in the sixth month. Melatonin can be used to avoid the negative effect of the Pomeroy method of tubal ligation on the ovary. According to our Pub Med search (melatonin, rat, Pomeroy tubal ligation), our study is the first of its kind, and original in this respect.

The Pomeroy method of tubal ligation may lead to damage such as ischemia reperfusion. Melatonin reduces neutrophil infiltration and destructive tissue effects of
neutrophils during ischemia reperfusion, and particularly in reperfusion [17-20]. The fact that the damage in G3 was less than the damage in G2 can be attributed to the effect of melatonin. Our results are consistent.

It was found that changes in angiogenesis in the corpus luteum did not regress in G2. In normal rat ovaries, capillaries that emerge in the corpus luteum regress. Vascular endothelial growth factor (VEGF) has a major role in the emergence of these structures in the corpus luteum. One of the main stimulants of VEGF is hypoxia [21, 22]. As we impaired the blood flow in utero-ovarian anastomosis during the left Pomeroy method of tubal ligation, hypoxia resulted in the ovary [5], and this probably led to an increase in angiogenesis in the corpus luteum, and a decrease in regression of angiogenesis through VEGF. This may explain why regression of angiogenesis was lower in G2, relative to G1 and G3.

Hypoxia-induced factor-1 (HIF-1) is activated in both the ovary and other organs in case of acute or chronic hypoxia [23, 24]. HIF-1 alpha and hypoxic environments bring about regression and apoptosis in follicles, and result in an increase in atretic follicles and a decrease in follicular reserve [23]. The increase in atretic follicles and fibrosis observed in G2 may be associated with the apoptotic and degenerative effects of chronic hypoxia and lipid peroxidation products [23]. Our results are consistent.

Melatonin has a favorable effect on microvascular perfusion as it supports the endothelium [25]. Restoration of microvascular perfusion will reduce the effect of hypoxia (HIF-1 alpha, VEGF). This may be one of the reasons why there was no damage in the melatonin group.

HIF-1 alpha also increases VEGF secretion. VEGF helps angiogenesis, increases vascular permeability and 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 [21, 22, 26, 27]. Furthermore, VEGF directly stimulates collagen synthesis [27, 28]. The increase in fibrosis and follicle cysts in G2 may be explained by VEGF.

Although there was no ovarian fibrosis in G1 and G3, it was found to be significantly higher in G2. In case of blood or lymphatic circulation impairment, collagen neoformation is stimulated [29]. Uterine and tubal lymphatics are very close to each other on the broad ligament [29]. Lymphatic circulation may be damaged during the left Pomeroy method of tubal ligation, which may cause an increase in collagen formation. Our results are consistent with G2.

Melatonin can reduce fibroblast proliferation and collagen synthesis. Increased collagen levels in both intact skin and wounds have been observed following pinealectomy, whereas exogeneous application of melatonin caused the opposite effect [30]. Fibrosis caused by lipid peroxidation and its products decreases after the administration of antioxidants [melatonin, vit E] in animal models [31-33]. Therefore, the melatonin group has no fibrosis. The protective action of melatonin may be related with its antioxidant activity.

Conclusion
The left Pomeroy method of tubal ligation reduces regression of angiogenesis in the corpus luteum, and increases atretic follicle and fibrosis development. Melatonin use restores these harmful effects. Melatonin can be used to refrain from the negative effect of the Pomeroy method of tubal ligation on the ovary.

References


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Effect of fibrin glue and comparison with suture on experimental induction of endometriosis in a rat endometrial autograft model

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Summary

Objective: The effects of fibrin glue (FG) and suture were investigated and compared with experimental induction in an endometriosis model. Material and Methods: A randomized, controlled, and double-blind study was performed with 25 adult female Wistar Albino rats. Two autologous endometrial grafts were obtained from each of the rats. The endometrial grafts were transplanted by gluing with FG on the right abdominal wall and suturing with only 5/0 prolene on the left in ten rats. Gluing+sutting and after suturing over the covering with FG of the endometrial graft were performed, respectively, on the right and left in another ten rats. Covering with FG glue of the endometrial graft was performed in another five rats. The endometriosis-like lesions and intra-peritoneal adhesions were evaluated macroscopically and histopathologically. Results: The mean volume (31.4 ± 17.3), adhesion (0.8 ± 0.7) and inflammatory reaction (1.2 ± 0.7) score of the implants in the group using only FG were significantly lower in the group using suture [respectively, (49.2 ± 20.6), (2.4 ± 0.8), (2.2 ± 0.8)] (p < 0.05). Conclusions: Our results demonstrate the general feasibility of reproducible and reliable endometrial graft fixation with FG onto the inner abdominal surface in rats. Furthermore, several advantageous characteristics could be demonstrated such as less inflammation and fewer adhesions.

Key words: Fibrin glue; Suturing; Animal model; Endometriosis induction.

Introduction

Fibrin sealants can be used for hemostasis, wound closure, and tissue sealing and they are not associated with inflammation, foreign body reactions, tissue necrosis, or extensive fibrosis [1]. Fibrin sealants contain the active components thrombin and fibrinogen that, when mixed together, form a fibrin clot. The wound healing properties of fibrin sealant may be attributed to the ultrastructure of the fibrin sealant matrix which, like a normal fibrin clot, allows for diffusion of nutrients and cytokines and subsequent vascularization [2, 3]. Fibrin sealants which have been established include cardiovascular surgery, thoracic surgery, neurosurgery, plastic surgery, and dental surgery. There are, as yet, few clinical reports of the application of fibrin sealants to endometriosis surgery [4, 5].

The conventional method of an animal endometriosis model after endometrial excision is mostly the fixation of endometrial grafts using suture [6]. Sutures provide point fixation of the graft but not continuous adherence to and between endometrial and peritoneal surfaces. This situation draws attention to the use of fibrin glue (FG) for skin grafts [7]. On the other hand the use of FG for induction of endometriosis is interesting for us. This study was designed to observe the possible effect of FG on induction of endometriosis in an animal model.

Materials and Methods

The Cumhuriyet University Committee on the Use and Care of Animals approved the experiments, and all investigations complied. Twenty-five mature, female, non-pregnant Wistar Albino rats weighing between 220 and 280 g were used. Animals were provided by Cumhuriyet University Animal Reproduction Centre and housed in the Animal Laboratory of Cumhuriyet University. They were caged in a controlled environment at 22°C with 12 h light/dark cycles. Standard rat feed and water were provided ad libitum. All rats were allowed to acclimatize to this environment for one week before the experiment.

Rats were anesthetized by intraperitoneal administration of 60 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey). Before surgery, the abdominal skin was shaved and antisepsis was obtained with 10% povidone iodine solution. Using a sterile technique, a 3-4 cm ventral vertical incision was made to expose the reproductive organs. A 12 mm segment of the right uterine horn was excised after devascularization and ligature using 4-0 prolene and placed in sterile phosphate-buffered saline (PBS) at 37°C. Ectopic endometrium was induced surgically in rats by transplanting an autologous frag-
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Placement of endometrial tissue onto the inner surface of the abdominal wall. Five mm in diameter circular endometrial pieces (surface area = 19,625 mm²) were made by a cutting device without removing the myometrium (Figure 1a). The cutting device was designed by one of the authors and made by a gun repair workshop in Sivas. In ten rats, two pieces of uterine tissue were transplanted respectively, by gluing with FG (PFG group) and only suturing (OS group) with single non-absorbable 5-0 prolene sutures onto a vascular area of the inner surface of the right and left abdominal wall with the endometrial surfaces (Figure 1b-c). In another ten rats, endometrial grafts were transplanted with FG+suturing (PFG-S group) on the right and transplanted then by suturing over the covering with glue (S-OCFG group) on the left. In five rats, endometrial grafts were transplanted over the covering with FG (OCFG group) on the right and left abdominal inner wall. The vertical abdominal incision was closed with the use of two-layer polyglactin sutures. After the operation, all rats were observed for 14 days without medication.

Rats were euthanized by ketamine anesthesia and a second laparotomy was performed on day 15. Then all the observations about adhesions were scored according to Blauer’s scoring system [8]: 0 = no adhesion; 1 = thin, easily separable adhesions; 2 = thick adhesions limited to one area; 3 = thick and widespread adhesions; 4 = thick and widespread adhesions plus adhesions of viscera to the anterior/or posterior of the abdominal wall. During laparotomy, implant volumes were calculated in vivo by measuring their dimensions (length, width, height) with a micrometer and using the ellipsoid volume formula (π/6 x length x width x height). The implants were then excised and fixed in 10% formalin for histopathological examination. All operations, scoring of adhesions and measurements were performed by physicians blinded to the study treatment.

Samples from the endometriosis areas were fixed in 10% formaldehyde for a minimum of 12 h. After routine procedures, specimens were embedded in paraffin and cut into 5 µm sections. Sections were stained with hematoxylin and eosin according to standard laboratory procedures. The amounts of glandular tissue (GT) and stromal tissue (ST), inflammatory reaction (IR) and angiogenesis in the implants were histopathologically examined. The GT and ST was scored from 0 to 3. The ST score was zero if there was no ST per 10 hpf, 1 in < 25%, 2 in 25-50%, 3 in 50-75%...
Figure 2.
(a) Endometriosis-like lesion with light inflammation in group PFG (H-E; x 40). (b) Endometriosis-like lesion with moderate inflammation in group OS (H-E; x 100). (c) Massive inflammation that destructs the glandular tissue of endometriosis-like lesion in group OS (H-E; x 40).

Table 1. — Comparison of macroscopic and histopathologic results.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PFG (N: 10)</th>
<th>OS (N: 10)</th>
<th>PFG-S (N: 10)</th>
<th>S-OCFG (N: 10)</th>
<th>OCFG (N: 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume mm³</td>
<td>34.4±16.6</td>
<td>54.7±20.9</td>
<td>48.4±19.444.5</td>
<td>22.2±28.3</td>
<td>18.4</td>
</tr>
<tr>
<td>Adhesion scores</td>
<td>1±0.8</td>
<td>2.6±0.9</td>
<td>2.2±0.7</td>
<td>2.5±0.9</td>
<td>0.7±0.6</td>
</tr>
<tr>
<td>IR scores</td>
<td>1.5±0.7</td>
<td>2.6±0.6</td>
<td>2.2±1.0</td>
<td>1.9±0.8</td>
<td>1±0.6</td>
</tr>
<tr>
<td>GT scores</td>
<td>1.8±0.7</td>
<td>1.1±0.8</td>
<td>1.6±0.0</td>
<td>1.7±0.9</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>ST scores</td>
<td>0.9±0.5</td>
<td>1.4±0.8</td>
<td>1.2±0.9</td>
<td>1.6±0.8</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Microvessel density</td>
<td>12.9±3.3</td>
<td>11.0±4.1</td>
<td>13.0±4.3</td>
<td>12.2±5.0</td>
<td>11.7±4.7</td>
</tr>
</tbody>
</table>

N: number of endometriotic lesions; GT: glandular tissue; ST: stromal tissue; IR: inflammatory reaction; * p value for Kruskal–Wallis; * p = 0.03; ** p = 0.001; *** p = 0.002.

Table 2. — Groups created based on the use of suture. Comparison of the macroscopic and histopathologic results.

<table>
<thead>
<tr>
<th>Group</th>
<th>FG (N: 20)</th>
<th>S (N: 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume mm³</td>
<td>31.4±17.3</td>
<td>49.2±20.6</td>
</tr>
<tr>
<td>Adhesion scores</td>
<td>0.8±0.7</td>
<td>2.4±0.8</td>
</tr>
<tr>
<td>IR scores</td>
<td>1.2±0.7</td>
<td>2.2±0.8</td>
</tr>
<tr>
<td>GT scores</td>
<td>1.6±0.7</td>
<td>1.4±0.8</td>
</tr>
<tr>
<td>ST scores</td>
<td>1±0.5</td>
<td>1.4±0.8</td>
</tr>
<tr>
<td>Microvessel density</td>
<td>12.3±4</td>
<td>12±4.4</td>
</tr>
</tbody>
</table>

N: number of endometriotic lesions; GT: glandular tissue; ST: stromal tissue; IR: inflammatory reaction; * p value for Kruskal–Wallis; * p = 0.03; ** p = 0.001; *** p = 0.002.

and 3 in > 50%. At magnifications of x10 and x100 the IR was scored as mild (score 1), moderate (score 2) or severe (score 3).

To evaluate angiogenesis, vessels were immunohistochromically highlighted using CD31 antibody. Immunostaining was performed on 5-µm-thick, formalin-fixed, paraffin-embedded tissue sections of endometriosis. Sections were deparaffinized in xylene and dehydrated through graded concentrations of ethanol. After blocking of endogenous peroxidase activity with 3% hydrogen peroxide for 15 min, the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 min. The slides were allowed to cool to room temperature, and non-specific binding was blocked with normal horse serum for 30 min. The sections were then stained using the avidin-biotin complex (ABC) immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA), for demonstration of binding sites where AEC chromogen was applied. Phosphate buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer’s hematoxylin. Microvessel density was determined by counting the number of CD31 positive microvessels in an entire 1-mm core. A microvessel was defined as any endothelial cell or endothelial cell cluster staining positive for CD31.

All tissues were evaluated by the same pathologist, who was blinded to the origin of the samples.

Statistical analysis: for statistical analysis the Kruskal–Wallis test and one-way analysis of variance (ANOVA) Tukey’s method were used.

When the groups were created based on the use of suture, two groups were formed. One was the FG group (FG group = group PFG+ group OCFG) and the other was group S (group S = group OS+ group PFG-S + group S-OCFG). The data of these groups were analyzed by the Mann–Whitney test. Spearman’s test was used for correlations. Values are expressed as mean ± SD; significance was defined as p < 0.05.

Results

The standardized surgical procedures were well tolerated by all animals and none of them died. There was an infected area on one laparotomy site in the form of an abscess. In the group OCFG, using fibrin adhesive without sutures, one endometrial graft did not remain attached to the abdominal wall. It was found near the cecum whereas the rest of the grafts remained stable in groups PFG and OCFG.

There were significant differences between groups for volume, adhesions and inflammation reaction (IR). There were no differences between groups for GT, ST and microvessel density (Table 1). When the five groups were compared in pairs the adhesion score of the implants was lower in group PFG and OCFG than in all other groups (p < 0.05). There were no other differences between group PFG and OCFG and between each other group.

The mean volume of the implants in group OCFG was significantly lower than in group OS (respectively 28.3 mm³ and 54.7 mm³) (p < 0.05). The IR score was significantly lower in Group OCFG than in group OS and group PFG-S (p < 0.05). There was no correlation for data in these five groups.

When the groups were created based on the use of sutures, the mean adhesion score, volume and IR score of the implants was lower in group FG than in group S (p <
There was a positive correlation between adhesion score and IR score in group FG (R = 0.53, p < 0.05). Also when the data were not grouped there was a positive correlation between adhesion score and IR score (R = 0.51, p < 0.001). We observed more glandular tissue in endometriosis-like lesions in the FG group than in the S group (respectively, 1.6 ± 0.7, 1.4 ± 0.8), and vice versa for stromal tissue (respectively, 1 ± 0.5, 1.4 ± 0.8) (Figures 5/6). Also we observed that nearly all glandular tissue could be destroyed in highly inflammed lesions (Figure 7). Thus we noted endometriosis-like lesions to be histopathologically cleaner in the FG group than in group S. However, this finding was observed histopathologically.

Discussion

In this study, it was observed that foci of endometriosis obtained by using only FG were smaller, clearer, less inflammed and less adherent to other intraabdominal tissue than by suturing (Figure 4). Autologous fragments of endometrial tissue were transplanted onto inner surfaces of the abdominal wall by FG and/or suturing. Thus, two endometriosis foci were obtained by two different methods in one rat. Transplanted endometrial fragments were achieved in a circular form and, as well, as exactly equal by instrument design. With these methods, the numbers of subjects used in the study were reduced, while at the same time in every way equal to the creation of a model of endometriosis. These methods made it easy to compare and evaluate the results of the experiment.

Most homologous murine models of endometriosis are based on surgical implantation of endometrial tissue at different sites within the peritoneal cavity of recipient animals [6]. Implantation of endometrial tissue on peritoneal surfaces is done mostly with the help of a suture. Endometriosis foci can be obtained via intraperitoneal injection or by subcutaneously placing endometrial fragments without suturing [9, 10]. However, the purpose of suture is to determine in advance the location of the focus of endometriosis. On the other hand suturing provides only point fixation, if there is a large endometrial fragment, and it may fully prevent “touching” by between the endometrium and peritoneum.

Gibran et al. [7] used fibrin sealant for sheet skin grafts in patients with burns thus drawing attention to this situation, and continuous adherence to the wound bed which is optimal for adhesions and vascularization. In this study endometrial fragments were designed as circular and their surface area was 19,625 mm². In some studies, endometrial fragments were designed as square and sized 25 mm² [11]. Use of endometrial fragments in that form and prolene suture may create “touching” problems in corners.

Hiratru et al. [12] drew attention to the fact that in homologous and heterologous types of animal models, endometriotic-like lesions which are identified histologically are sometimes too unclear to distinguish from surrounding normal tissue. We concluded that inflammation contributes to this situation. On the other hand, inflammation may cause difficulty for histopathological evaluation. In this study histopathological examinations showed more intense adhesions and inflammation in all groups in which suturing was used and these findings suggest that suturing induces inflammation and adhesions. We concluded that the reason that larger implants were obtained when suturing was used induced more inflammation. In groups, in which only FG was used, there was no adhesion in seven of 20 subjects. Grade 1 adhesion was observed in nine and grade 3 and 4 adhesions were not seen in those subjects. The findings of the study supports the argument that FG may be a barrier to adhesions, as proposed in other experimental studies [13, 14]. Another conclusion extracted from the results of the study is that FG may have an adhesion preventing effect, at least it does not induce adhesions.

Although Brown et al. [2] reported that FG does not impair the supply and vascularization in wound healing, it was uncertain whether attachment of endometrial and peritoneal surfaces via FG impaired the supply and vascularization in endometrial grafts. Findings of the study showed that endometriosis-like lesions may successfully be obtained by using FG. Microvessel density was similar in all groups, which means that FG does not have a deleterious effect on angiogenesis and vascularization.

Two clinical studies were reported that had reduction of adhesions with FG after laparoscopic excision of large ovarian endometriomas [4, 5]. However two reviews about clinical use of FG did not mention the use of FG in gynecology [15, 16]. Endometriosis surgery is a difficult operation and it may cause excessive bleeding, whereas postoperative adhesions create major problems in infertility. When considering the hemostatic features of FG, it can be concluded that it will have more use in endometriosis surgery.

Conclusion

Finally, endometriosis-like lesions can be obtained by FG in animal models. The use of FG does not impair the supply and vascularization of endometrial grafts. The endometriosis-like lesions obtained via this method were smaller, cleaner, and included fewer inflammatory reactions and adhesions to other organs. Therefore wider clinical use of FG for endometriosis surgery may be possible.

References

Effect of fibrin glue and comparison with suture on experimental induction of endometriosis in a rat endometrial autograft model


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Comparison of diclofenac sodium with indomethacin suppositories for mediolateral episiotomies

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Summary

Objective: The study was carried out to compare the analgesic effect of diclofenac sodium and indomethacin suppositories for management of right mediolateral episiotomy repair. Method: A total of 70 patients who gave birth vaginally with right mediolateral episiotomy were randomly assigned to receive 100 mg diclofenac sodium suppositories/day (G1, n = 35) or 100 mg indomethacin suppositories/day (G2, n = 35) after episiotomy repair and postpartum for three days. Pain ratings were recorded before, the first hour and 24 hours after medication. The verbal rating scale (VRS) and visual analog scale (VAS) were used for pain recording. The independent T test, Mann-Whitney U and Wilcoxon rank test were used for statistical analysis and Spearman correlation analysis was used for comparison between VRS and VAS. Results: Diclofenac sodium was a more effective analgesic than indomethacin suppositories for right mediolateral episiotomy pain. For G1 the first hour VRS was 2.6 ± 0.5 points and VAS 4.9 ± 0.8 points; for G2 the first hour VRS was 3.4 ± 0.6 points VAS 6.6 ± 1.2 points; this difference was statistically significant (p < 0.05). For G1 at the 24th hour VRS was 1.2 ± 0.4 points and VAS 2.4 ± 0.9 points; for G2 at the 24th hour VRS was 2 ± 0.7 points and VAS was 3.4 ± 1.3 points; the difference was statistically significant (p < 0.05). A positive correlation was obtained between the first and 24th hour VRS and VAS by Spearman correlation analysis (rs = 0.9, n = 70, p = 0.000). Conclusion: The two analgesics were effective after episiotomy repair, however diclofenac sodium suppositories may be the preferred choice because they were more effective.

Key words: Diclofenac sodium; Indomethacin; Mediolateral episiotomy; Pain.

Introduction

Severe perineal pain during vaginal delivery is associated with lacerations or episiotomy. Treatment is usually by local anesthesia and oral or rectal analgesics [1-5]. Perineal pain, especially three days after vaginal delivery, can poorly effect the daily (movement, micturation, defecation, lactation) activities of women [6]. Both diclofenac sodium and indomethacin are nonselective nonsteroidal anti-inflammatory drugs (NSAID) that inhibit prostaglandine synthase via cyclooxygenase 1 (COX-1) and 2 (COX-2) [7, 8]. Inhibition of prostaglandin synthase leads to anti-inflammatory and antinociceptive effects [9, 10]. Odigie [4] et al. used 2 x 100 mg of indomethacin suppositories for post episiotomy pain and found them effective in their study. Searle and Pring [5] reported effective usage of 100 mg diclofenac suppositories for second degree perineal tears or episiotomy.

Our purpose in designing the study was to compare the analgesic effect of diclofenac sodium and indomethacin suppositories for right mediolateral episiotomy (MLE) that are often used for analgesia however have different action mechanisms.

Material and Methods

Seventy patients with spontaneous vaginal deliveries and MLE were randomly (randomized number table used), one-side blinded, and prospectively divided into two groups. Group 1: (n = 35) was composed of patients medicated by diclofenac sodium suppositories (Voltaren, 100 mg diclofenac sodium, Novartis, Istanbul, Turkey), Group 2: (n = 35) was composed of patients medicated by indomethacin suppositories (Endol, 100 mg indomethacin, Deva, Istanbul, Turkey). Permission was given by the Firat University Medicine Faculty Ethical Committee (ethical standards declared in Helsinki in 1983 were followed) for the study. All patients were informed about the study and gave written permission.

Exclusion criteria of the study were chronic renal failure, coagulopathy, non steroidal anti-inflammatory intolerance or allergy, episiotomies longer than 5 cm, forceps delivery and perineal tears. Age, length, gravida, abortus, length of the episiotomy, reoperation duration and presence of hematoma in the MLE region were recorded. Right after MLE reoperation 100 mg rectal suppositories were administered. The verbal rating scale (VRS) and visual analog scale (VAS) were used for pain score at rest, the first and 24th hour after medication. VRS scoring: No pain = 0 point, mild = 1 point, medium = 2 points, severe = 3 points, very severe = 4, intolerable = 5 points. A 100 mm ruler was used for VAS: 0 mm = no pain and 100 mm = very severe pain [11]. Control pelvic examinations were performed on postpartum day 1 and patients were released and prescribed 100 mg suppositories daily for two more days.

For statistical analysis the SPSS 9.0 computer programme (Microsoftware, Chicago, IL USA) was used. Continuous and ordinal data were established as mean ± standard deviation (SD). For comparison of independent groups, the Mann-
Comparison of diclofenac sodium with indomethacin suppositories for mediolateral episiotomies

Whitney U test was used for ordinal data and continuous data were determined by the independent T-test. The chi-square test was used for nominal data, and repeated measurements in the groups were analyzed by the Wilcoxon-rank test; \( p < 0.05 \) was considered as statistically significant. Spearman correlation analysis was used for analysis between VRS andVAS (\( r_s , n, p \)).

Results

Sociodemographic characteristics of the patients were similar (Mann-Whitney U test) in both groups. MLE length and MLE reoperation duration of the patients were also similar (independent T test) in both groups (non significant).

Primigravidas were 60% in Group 1 (G1) and 74% in Group 2 (G2) and no statistically significant differences were obtained (X² test) (Table 1).

No hematoma or complications were seen in MLEs.

For G1 one-hour VRS was 2.6 ± 0.5 points and VAS was 4.9 ± 0.8 points; for G2 one-hour VRS was 3.4 ± 0.6 points and VAS was 6.6 ± 1.2 points and this difference was statistically significant (\( p < 0.05 \), Mann-Whitney U test).

For G1 the 24th hour VRS was 1.2 ± 0.4 points and VAS was 2.4 ± 0.9 points; for G2 VRS was 2 ± 0.7 points and VAS was 4 ± 1.3 points (\( p < 0.05 \), Mann-Whitney U test).

The first and 24th hour pain scores (VAS1-VAS24, VRS1-VRS24) were decreased dramatically for both groups (\( p < 0.05 \), Wilcoxon rank test).

A positive correlation was obtained between the first and 24th hour for VRS and VAS scores by Spearman’s correlation analysis (\( r_s = 0.9, n = 70, p = 0.001 \)).

Discussion

Diclofenac sodium suppositories had more effective analgesia than indomethacin suppositories for early- and late-term perineal pain occurring after MLE. Consequently diclofenac sodium suppositories may be proposed for post MLE pain.

We quantified our study by using both VRS and VAS scales that are generally used in analgesia studies. A positive correlation was obtained between VRS and VAS by Spearman’s correlation analysis.

Odigea et al. [4] administered 2 x 100 mg indomethacin suppositories to 30 patients and placebo to another 30 patients and evaluated the pain at 15, 30, 60 and 90 minutes. No patients complained about pain after MLE reoperation in the study group, however in the placebo group patients felt variable pain. Our first hour findings correlated with this study. We thought that pain after MLE would poorly effect the quality of life so we did not design any placebo group.

Seckin et al. [12] administered 100 mg indomethacin suppositories vaginally and could not establish any analgesia pain at 15, 30, 60, and 90 minutes when compared with placebo. The administration way of drugs and maximum pain point on VRS (3 points) differed from our study and that may have altered the results. In our study the administration route was rectal and maximum points on VRS were 5. Searles and Pring [5] administered 100 mg diclofenac suppositories to 100 cases and found that average pain score markedly decreased on the first, second and third days when compared with a control group, which is compatible with our findings. Both diclofenac sodium and indomethacin are non selective non steroidal anti-inflammatory drugs. They inhibit prostaglandin synthesis and thus act as anti-inflammatory analgesics [7, 8].

Diclofenac and other nonsteroidal anti-inflammatory drugs (ketorolac, metamizole, nimesulide and meloxicam) have analgesic effects independent from prostaglandin, which is why these analgesics have different analgesic properties.

Diclofenac activates the L-arginine-NO-cGMP-potassium channel and inhibits the hydrogen channel [13-16]. Potassium channel opening leads to hyperpolarization and increased intracellular cGMP. Hyperpolarization causes desensitization and cGMP has an analgesic effect. Indomethacin does not act in this way, which may be the explanation as to why diclofenac sodium suppositories were more effective analgesics in our study.

Inhibition of hydrogen ion channels show an analgesic effect especially when topical medicaments (suppositories) were applied [13]. However, indomethacin does not have any effect on these channels.

These two medications have been compared especially in rheumatoid arthritis cases. Wafin et al. [17] reported similar analgesic effects of 100 mg diclofenac and 100 mg indomethacin suppositories. However these were for joint pain (hand, elbow, finger and knee), not perineal. Topical diclofenac sodium has an analgesic effect on the perineum. Diclofenac sodium increases both plasma and brain \( \beta \)-endorphin levels [18, 19]. Increased \( \beta \)-endorphin decreases pain [20]. Thus analgesia effects of diclofenac sodium were marked in our cases.

In conclusion, after vaginal deliveries with right mediolateral episiotomies diclofenac sodium suppositories may be the first choice as a more effective analgesic than indomethacin suppositories.
References


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Early abdominal pregnancy with an unexpected and misleading location. The ultrasonographic interpretation

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Maternidade Dr. Alfredo da Costa, Lisboa (Portugal)

Summary
Abdominal pregnancy is a rare condition defined as an ectopic pregnancy that implants in the peritoneal cavity and is associated with important morbidity and mortality. We report a case of a 35-year-old woman with an ultrasonographic diagnosis of a left extraterine pregnancy located proximally in the isthmus or distally in the interstitial region. In the exploratory laparotomy a gestational sac implanted anteriorly on the uterine serosa was found, with no signs of uterine perforation or tubal abortion. The pathologic examination confirmed the diagnosis of an early first trimester abdominal pregnancy. This case illustrates the importance of an early sonography in the diagnosis of an abdominal pregnancy as well as a high index of suspicion.

Key words: Primary abdominal pregnancy; Hemoperitoneum; Early ultrasound.

Introduction
Abdominal pregnancy is a rare condition defined as an ectopic pregnancy that implants in the peritoneal cavity either primarily or secondarily. It is associated with important morbidity and mortality and the estimated incidence is one in 10,000 in the USA [1], representing 1.4% of all ectopic pregnancy cases [2, 3]. The risk factors studied are the same as for ectopic pregnancies in general, therefore assisted reproductive technology (ART) increases the risk of an abdominal pregnancy [2, 4-6].

Many cases have been described in the literature and the implantation sites reported include the omentum, pelvic side wall, broad ligament, cul-de-sac and abdominal organs (spleen, bowel, liver), large pelvic vessels, diaphragm and the uterine serosa [3-5, 7-9]. Various hypotheses have been proposed to explain this rare phenomenon, the most probable being secondary implantation from an aborted tubal pregnancy. Primary implantation, i.e., intraabdominal fertilization of sperm and ovum, is an extremely rare event according to the literature [10]. In the context of infertility, there are reports of different mechanisms to explain the pathogenesis of an abdominal pregnancy, including uterine perforation by the embryo transfer catheter, migration of an embryo through a microscopic fistulous tract in the interstitial portion of the tube and subsequent implantation in the abdominal cavity, and migration of an oocyte to the abdominal cavity where it is fertilized by spermatozoa entering through a cornual fistulous tract (after follicular aspiration) [2].

The diagnosis of an early abdominal pregnancy may be a difficult challenge. A high index of suspicion is required due to the non specificity of clinical history, physical examination, as well as laboratory and ultrasonographic findings. The clinical manifestations are frequently non specific and vague, depending on the pregnancy location and gestational age.

Ultrasound (US) examination is the gold standard diagnostic tool. However, according to Costa and associates, a sonographic diagnosis of abdominal pregnancy is missed in half the cases [11]. In the particular case of an early pregnancy, it may be difficult to distinguish an abdominal from a tubal pregnancy if it implants near the adnexa [4].

Case Report
A 35-year-old woman, nullipara, was referred to our infertility unit in July 2009, due to a history of primary infertility of two years duration, with a diagnosis of ovarian endometriosis. The patient had been previously examined in another hospital, where she was submitted to an exploratory laparoscopy in March 2009. During this intervention an endometrioma of the left ovary was removed and many implants of endometriosis on the uterosacral ligaments and peritoneum were detected and electrocoagulated. The patency of both fallopian tubes was demonstrated and subsequently the pathologic examination confirmed the diagnosis of endometrioma.

In our institution, we studied the male factor as a possible infertility cause, and found an astenoteratozoospermia. The patient study did not reveal additional pathology and she was waiting for an in vitro fertilization cycle.

In October 2009, the woman was admitted to our emergency room, complaining of intense abdominal pain, predominantly in the pelvic region, accompanied by an episode of loss of consciousness. Vital signs were normal and physical examination revealed a distended abdomen and diffuse tenderness in the lower quadrants, although with no rebound tenderness. Transvaginal ultrasound scan showed a thick endometrium and an empty uterus with no evidence of a gestational sac. A small amount of free fluid was noted in the pelvis and the ovaries both

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had augmented size – the left one due to a corpora lutea and the right one owing to an apparently multiseptated cyst. Blood and urine analyses were requested and indicated the existence of a possible pregnancy, with a beta HCG level of 7,271 mUI/ml and progesterone level of 7.8 ng/ml. The hemoglobin was 10.5 g/dl.

We decided to repeat the US scan a few hours after admission. This was performed in our ultrasonography unit in order to obtain a more detailed and precise exam. The transvaginal scan (Figures 1-4) confirmed the previous findings and additionally noted an anechoic image in the left adnexa, just close to the uterus, 24 \times 23 \text{ mm} in diameter, surrounded by an echoic hale. In its interior, we found an embryo with cardiac activity and a crown-rump length (CRL) of 9 mm. Free echoic fluid in the pelvis was estimated as 150 ml. Therefore, the US findings were compatible with a left extrauterine pregnancy located proximally in the isthmus or distally in the cornual region.

Blood analyses were repeated and showed a decrease in the hemoglobin level to 9.4 g/dl, normal platelet count and normal coagulation tests. Anemia was corrected by a transfusion of one unit of red blood cells.

After evaluating the Fernandez score [12] the patient underwent surgery (Table 1). Exploratory laparotomy showed moderate intraabdominal bleeding (hemoperitoneum) and a gestational sac implanted anteriorly on the uterine serosa, between the left uterine cornu and the origin of the left round ligament. After removing the ruptured gestational sac, the implantation site, which showed no signs of uterine perforation or tubal abortion, was electrocoagulated and the hemorrhage ceased. The uterus and both ovaries and tubes were carefully inspected. They appeared normal and no signs of uterine perforation or bleeding from either fimbriae were observed. A transfusion of

<table>
<thead>
<tr>
<th>Score</th>
<th>Weeks of amenorrhea</th>
<th>( \beta )hCG (mUI/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Abdominal pain</th>
<th>Hematosalpinx diameter</th>
<th>Hemoperitoneum volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7 weeks + 1 day</td>
<td>7271</td>
<td>7.8</td>
<td>spontaneous</td>
<td>24 \times 23 \text{ mm}</td>
<td>150 ml</td>
</tr>
</tbody>
</table>

Table 1. — Fernandez Score [12].
Early abdominal pregnancy with an unexpected and misleading location. The ultrasonographic interpretation

In conclusion, although abdominal pregnancy is a rare event, awareness of this condition is very important in reducing the associated morbidity and mortality.

References


maal285-31 - Case Reports:1648_29 Incidence of multiple 21/02/12 14:01 Pagina 117

one unit of erythrocyte concentrate was made intraoperatively. The right ovary had two cysts – one simple and the other hemorrhagic – which were removed. The pathologic examination confirmed the diagnosis of a first trimester abdominal pregnancy. The postoperative course was uneventful and the patient was discharged on the fourth postoperative day. Hemoglobin at the discharge was 10.4 g/dl and beta hCG was 480.10 mUI/ml. Posterior clinical evolution was favorable, without intercurrences, and 17 days postoperative the beta hCG was almost negative (14 mU/ml). The patient was therefore referred to the infertility unit.

Discussion

Abdominal pregnancy is a rare event, having an incidence of one in 10,000 pregnancies in developed countries. It occurs either as a result of tubal abortion or rupture (secondary abdominal pregnancy) or, more rarely, as a direct implantation on the peritoneum, with normal fallopian tubes, normal ovaries, and no tubal fistula (primary abdominal pregnancy) [13]. Since maternal morbidity and mortality is very high, the diagnosis of this condition in early gestation is extremely important to avoid massive hemorrhage [14].

Diagnosis of an abdominal pregnancy is a difficult challenge and only 40% of cases are correctly identified before surgery. A high index of suspicion is required and knowledge of abdominal pregnancy risk factors, the same as those for ectopic pregnancy, is essential. Initial clinical findings are also the same as those of ectopic pregnancy, the most frequent ones being abdominal pain and vaginal bleeding.

Early transvaginal US is valuable in diagnosing abnormalities such as anembryonic gestation, viable intrauterine or extrauterine gestation, and it is currently the imaging method of choice [15]. In this particular case, it is essential to notice the rare and misleading location, since it was this feature that led us to consider the hypothesis of the US diagnosis of a left extrauterine pregnancy located proximally in the isthmus or distally in the cornual region. US is the only available non invasive method which can detect a peritoneal pregnancy, although the differential diagnosis with tubal pregnancy has yet been unsatisfactory [16].

Laparotomy has been the treatment of choice in abdominal pregnancy with concurrent intraabdominal hemorrhage. The development of efficient laparoscopic instrumentation and accumulating experience and skills of laparoscopic surgeons have led to recent reports of successful management of abdominal pregnancy by laparoscopy. However, successful treatment of this condition associated with severe hemoperitoneum has rarely been reported [17]. In our case report, the patient was hemodynamically unstable, which led to the option of performing a laparotomy. Nevertheless, laparoscopy must be considered today as the gold standard treatment for early abdominal pregnancy, even with concurrent intraabdominal bleeding [18].
First trimester diagnosis of 13q-syndrome associated with increased fetal nuchal translucency thickness. Clinical findings and systematic review

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Summary

13q-syndrome is a rare chromosomal disorder caused by partial deletion of the long arm of chromosome 13 with variable phenotypic presentation. Further sonographic features involve fetal growth restriction, bradycardia, encephalocele, facial dysmorphism and upper extremity deformity. We report a case of 13q-syndrome presenting as increased nuchal translucency diagnosed by chromosome studies and confirmed by array comparative genomic hybridization (CGH) analysis in the first trimester of pregnancy. Pregnancy was terminated at 14 weeks’ gestation. The parents did not give consent for a postmortem examination. Furthermore we performed a systematic review of the international literature on previous cases of 13q-syndrome diagnosed prenatally. Our case emphasizes the importance of a detailed 11-14 week ultrasound assessment in diagnosing fetal chromosomal aberrations in combination with the modern aspects of array CGH, thus providing more precise and rapid prenatal diagnosis.

Key words: 13q deletion; Nuchal translucency; Prenatal diagnosis, Comparative genomic hybridization.

Introduction

Partial deletion of the long arm of chromosome 13, also known as 13q-syndrome, is an exceedingly rare chromosomal aberration which is related to mental and growth retardation and various congenital malformations [1]. Clinical features include moderate to severe developmental delay, growth retardation, craniofacial dysmorphism (microcephaly, hypertelorism, broad nasal bridge, micrognathia), hand and foot anomalies (hypoplastic or absent thumbs), hypoplastic kidneys, and ambiguous genitalia. Central nervous system anomalies such as neural tube defects, holoprosencephaly and agenesis of the corpus callosum, and malignant neoplasms like retinoblastoma have also been reported [2, 3]. Herein we report a case of 13q deletion diagnosed prenatally together with a systematic review of the literature.

Case Report

A 24-year-old Caucasian woman, gravida 1, para 0, with no remarkable previous obstetric or family history, presented in the first trimester of pregnancy. An ultrasound (US) examination at 13 weeks of gestation revealed an increased nuchal translucency (NT) of 3.4 mm. The adjusted risk for trisomy 21 was one in 29 whereas the adjusted risk for trisomy 18 and 13 was one in 131. Although the woman was sure about the first day of her last menstrual period and had had regular cycles, fetal crown-rump length (CRL) was 49.6 mm showing an obvious discrepancy between fetal size and gestational age, suggesting early fetal growth restriction. Fetal heart rate was measured as 140 beats per minute (below the fifth percentile for the gestational age). A midsagittal view of the face revealed a dysmorphic appearance (micrognathia, distinctive flat profile); additionally, a small parietal encephalocele was documented.

Assessment of fetal extremities showed fixed arms with lack of elbow extension and suspicion of bilateral clinodactyly. Genetic counseling was performed and the parents opted for invasive prenatal diagnosis by chorionic villus sampling (CVS). Fetal DNA was extracted and a multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) analysis was performed. The QF-PCR products were analyzed by capillary electrohoresis on an ABI 3130 automated DNA sequencer. All short tandem repeat markers for chromosomes 18, 21 and X were observed in a normal diallelic pattern. The three markers used for chromosome 13 were monoallelic (D13S634, D13S631, D13S258) and were unusually lower in peak height and peak area compared to the other markers thus indicating a possible monosomy.

The QF–PCR report for chromosome 13 was given as inconclusive. The chorionic villi were then cultured and GTG banding (300-400 bands) of chromosomes revealed a deletion of chromosome 13 in two different cultures [fetal karyotype: 46,XX,del (13)(q22.2qter)].

Further analysis by array-CGH1 confirmed the diagnosis revealing a deletion of about 40 MB of the distal long arm of chromosome 13 [del(13)(q22.2qter)] with the proximal breakpoint between 74,497 MB (last normal oligonucleotide) and 74,620 MB (first deleted oligonucleotide). The last oligonucleotide present in the array at the 13q position, 114,077 MB was deleted (Figure 1). Both parents had normal karyotype. Based on US findings and cytogenetic analysis the prenatal diagnosis of 13q-syndrome was established. After genetic counseling, termination of pregnancy was carried out at 14 weeks. Autopsy was not performed due to lack of parental consent.
Table 1. — Clinical characteristics of the studies with prenatal diagnosis of 13q deletion.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Maternal age</th>
<th>Ultrasound features (wks)</th>
<th>Invasive testing</th>
<th>Cytogenetic analysis karyotype-outcome</th>
<th>Gender-pathology report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santolaya et al.</td>
<td>USA</td>
<td>18</td>
<td>G1PO 18 weeks - Holoprosencephaly bilateral talipes, hydrocephalus</td>
<td>Aminocentesis</td>
<td>Giemsa banding 46 XY,del(13) IUD</td>
<td>Male - Hydrocephalus, bilateral talipes, atrial septal defect</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>Taiwan</td>
<td>28</td>
<td>G3P2 27 weeks - IUGR cardiomegaly microcephaly, encephalocele</td>
<td>Aminocentesis</td>
<td>Giemsa banding 46,XX,del(13) (pter-q21)</td>
<td>Male - Microcephaly, encephalocele, microphthalmia, hypoplasia, micrognathia</td>
</tr>
<tr>
<td>Lam et al.</td>
<td>Hong Kong</td>
<td>37</td>
<td>NS 12 weeks - Exencephaly abnormal fetal cranium</td>
<td>NP - material analysed after curetag</td>
<td>Giemsa banding of placental tissues 46,XX,13q</td>
<td>Post-mortem examination not possible</td>
</tr>
<tr>
<td>Gutierrez et al.</td>
<td>Chile</td>
<td>29</td>
<td>G3P0 32 weeks - IUGR polyhydramnios holoprosencephaly micrognathia unilateral talipes</td>
<td>Cordocentesis</td>
<td>Giemsa banding 46,XY,del(13) (q22 -qter) IUD in 33 wks</td>
<td>Male - Holoprosencephaly micrognathia, microphthalmia, unilateral clubfoot, agenesis of thumbs, ambiguous genitalia</td>
</tr>
<tr>
<td>Widenschwendter et al.</td>
<td>Austria</td>
<td>27</td>
<td>G1PO 24 weeks - Asymmetric IUGR - encephalocele retrognathia hypoplastic thumbs unilateral hypoplasia</td>
<td>Aminocentesis</td>
<td>Rapid-FISH Culture-fetal lymphocytes 46,XY/46,XY,del(13) (q13.3)</td>
<td>Male - Meningoencephalocele microphthalmia, micrognathia, syndactyly</td>
</tr>
<tr>
<td>McCormack et al.</td>
<td>USA</td>
<td>30</td>
<td>G3P1 NS-IUGR</td>
<td>Aminocentesis</td>
<td>Giemsa banding 46,XY,d(13) t(1;13)(q43;q21)</td>
<td>Male - Microcephaly, holoprosencephaly, aplasia of organs</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>Taiwan</td>
<td>28</td>
<td>G2P1 17 weeks - Holoprosencephaly premaxillary agenesis hypoplastic left heart hexadactyly</td>
<td>Aminocentesis</td>
<td>Giemsa banding 46,XX,del(8);(8;13) (p23.q32;q21)</td>
<td>Female - Holoprosencephaly, premaxillary agenesis, hexadactyly</td>
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<tr>
<td>Alanay et al.</td>
<td>Turkey</td>
<td>27</td>
<td>G1PO 21 weeks - IUGR DWM-limb deformities</td>
<td>Cordocentesis</td>
<td>Giemsa banding 46,XYdel(13) (q14 -qter)</td>
<td>Male - Absence of fetal vermis DWM, corpus callosum agenesis, renal agenesis lobe anomaly</td>
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<td>Gul et al.</td>
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<td>25 weeks - IUGR microcephaly, microphthalmia, oligodactyly, DWM</td>
<td>Cordocentesis</td>
<td>Giemsa banding 46 XY del (13) 13q31.2 /q32.13qter</td>
<td>Male - IUGR, DWM, microphthalmia, micrognathia, oligodactyly</td>
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<td>Araujo et al.</td>
<td>Brazil</td>
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<td>G3P1 23 weeks - IUGR-fusion of the lateral ventricles, agenesis of left kidney, hypoplasia</td>
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<td>Giemsa banding 46,XX,del(13) (pter-31.3)</td>
<td>Female - Pathology, findings consistent, with US findings</td>
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<td>Hindryckx et al.</td>
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<td>41</td>
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<td>NS - DWM, parietal encephalocele, agenesis of corpus callosum, renal dysplasia</td>
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<td>Aminocentesis</td>
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<td>Female - Multiple cord lacerations, Pathology NS</td>
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<tr>
<td>Cain et al.</td>
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<td>Male - Post-mortem examination not performed</td>
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Discussion

We report a case of 13q-syndrome diagnosed in the first trimester of pregnancy manifesting with increased NT. Increased NT in the first trimester can be associated with numerous chromosomal abnormalities, fetal structural defects, congenital heart defects, and genetic syndromes [4, 5]. It can also be a variant of normal, documented by spontaneous resolution as the pregnancy advances beyond the first trimester [6]. The clinical characteristics of 13q syndrome have been known since 1963 [3]. Up to now approximately 100 cases have been reported and most of them were diagnosed in individuals with mental retardation associated with several congenital anomalies [1]. However in the international literature there are few cases diagnosed prenatally.

We performed a search of Medline (1950-March 2010) electronic database. The Mesh (Medical subject Headings) we used were the following: ‘prenatal diagnosis’, ‘13 q deletion’, ‘13 q syndrome’. We included in the review only studies associated with in utero prenatal diagnosis of 13q syndrome. Studies that reported clinical diagnosis of 13q syndrome post partum and during early and late childhood were excluded from the review. The articles were all written in the English language and published during the time period of July 1993 to September 2008. The clinical characteristics of the selected studies are summarized in Table 1.

A total of 15 patients, including our case were enrolled in the review. Six studies derived from Asia [7-12] five studies were reported from America [13-17], and three studies were from Europe [18-20] The median maternal age was 29.61 years with a range of 18-41 years (standard deviation = 6.03). Median maternal gravidity was 3 (standard deviation = 2.4). Median weeks of diagnosis was 18 with a range of 12-32 weeks [13-17]. With regard to the aspect of prenatal invasive diagnosis, amniocentesis was the most common procedure performed in eight cases (53.3%) [7, 9, 10, 12, 13, 15, 16, 19]; CVS in five cases (33.3%) [17, 18, 20]; cordocentesis in four cases (26.6%) [11, 14, 16, 19]. IUGR (intrauterine growth retardation) was the most common US feature notified in eight cases (53.3%) [7, 10, 11, 14-16] including our case as well.

The most common structural abnormality reported was encephalocele, present in five cases (33.3%) [7, 17, 19, 20], followed by holoprosencephaly in four cases (26.6%) [13-15, 19].

In three cases (20%) of 13q deletion we observed a combination with Dandy-Walker malformation [10, 11, 20]. Multiple limb deformities were visualized, most often localized on the hands, and in six cases (40%) these were anomalies such as synactyly, oligodactyly, agenesis of thumbs and other morphological abnormalities [9-11, 17, 19].

Cytogenetic diagnosis with FISH (fluorescent in situ hybridization) was confirmed in eight patients [9, 11, 17-20] and CGH array was utilized in three cases [17, 20], including our patient.

Termination occurred in ten pregnancies (66.6%) [7-9, 11, 17-20] and IUD (intrauterine death) in three pregnancies (20%) [13, 14, 16]; three neonates (20%) expired after delivery [10, 12, 15]. Nine fetuses had male gender and five had female gender.

Brown et al. [1] suggested a new classification in which the individuals with deletion of the chromosome 13 long arm should be subdivided in three groups according to the location of the deletion. Individuals with distal deletions would be the most severely affected, while those with proximal deletions would have fewer major anomalies. The first group would include those with proximal deletion, generally not reaching band 32q, presenting mild to moderate mental retardation, varied dysmorphism and growth restriction. The second group would include those with distal deletion reaching band 32q, with a larger number of major anomalies such as severe microcephaly, occipital encephalocele, holoprosencephaly, lack of the thumbs or other anomalies of the distal limbs, severe microphthalmia, coloboma, malformations of the genitourinary and gastrointestinal tract and severe mental retardation with growth restriction. The third group would include those with a more distal deletion reaching bands 33 and 34q, with severe mental retardation but not with major anomalies and usually without growth restriction. The authors suggested that there is a critical area on band 32q that contains a gene or genes which are essential for brain, finger and other organ development.

In our case the most remarkable finding was the early detection of increased NT in the first trimester scan in association with IUGR, encephalocele, clinodactyly and facial dysmorphism. Three studies achieved early prenatal diagnosis, the first by Lam et al. [8], the second by
First trimester diagnosis of 13q-syndrome associated with increased fetal nuchal translucency thickness. Clinical findings and etc. 121

Hindryckx et al. [20] and the last by Cain et al. [17]. Our findings are consistent with those of Hindryckx et al. [20], where the fetus exhibited increased NT at 13 weeks of gestation.

Identification of small unbalanced translocations is one of the most difficult tasks in prenatal cytogenetics. It often requires parental karyotyping to search for a familial translocation, but frequently the unbalanced rearrangement will be a de novo event or a key family member will not be available for karyotyping. Until now, FISH has been the method of choice for uncovering these translocations. Probes are selected based on the phenotype, or in some cases, an analysis of all the subtelomeric regions is necessary. The process is time-consuming, expensive and not always successful. For these reasons array CGH appears to be an attractive alternative to traditional FISH, providing information on copy number changes at subtelomeric regions and loci known to be involved in genetic disease in one, rapid assay [17].

Our case emphasizes the importance of a detailed 11-14 week US assessment in diagnosing fetal chromosomal aberrations, using a further type of structural chromosomal abnormality diagnosed in the first trimester of pregnancy presenting with increased NT. CGH-array is a modern and precise diagnostic tool which will complement and enhance current methods of detecting chromosomal translocations, adding a further type of structural chromosomal aberrations. Probes are selected based on the phenotype, or in some cases, an analysis of all the subtelomeric regions is necessary. The process is time-consuming, expensive and not always successful. For these reasons array CGH appears to be an attractive alternative to traditional FISH, providing information on copy number changes at subtelomeric regions and loci known to be involved in genetic disease in one, rapid assay [17].

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Complicated abdominal hysterectomy subsequent to uterine embolization for large fibroids

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Introduction

Uterine fibroids are a common finding among women of reproductive age with an estimated incidence of 20-25% but their prevalence is estimated to be as high as 77% [1]. In recent years UAE has been offered as an alternative to surgical procedures or hormonal therapy and is associated with reduction in uterine volume and decrease in excessive uterine bleeding [2]. However, by decreasing the uterine blood supply this technique might predispose to intraabdominal adhesion formation as a result of tissue necrosis. It appears that the larger the myomas the higher the possibility of creation of dense adhesions since the necrotic tissue is also enlarged. Adhesions should be expected between the uterus and the surrounding organs like adnexes, small bowel, omentum and peritoneum and could complicate a routinely performed hysterectomy.

Case Report

A 41-years-old patient presented at the Fibroid Clinic of our Unit complaining of menorrhagia and pressure symptoms associated with a large fibroid uterus. The patient was nulliparous with no obstetrical history and no previous abdominal surgery. Concerning gynecological history, one year earlier the patient presented with similar symptoms and was offered all the options for treating fibroids. She had no interest in infertility issues or future pregnancy but rejected abdominal surgery (myomectomy or hysterectomy) and decided to proceed to UAE. She did not present for follow-up, thus fibroid volume reduction was not estimated.

On clinical examination the uterus was enlarged with fibroids palpated. The patient mentioned that the symptoms returned six months after UAE and deteriorated the last month. Magnetic resonance imaging (MRI) revealed multiple fibroids including two large myomas, one of which was situated in the lower segment of the uterus and the other at the uterine fundus (Figure 1). Unfortunately previous MRI findings were not available so as to compare the number and size of fibroids. After counseling, the patient decided to undergo hysterectomy considering her decision that future pregnancy be excluded.

Hysterectomy was started via a subumbilical midline laparotomy. As it was not possible to exteriorize the uterus, the incision was extended cephalad. Despite releasing relatively minor adhesions between the uterus and small bowel, it was still not possible to maneuver the uterine fundus into the laparotomy incision. Palpating the uterus inside the abdomen, we noted a solid, dense mass between the fundus and the peritoneum which was obviously restricting uterine mobility. Rather than extending the incision up to the xiphisternum, we decided to proceed to hysterectomy without freeing the uterine fundus. The surgery was performed with some difficulty. Once the cervix had been separated from the vagina, we were able to elevate the uterus towards the chest and free a mass of dense adhesions measuring approximately 8 x 2 cm which was attached to the fundal fibroid (Figure 2). Reviewing the patient’s MRI films, we considered that the mass most probably corresponded to adhesions created around a large fundal fibroid following UAE. The patient’s postoperative course was uneventful.

Discussion

Large fibroids do not seem to be a contraindication for UAE and these patients have similar response with respect to menorrhagia to their counterparts with small fibroids. Compared with surgical treatment the quality of life score of UAE at one year is similar [3]. However, large fibroids are more difficult to treat, while fibroid size (more than 10 cm) and volume are considered predictive factors for subsequent surgery after UAE [4, 5]. It is reported that 12 months after UAE, approximately 10% of patients undergo surgery, mainly hysterectomy [6].

It has been suggested that embolization of uterine myomas might result in tissue necrosis predisposing to
Complicated abdominal hysterectomy subsequent to uterine embolization for large fibroids

adhesion formation. Although other causes may operate in some patients (e.g., previous surgery, adnexal torsion, infection, malignancy), it is widely felt that embolization of large subserous myomas itself can play a role in the development of de novo intraabdominal adhesions between the uterus and the adnexae, bowel, peritoneum and/or omentum through thrombosis and necrosis of the tissue [1].

A recent case controlled study [6], has confirmed the impression gained from isolated case reports [7, 8] and concluded that UAE can be associated with the formation of intraabdominal adhesions, with large myomas in particular predisposing to such adhesions due to increased volume of the necrotic area.

Our patient, who had no risk factors for intraperitoneal adhesions apart from having undergone UAE a year earlier, was found to have extensive adhesions involving the uterus, including very dense adhesions on to a large fundal fibroid. As a result we carried out the hysterectomy with the uterine fundus still adherent inside the abdomen and only freed the fundus by ultimately reflecting the uterus towards the sternum to gain access to the fundal adhesion. With this surgical maneuver there were no serious sequelae (incision extension or severe bleeding), but it has to be noted that the operation was completed with considerable difficulty.

Our experience, and that of others, shows that women who undergo hysterectomy after UAE, especially in the presence of relatively large fibroids, should be warned about potential surgical problems during surgery such as extension of the initial incision, bleeding, and bowel injury, as a result of the development of UAE-associated adhesions.

References

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Removal of a large bizarre uterine leiomyoma by operative hysteroscopy. Case report and review of the literature

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Introduction

In the current World Health Organization (WHO) classification, leiomyoma with bizarre nuclei (LBN) is grouped under the heading ‘atypical leiomyoma’ [1]. An atypical leiomyoma by definition is a smooth muscle tumor that shows cellular atypia and may sometimes have increased mitotic rates. Once regarded as in-situ leiomyosarcoma, [2] this tumor is now generally accepted as a clinically benign variant of leiomyoma, despite microscopic features of malignancy [3, 4]. In the earlier edition of the WHO classification, LBN was defined as ‘a leiomyoma containing giant cells with pleomorphic nuclei with little or no mitotic activity’ [5]. Symplastic leiomyoma, bizarre leiomyoma, and pleomorphic leiomyoma are older synonyms for atypical leiomyoma. We present a case of an atypical uterine leiomyoma which was diagnosed and removed by operative hysteroscopy in our department, together with a review of the literature.

Case Report

The patient, a 49-year-old, gravida 3, para 3 perimenopausal Greek woman presented with a complaint of dysmenorrhea and episodes of menometrorrhagia during the last six months. Her past medical and surgical histories were unremarkable. For the last three months the patient had received gonadotropin-releasing hormone agonists. The gynaecologic examination was normal and Papanicolaou examination of cervical, vaginal smears was also normal. Transvaginal ultrasound (TVS) revealed a large submucosal leiomyoma at the fundus of the uterine cavity. The endometrium had also a width of 16.8 mm, with multiple blood vessels at the Doppler examination.

Hysteroscopy was then performed using saline infusion as a distension medium. A large, submucosal uterine leiomyoma 4 x 5 cm, that filled up all the uterine cavity, was detected. Myomectomy by operative hysteroscopic resection and uterine curettage under general anaesthesia were undertaken. The mass was entirely removed and the specimens were sent for histological examination. The tumour had a solid, soft, multinodular, yellow-tan surface, but without any other signs to the naked eye that may have helped in the differential diagnosis from the usual type of leiomyoma.

Histologic evaluation of the specimens revealed an atypical uterine leiomyoma. Large cells with eosinophilic cytoplasm and bizarrely shaped, multilobated, hyperchromatic nuclei, that characterize this tumor, were present. There was no mitotic activity or necrosis. Follow-up six months after surgery with TVS showed no evidence of recurrence.

Discussion

The aetiology and pathogenesis of atypical leiomyomas remain unclear. The question why bizarre cells develop in these leiomyomas has not yet been answered [6]. A few cases have occurred in patients receiving synthetic progesterins [7, 8], but a causal relationship has not been proven. Symplastic-type giant cells often have been attributed to degeneration, a theory supported by the cytologic features of some of the bizarre cells and by at least one ultrastructural study [9]. However, the cause for such degeneration is not apparent. There is also no known relationship of bizarre cells in leiomyomas to preoperative – as in our case – treatment with gonadotropin-releasing hormone agonists, such as leuprolide acetate [10].

The main symptoms and clinical findings in cases of atypical leiomyomas are irregular vaginal bleeding, pain and pelvic tumour [10]. Most LBNs are less than 5.5 cm in diameter [6]. This contrasts with the relatively large size of most leiomyosarcomas in which the mean maximum dimension is about 10 cm [11, 12]. In our case the patient presented because of menometrorrhagia and dysmenorrhea and the tumour was less than 5 cm in maximum diameter.

Summary

Background: Atypical leiomyomas are relatively uncommon in the general practice of gynaecology. We present a case of a large uterine bizarre leiomyoma removed by operative hysteroscopy and review of the literature. Case: The patient, a 49-year-old, gravida 3, para 3, perimenopausal Greek woman presented to our Department because of dysmenorrhea and abnormal vaginal bleeding. She underwent hysteroscopy in which a large submucosal leiomyoma was detected and entirely removed in one session. The histopathology revealed bizarre uterine leiomyoma. Discussion: There is no evidence to indicate that hysterectomy is necessary, if the diagnosis of atypical leiomyoma has been firmly established.

Key words: Atypical leiomyoma; Hysteroscopy; Bizarre leiomyoma.
Removal of a large bizarre uterine leiomyoma by operative hysteroscopy. Case report and review of the literature

The gross appearance of atypical leiomyomas usually resembles that of typical leiomyomas, but yellow to tan areas, softening, and cysts are seen in a minority of cases. The cardinal microscopic feature is the presence of large cells with eosinophilic cytoplasm and bizarrely shaped, multilobated or multinucleated, hyperchromatic, often ‘smudged’ nuclei, but an absence of other worrisome histological features. The nuclei often contain cytoplasmic pseudoinclusions. In our specimens these large bizarre, pleomorphic tumour cells with atypical nuclei and eosinophilic cytoplasm were present.

LBNs are reported to be clinically benign. The major criteria of malignancy are mitotic index, nuclear atypia and necrosis [13, 14]. Mitotic activity is low compared to leiomyosarcomas. Downes and Hart studied 24 LBNs and found 0-7 mitotic figures (MFs) per 10 high-power fields (HPFs) using the highest count method (mean 1.6 MFs/10HPFs), or 0-2.8 MFs/10HPFs using the average count method (mean 0.8 MFs/10HPFs). Although one of the tumors had 7 MFs/10HPFs, none of them recurred after a mean follow-up of 11.2 years [6]. In our case there was no mitotic activity or necrosis and no evidence of recurrence has been detected during this six-month follow-up period.

When an atypical leiomyoma is discovered in a hysterectomy specimen, no further treatment is required. Despite its good reputation, cases of recurrence following conservative surgical treatment for atypical leiomyoma with myomectomy have been reported. With an increasing number of patients being treated by myomectomy, however, the frequency of LBNs in myomectomy specimens will undoubtedly increase. Although the number of reported cases in which myomectomy only was performed and long-term follow-up remains small, there is currently no evidence to support that hysterectomy is necessary, once the diagnosis of LBN has been firmly established. However a much more cautious approach must be adopted in the management of atypical...
leiomyoma following myomectomy, especially when the atypia is diffuse or the tumour is large, by recommending follow-up with non-invasive imaging in order to exclude regrowth in the first several years.

Hysteroscopic diagnosis and treatment of atypical submucosal uterine leiomyomas is a safe minimally invasive surgery method. Hysteroscopy does not increase the risk of microscopic intraperitoneal spread even in cases with malignancy compared with diagnosis by curettage or endometrial biopsy. It is a method of diagnosis and treatment under direct view, with a high therapeutic outcome and a mean hospitalisation time of less than eight hours.

Conclusions

Atypical uterine leiomyoma is a challenging diagnosis. Careful evaluation of the microscopic features will assist the pathologist in determining the benign nature of the neoplasm. There is no evidence to indicate that additional therapy is needed once the diagnosis of atypical leiomyoma in myomectomy specimen has been firmly established.

References


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Pancreatic cancer with liver metastases in a pregnant patient: case report and review of the literature

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Summary

In this case report, the authors discuss clinical presentation, surgical procedure and early results of chemotherpay of pancreatic carcinoma with liver metastases diagnosed a few days after delivery. Pancreatic adenoocarcinoma occurs infrequently in pregnant and childbearing women: only ten cases have been reported in the literature. The early diagnosis of pancreatic cancer is difficult because symptoms appear when cancer is about to reach an advanced stage. In pregnancy, it is even more difficult because symptoms like dyspepsia, vomiting and epigastric pain may result confusing. The authors outline the difficulties in diagnosis and treatment of this kind of disease during pregnancy.

Key words: Pregnancy; Pancreatic adenocarcinoma; Surgical palliation; Chemotherapy; Liver metastases.

Introduction

Each year, more than 29,000 new cases of pancreatic adenocarcinoma are diagnosed in the USA (2% of the tumors detected every year) and about 25,000 patients die within 12 months of diagnosis. Radical surgery is reserved only to 10-20% of patients and this sort of cancer is the fifth most common cause of cancer-related death [1, 2]. This cancer is a common disease in men of advanced age, but is rare in childbearing and pregnant women.

We report a case of pancreatic adenocarcinoma in a pregnant patient focusing on diagnosis and therapy difficulties.

Pregnancy brings to some significant physiological and anatomical changes in the abdominal cavity. The diagnosis of certain gastrointestinal tract disorders may then be impaired, leading to a difficult pathology analysis in case of a vague symptomatic presentation.

Furthermore, optimizing both maternal and fetal outcomes is particularly challenging when a surgical procedure can be accomplished with the intention to cure.

Case Report

A 36-year-old Italian woman at 35 weeks of gestation was admitted to the Gynecologic and Obstetrics Department in February 2010. The patient’s medical history revealed hepatitis C infection (diagnosed 5 years before and treated with interferon), cigarette smoking addiction (40/day before pregnancy, 4-5/day during pregnancy) and past cocaine use.

Her obstetric history was positive for five previous spontaneous abortions and one ectopic pregnancy treated by methotrexate.

The patient had been hospitalized at our clinic after 20 days of epigastric abdominal pain, vomiting and weight loss. At admittance she showed a recent abdominal ultrasound (US) analysis report (performed in a different hospital). The exam result was negative for any sort of lesion. We repeated abdominal US and the exam was still negative for any sort of lesion.

The hematologic exams revealed increased hepatic and pancreatic enzymes, impaired renal function, anemia and hypokalemia. Obstetric US scan showed a single fetus with normal biometry, Doppler velocimetry and amniotic fluid index. Significant laboratory data were: Alanine transaminase (ALT: 593 U/l), lactate dehydrogenase (LDH: 608 U/l), total bilirubin (1.92 mg/dl), glycemia (149 mg/dl), creatinine (2.19 mg/dl), uric acid (18.3 mg/dl) and electrolytes (sodium, Na+: 133 mEq/l; potassium, K+: 2.5 mEq/l; chloride, Cl-: 77 mEq/l). The patient was treated with rehydration (Ringer’s lactate solution and potassium chloride solution, KCl), furosemide and metoclopramide.

The patient’s condition dramatically worsened and as well acute renal failure occurred (due to hypovolemia). It was therefore necessary to carry out a cesarean delivery, deferring the hepatic and pancreatic dysfunction diagnosis to the postpartum.

We administered two 12 mg doses of betametasone in a 12-hour interval to prevent neonatal respiratory disease and we planned the cesarean section (CS) after its completion. The patient underwent a low transverse CS via a Pfannenstiel incision under general anesthesia and she delivered a 2,520 g female with Apgar scores of 8 and 9 at 1 and 5 min. After delivery, clinical condition and laboratory values were improving: total bilirubin 0.87 mg/dl, ALT 395 U/l, LDH 438 U/l, creatinine 1.93 mg/dl; nevertheless hypokalemia, hyponatremia and hypochloremia persisted.

Four days after CS, the patient’s condition worsened: vomiting, retrosternal pyrosis and epigastric pain radiating to the back. Moreover, no relief was achieved with the proper therapy (lansoprazole, magnesium hydroxide, metoclopramide and ketorolac).

A new abdominal US showed a solid mass of 4 cm in the pancreatic uncinate process, compressing the duodenum and causing fluid retention in the head of the pancreatic duct; a hyperenhogenic angiomatous-like lesion was found in the right hepatic lobe.

Computed tomography (CT) of the abdomen revealed a...
hypodense pancreatic mass with fringed borders, leaning toward the mesenteric vessels and to the inferior vena cava. Moreover, CT revealed three hepatic masses of uncertain significance; gastrohepatic ligament lymph nodes were not significantly enlarged (< 1 cm) (Figure 1).

Esophagogastroduodenoscopy (EGD) did not reveal gastric involvement despite a duodenal obstruction due to edema and external compression.

The patient underwent surgery in order to heal the gastric obstruction and the jaundice, reducing the tumor burden and bearing out the hypothesis of metastatic liver disease. After a midline incision was made, no ascites were found entering the peritoneal cavity.

Inspection and palpation of the liver showed metastatic lesion in segment 3 and another nodule was present deep between segments 4 and 5.

Palpating the pancreas, a firm mass (about 5 cm in diameter) was identified on its head, extending to the uncinate process. The tumor partially infiltrated the superior mesenteric vein. The veins of the greater omentum and mesentery of the transverse colon were dilated. Enlarged lymph nodes around the hepatoduodenal ligament and close to the celiac vessels were present; due to a possible upward extension of the tumor, it was impossible to free and expose the right hepatic artery.

The duodenum compression was caused by two factors: the direct tumor growth into the third portion of the intestinal wall and mechanical obstruction of the duodenum itself due to the pancreatic mass.

After this staging evaluation was made during surgery, no attempt at pancreatic resection for optimal palliation was made. The decision was a selection of surgical procedures for palliative treatment and preparation for postoperative understaging chemotherapy with or without radiation regimens.

Several biopsies of pancreatic, peripancreatic tissue and from mesenteric lymph nodes were obtained. The gallbladder was removed and the patient received both a retrocolic gastrojejunostomy and a biliary bypass (double bypass).

The surgical procedure was completed resecting the two metastatic lesions on the liver. Postoperative histology showed sclerosis and chronic inflammation in the pancreatic and peripancreatic specimens, while the resected liver and lymph node revealed moderately differentiated metastatic lesions of likely pancreatic origin.

After the surgery the patient began palliative systemic chemotherapy with gemcitabine (1000 mg/m²) and oxaliplatin (100 mg/m²) every two weeks, along with nutritional support. A restaging CT scan obtained after three cycles of treatment (June 2010) revealed stable disease (SD) (Figure 2). After a collegial discussion, we decided to keep on administering gemcitabine (1000 mg/m²) every two weeks. The decision was based on some considerations: the absence of indication for radiotherapy (non favorable cost/effectiveness approach related to colic dilatation and consequent iatrogenic risk of perforation), the hypersensitivity reaction to the last oxaliplatin including pruritus and erythema (rapid resolution with the suspension of therapy and use of steroids) and SD at CT scan.

Twelve months after the diagnosis, the clinical conditions were stable and the CT scan performed in December 2010 confirmed SD.

**Discussion**

Cancer during pregnancy is not a rare event: about one out of 1,000 pregnancies has tumoral complications [1].

Pancreatic adenocarcinoma occurs infrequently in pregnant and childbearing women: only ten cases have been reported in the literature (Table 1) [3-12].

Age of the patients ranged from 32 to 43 years old and gestational age from 16 to 30 weeks. Only a few had a cancer risk factor related to cigarette smoking, gastrointestinal tumor inheritance or use of oral contraceptives [3, 5, 8].

The patient presented in our study was 36 years old and in the 35th week of pregnancy. Her medical history revealed she had contracted hepatitis C five years earlier and was consequently treated with IFN for one year. It was reported that she had a past use of cocaine and a cigarette smoking addiction (40 cigarettes a day).

While traditional risk factors (advanced age, male gender, diabetes, obesity, chronic pancreatitis, smoking, hereditary factors) have been widely demonstrated, the association between use of cocaine and pancreatic adenocarcinoma has been suggested just in one study [15]. Our case report highlights the difficulties encountered diagnosing pancreatic adenocarcinoma during late pregnancy.
Pancreatic cancer with liver metastases in a pregnant patient: case report and review of the literature

Usually early diagnosis of pancreatic cancer is difficult because the symptoms appear when the cancer is about to reach an advanced stage; in pregnancy, it is even more difficult because some symptoms like dyspepsia, vomiting and epigastric pain may result confusing [5]. Physical examination, laboratory tests, tumoral markers (CEA, CA19-9) combined with abdominal US can help in most cases to recognize the presence of a gastrointestinal cancer. Along with a proper work-up and right timing, it can also succeed in detecting the cancer itself. Abdominal US is usually the front-line diagnostic exam because it is a simple and non-invasive procedure. Although the literature reports high sensitivity (90%) of ultrasounds, in some cases this test is unable to detect a pancreatic mass. In such case there are some risks associated with the eventual more invasive methods and to diagnostic delay [3, 10, 12].

Indeed, in other cases the diagnosis was performed early in the pregnancy period, when the uterus had not yet reached considerable size.

In our patient, the US exam was negative and the pancreatic mass was detected only after the delivery; the gravid uterus probably caused some difficulties during the echography process, interfering with abdominal viscera visualization.

Nevertheless, the diagnostic difficulties in detecting pancreatic cancer have been early described by other authors: in the case described by Kakoza et al., the US showed only a biliary sludge and the condition was ascribed to cholecystitis and gallstone pancreatitis. The mass was discovered only with duodenal mucosal biopsy during endoscopic retrograde cholangiopancreatography (ERCP) and with CT scan [4].

In the case reported by Blackbourne et al. all the tech-

### Table 1. — Review of the literature on pancreatic adenocarcinoma in pregnant patients.

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient age</th>
<th>Gestational age</th>
<th>Risk factors</th>
<th>Clinical features</th>
<th>Diagnosis</th>
<th>Management</th>
<th>Maternal outcome</th>
<th>Fetal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamburcella, 1984</td>
<td>37</td>
<td>24</td>
<td>–</td>
<td>Chondral burning, epigastric discomfort, weight loss</td>
<td>Made by ERCP and exploratory laparotomy before delivery</td>
<td>Cesarean section at 32 weeks and cholecystectomy (palliative)</td>
<td>Patient died 3 months after delivery</td>
<td>Healthy twins</td>
</tr>
<tr>
<td>Porcel et al., 1992</td>
<td>43</td>
<td>28</td>
<td>none</td>
<td>Epigastric pain, upper lumbar backache, nausea, vomiting, pre-eclampsia</td>
<td>Made by abdominal ultrasound and aspiration cytology of hepatic metastases after delivery</td>
<td>Cesarean section at 30 weeks</td>
<td>Patient died 35 days after delivery</td>
<td>Live newborn</td>
</tr>
<tr>
<td>Simchuk et al., 1996</td>
<td>39</td>
<td>16</td>
<td>–</td>
<td>Right upper quadrant pain and anorexia</td>
<td>Made during a Whipple procedure before delivery (at 20 weeks)</td>
<td>Choleccho-duodenostomy and jejunostomy (palliative bypass procedure) at 20 weeks. Cesarean section at 28 weeks.</td>
<td>Patient months after surgery</td>
<td>Healthy male</td>
</tr>
<tr>
<td>Sciscione et al., 1996</td>
<td>20</td>
<td>–</td>
<td></td>
<td>Asymptomatic</td>
<td>Detected accidentally during a routine obstetric ultrasound</td>
<td>Good outcome postoperatively</td>
<td>The baby died</td>
<td></td>
</tr>
<tr>
<td>Blackbourne et al., 1997</td>
<td>32</td>
<td>17</td>
<td>2-year history of smoking cigarettes</td>
<td>Back pain, nausea, vomiting, dark urine, scleral icterus</td>
<td>Made by exploratory laparotomy and aspiration cytology of the mass before delivery</td>
<td>Pyloric-preserving pancreatico-duodenectomy. Cesarean section at 28 weeks.</td>
<td>Patient outcome was good at 3 months after surgery (no other informations reported)</td>
<td>Normal fetal development at 3 months after surgery</td>
</tr>
<tr>
<td>Goticic et al., 2005</td>
<td>37</td>
<td>Second trimester</td>
<td>Family history for digestive tract carcinoma</td>
<td>Abdominal pain and frequent fatty stools alternating with constipation</td>
<td>Made by abdominal ultrasound, ERCP with biopsy, magnetic resonance before delivery</td>
<td>Cesarean section, hysterectomy and pancreatic resection</td>
<td>Patient alive at date of publication</td>
<td>Live female</td>
</tr>
<tr>
<td>Marinoni et al., 2006</td>
<td>38</td>
<td>27</td>
<td>Cigarette and combined oral contraceptives use for 10 years</td>
<td>Epigastric abdominal pain, right derrbral top gastritis, upper lumbar backache, nausea and vomiting</td>
<td>Made by abdominal ultrasound, ERCP with biopsy and intubilar stent (palliative)</td>
<td>Cesarean section at 30 weeks and intubilar stent (palliative)</td>
<td>Patient died 50 days after delivery</td>
<td>Live female</td>
</tr>
<tr>
<td>Su et al., 2006</td>
<td>37</td>
<td>22</td>
<td>–</td>
<td>Epigastric pain</td>
<td>Made by abdominal ultrasound and biopsy before delivery</td>
<td>Patient decided to terminate the pregnancy at 24 weeks and proceed with chemotherapy</td>
<td>Patient died 4 weeks after diagnosis</td>
<td>Healthy female</td>
</tr>
<tr>
<td>Kakoza et al., 2009</td>
<td>40</td>
<td>24</td>
<td>none</td>
<td>Epigastric pain, nausea, vomiting, early satiety, anorexia, reflux and weight loss</td>
<td>Made by ERCP with biopsy and computed tomography before delivery</td>
<td>Cesarean section at 28 weeks.</td>
<td>Patient died 6 months after surgery</td>
<td>Healthy female</td>
</tr>
<tr>
<td>Onuma et al., 2010</td>
<td>32</td>
<td>30</td>
<td>none</td>
<td>Pain and tenderness in the upper right quadrant</td>
<td>Made by surgical exploration</td>
<td>Cesarean section and pancreaticoduodenectomy at 34 weeks, followed by chemotherapy</td>
<td>Patient is disease-free at 2 years</td>
<td>Healthy male</td>
</tr>
</tbody>
</table>
niques used (abdominal US, ERCP with cytology, CT scan, exploratory laparoscopy) did not show the pancreatic tumor, which was discovered only during an exploratory laparatomy [5].

Onuma et al. was unable to identify pancreatic cancer by transabdominal US because the large uterus surrounded by the intestine obscured the pancreas and intestinal and colon gas interfered with US. A retroperitoneal mass was detected by CT but was confused with an appendicitis or perforation of the stomach and retroperitoneal abscess. The correct diagnosis was achieved only by means of surgical exploration [12].

Another aspect to consider is the proper management of this pathology in pregnancy. The choice of correct management is a crucial decision requiring counseling with the patient and collaboration between surgeons and gynecologists. The only curative therapy is surgery, but only 15-20% of patients have, at time of diagnosis, lesions that can be surgically treated. Those that can be treated do not present any metastatic disease, distant lymphatic involvement (i.e., celiac, aortocaval, iliac), superior mesenteric-portal venous confluence nor superior mesenteric artery or celiac axis involvement.

Since the patient in our study was at a gestational age of 35 weeks – which allowed the completion of delivery without particular risks for the newborn – we decided to perform a cesarean section and postpone the diagnosis until after the birth.

However, even if the diagnosis had been made before the birth, it would not have altered the prognosis of the patient nor the management itself.

In other cases early gestational age during diagnosis (16-28 weeks) presented many problems related to the correct patient management. Where it was possible to resort to surgery, the mother and the clinicians could choose among three options:

1. Immediate surgical resection with pregnancy termination risk in case of a too early gestation;
2. Delay the surgery until the maximum fetal outcome with disease progression risk for the mother;
3. Perform surgical resection at the gestational age (starting from 28 weeks) with good fetal survival chances.

In most cases the patients decided to postpone the surgery to a later gestational age in order to reach a good survival probability for the newborn [3, 4, 6, 7, 10, 11]. Unfortunately the prognosis for patients was poor: five patients died within four months after delivery.

These reports show how difficult the management of these cases can be during pregnancy, suggesting a greater aggressiveness of pancreatic cancer in pregnant women. This may be caused by the suppression of the immune system, typical of pregnancy. Some authors support the hypothesis that pancreatic cancer may be, in part, an estrogen-dependent disease, but in the literature there is no agreement on this issue [14].

Finally, even though pancreatic adenocarcinoma is rare during pregnancy, in a woman with symptoms like abdominal pain, vomiting, jaundice and impaired liver and pancreas function the presence of pancreatic adenocarcinoma should be excluded although the diagnosis may be difficult and require more than one diagnostic procedure.

Once the tumor is diagnosed, it is necessary to pay great attention to the therapeutic choice in order to ensure the best outcome for both mother and fetus.

References


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