Reproductive Biology Section

Serum levels of the progesterone induced blocking factor do not precipitously rise in women with gynecologic cancer in contrast to women exposed to progesterone


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J.H. Check, J.K. Choe, R. Cohen, D. Summers-Chase - Camden, NJ, USA

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J.H. Check, J.K. Choe, R. Cohen, D. Corley, D. Horwath - Camden, NJ, USA

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J.H. Check, A. Whetstone, J.K. Choe, R. Cohen - Camden, NJ, USA

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A.C. Collier, B.L.M. Sato, K.A. Milam, T.E. Wright - Honolulu, USA

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C. Gezer, A. Ekin, N.S. Gezer, I.E. Ertas, M.E. Aveci, I. Uyar, S. Ciftci, C.E. Taner - Izmir, TURKEY

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Reproductive Biology Section

Serum levels of the progesterone induced blocking factor do not precipitously rise in women with gynecologic cancer in contrast to women exposed to progesterone

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Summary

Purpose: To determine if an immunomodulatory protein (progesterone induced blocking factor [PIBF]) that is progesterone induced and found in higher concentration during pregnancy is similarly found with increased levels in women with gynecologic cancers.

Materials and Methods: A newly developed enzyme linked immunoabsorbent assay (ELISA) assay was used to measure PIBF in the sera of six women with various gynecologic cancers and compare them to five controls (three with benign tumors and two having gynecologic procedures for non-tumors).

Results: The PIBF levels in women with gynecologic cancer did not rise precipitously as historical controls of women or men exposed to progesterone. The two highest PIBF levels of the 11 subjects were in women with gynecologic cancer.

Conclusions: The data suggest that if PIBF helps cancer cells to evade immune surveillance, it probably operates through an intracytoplasmic presence. If an increase in sera PIBF could have been detected in women with gynecologic cancer, then this ELISA test could have been used to detect tumor recurrence. Future studies may concentrate on evaluating intracytoplasmic PIBF to possibly help determine which tumors may respond to progesterone antagonist receptors.

Key words: Progesterone induced blocking factor; PIBF; Gynecologic cancer; Pregnancy.

Introduction

One of the functions of progesterone during the luteal phase and throughout pregnancy is to help suppress immune rejection of the fetal semi-allograft. Immune rejection of the fetus occurs mostly through the cellular immune system and the two most important cytolytic cells that need to be suppressed are natural killer (NK) and cytolytic T cells. 

Early studies suggested that NK cell activity was predominantly inhibited during pregnancy by a 34 kDa protein which acted at least partially by stabilizing perforin granules in NK cells [1, 2]. This 34 kDa protein seems to be expressed by gamma/delta T cells [3, 4]. Since the use of the progesterone receptor modulator mifepristone was able to abrogate the immune suppression by these gamma/delta T cell, this suggested progesterone was needed to react with a progesterone receptor on these gamma/delta T cells to activate them [5-7]. The term coined for this 34 kDa immunosuppressive protein was the progesterone induced blocking factor (PIBF) [1,8].

The early studies did not have a pure 34 kDa protein to establish more sensitive assays, e.g., the enzyme linked immunoabsorbent assay (ELISA) and thus most studies used a less sensitive immunocytochemistry technique [9-11]. Using this less sensitive immunocytochemistry technique, early studies suggested that the actual pregnancy state was responsible for the increased sensitivity of the pregnancy lymphocytes to react to progesterone to secrete PIBF since some studies suggested that there was a need to increase the progesterone concentration 100 fold to obtain the same suppressive effect on NK cell activity by non-pregnant vs. pregnant lymphocytes [7, 9]. This led to the concept that the allogeneic stimulus of the fetus may cause an increase in progesterone receptors in gamma/delta T cells. This was supported by the demonstration that the allogenic stimulus of lymphocyte immunotherapy not only increased progesterone receptor expression in pregnancy lymphocytes, but it also increased PIBF secretion [12, 13].

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For a malignant tumor to proliferate it must borrow mechanisms already existent to allow cell growth while at the same time suppress mechanisms that signal cells to stop growing to accomplish this. The oncogenes will cause certain proteins on the tumor cell to be present which will not be normally present on the cell and thus make the tumor cells somewhat immunogenic and thus prone to immune rejection by the host.

The continued proliferation of the tumor cells then requires escape from immune surveillance. There are at least six different mechanisms by which tumor cells may evade immune rejection: 1) downregulation of major histocompatibility complex class I expression, 2) lack of co-stimulatory molecules that are needed to activate T cells, 3) immunoselection of tumor cells with weak immunogenicity, 4) failure of the host to respond to tumor antigens, i.e., tolerance because of neonatal antigen exposure, 5) induction of suppressor T cells, and 6) suppression of immune response by tumor secreted or directed production of certain cytokines, prostaglandins, soluble antigenic material, and even hormones [14].

Immune methods to suppress tumor growth would not require inhibiting all six of the aforementioned methods of escape (there could be more then six) but theoretically any one of these mechanisms. A model for potential tumor immunotherapy was proposed suggesting tumors may be able to direct gamma/delta T cells to the tumor microenvironment to secrete PIBF and thus inhibit NK cell cytolysis and allow cancer cells to proliferate. The model suggested a mechanism for cancer cells to secrete P and thus interact with P receptors in gamma/delta T cells in the tumor microenvironment [15]. The hypothesis suggested that if this mechanism was operative, blocking P action by treating with P receptor antagonists, e.g., mifepristone could inhibit tumor cell growth [15]. Though possibly not as high as seen with pregnancy some of the PIBF made by the gamma/delta T cells in the tumor microenvironment may spill over and cause a perceptible rise in serum PIBF.

Thus this hypothesis suggested that possibly by secreting hCG some tumor cells can secrete enough progesterone to interact with the progesterone receptors and the gamma/delta T cells and consequently make PIBF [15, 16]. The PIBF, in turn, could inhibit NK cells in the tumor microenvironment from attacking the tumor cells despite foreign oncofetal antigens [15].

Support for the possible role of PIBF in helping cancer cells to evade immune surveillance was provided by the demonstration that all 29 human leukemia cell lines evaluated were found to produce a considerable amount of mRNA for PIBF [17]. Furthermore four of ten leukemia cell lines tested by the less sensitive immunocytochemistry technique for PIBF was found to express the PIBF protein. Interestingly following the addition of mifepristone to the media PIBF protein expression was down-regulated [17].

The hope of detecting a relationship with PIBF and cancer cells is that the information could possibly be used in the treatment or the prevention of cancer. The hypothesis is that progesterone plays a continual role in the production of PIBF with cancer cells similar to the pregnancy state. However it is possible that PIBF could be made or directed by cancer cells through another mechanism that does not require progesterone. However, if progesterone is involved there are already drugs on the market, i.e., progesterone receptor antagonists, that could inhibit PIBF production and thus remove theoretical suppression of NK cell immune rejection.

Indeed treatment of a variety of murine and human cancers not known to be associated with progesterone receptors with mifepristone resulted in considerable palliation from their cancers [18-22]. These data lent support for the hypothesis that some cancers may escape immune surveillance from NK cells through the stimulation of increased production of the immunomodulatory protein PIBF [23]. The inhibition of the production of the 34 kDa PIBF protein found in the circulation of pregnant women could be inhibited from being produced by gamma/delta T cells in the tumor microenvironment as originally hypothesized [15]. However, another possibility exists as to the location as to where the main inhibitor of PIBF takes place and that is in the tumor cell itself rather than the microenvironment.

Using a Western blot analysis more information accrued about the nature and origin of PIBF in 2003 and 2004 [24, 25]. The parent compound actually resides in the nucleus at a centrosomal position [24]. Interestingly the PIBF protein seems to be unique showing no amino acid sequence homology with any known protein [25]. The full length protein consists of 757 amino acid residues and is encoded by PIBF1 CDNA [25]. The 48 kDa N terminal part of PIBF is biologically active [25].

The parent and dominant 90 kDa form of PIBF has been found to be present in most rapidly growing cells especially cancer cells as evidenced by Western blot analysis using PIBF specific antibodies [24]. There has been identification of the exon 1-5+17-18 transcript encoding for a 35 kDa protein [24]. The deletion observed in this transcript preserves the open reading frame for the full length PIBF protein [24]. Translation of the transcript results in a 35 kDa isoform of PIBF containing the N terminal 222 and C terminal 75 amino acids [24].

The PIBF gene has been identified on chromosome 13 in the vicinity of BRCA1 and BRCA2 mutations [24]. RNA expression analysis has shown that centrosomal PIBF is overly expressed in rapidly proliferating cells irrespective of whether they have been shown to be positive or not for progesterone receptors [24].

Immunofluorescence microassay demonstrated a 35 kDa form of PIBF localized to the cytoplasm of tumor cells [24]. Since this split isoform of the parent compound has a similar size to the circulating immunomodulatory protein in
the serum of pregnant women, it may be that PIBF may confer immunoprotection to the tumor cell itself (or for that matter fetal cells or trophoblast cells) by this intracytoplasmic position and may not need circulating PIBF to suppress NK cell attack. Since both the fetal placental unit and cancer cells are rapidly growing, thus the original hypothesized mechanism of suppressing NK cell activity in the tumor microenvironment by secretion of PIBF by gamma/delta T cells may not be the main operative mechanism of immune suppression. It is possible that the intracytoplasmic presence could also confer a degree of immune protection, but possibly because the fetal semi-allograft is more immunogenic than cancer cells, perhaps extra PIBF secreted externally and concentrated at the maternal-fetal interface is needed to allow the fetus to grow.

These new data suggest that the tumor cells themselves may actually produce the PIBF. Thus instead of inhibiting NK cells in the tumor microenvironment the intracytoplasmic location itself may directly confer immune protection to the tumor cell by suppression of NK cell immunosurveillance.

The PIBF protein has been purified and synthesized by recombinant DNA technology [26]. A purified protein is required to develop a monoclonal antibody and this was achieved [26]. The protein must be soluble which has been established [27].

With these new medical advances, the less sensitive immunocytochemistry technique using a polyclonal antibody to PIBF has been replaced with a much more sensitive ELISA test [28]. There is now found to be a marked difference in women whose PIBF levels are obtained in the follicular phase vs. three days after embryo transfer [28].

The objective of the present study was to obtain blood samples of patients with gynecological malignances prior to surgery to determine if an increased level of PIBF using a non-commercial ELISA assay for PIBF could be detected in women with a variety of gynecologic malignances.

Materials and Methods

Subjects

Serum was obtained from women about to have surgery for gynecologic problems including malignant and benign disorders. The samples would then be measured for PIBF using a new non-commercial ELISA assay for PIBF and for serum progesterone. Women with serum progesterone level greater than two ng/ml were eliminated.

Methodology of PIBF assay

A non-commercial ELISA was used to measure PIBF in serum. Serum specimens were stored at -20°C. Fifty microliters of recombinant PIBF standard was added to each pre-coated goat anti-rabbit antibody well in duplicate. The concentrations of the PIBF standard were S0 – 0, S1 – 3.2, S2 – 11.2, S3 – 40, S4 – 160, S5 - 802 ng/ml. The patient’s serum was then added to each well. Next 50 microliters of horse radish peroxidase conjugated PIBF antigen was added to each well except the zero standard. Next anti-PIBF IgG antibody was added to each well. The microtiter plate was then incubated in the dark for one hour at 37°C. After one hour, the wells were washed with PBS and decanted three times. Next 50 microliters of Substrate A (carbamide peroxide) and 50 microliters of Substrate B (tetramethyl-benzidine) were added. The microliter trays were then incubated in the dark at 37°C for 15 minutes. Next 50 microliters of stop solution was added whose main component is H2SO4. The plates were read within ten minutes using a microplate reader at 450 nm. The results were calculated using a four-parameter logistic curve fit.

Results

The PIBF levels (ng/ml) from lowest to highest in women with various gynecologic cancers (in all cases serum progesterone ≤ two ng/ml) were 10.06 (64-year-old woman with endometrioid type of adenocarcinoma of uterus), 17.35 (63-year-old woman with clear cell adenocarcinoma of ovary), 32.59 (72-year-old woman with papillary serous adenocarcinoma of uterus), 35.62 (66-year-old woman with primary peritoneal papillary serum cystadenocarcinoma), 54.7 (77-year-old woman with mucinous adenocarcinoma of the gastrointestinal tract), and 57.17 (68-year-old woman with a recurrent adult granulosa cell tumor). The average serum PIBF was 34.6 ng/ml.

There were three women with benign gynecologic tumors and the serum PIBF was 14.76 (45-year-old woman with leiomyomata), 15.7 (58-year-old woman with mucinous cystadenoma of the ovary), and 36.64 (57-year-old with adenomyosis and leiomyomata). Their average serum PIBF was 22.5 ng/ml.

There were two women with no tumors having gynecologic surgery and their serum PIBF levels (ng/ml) were 9.56 (21-year-old) and 35.27 (48-year-old with cervical dysplasia) with an average of 22.4 ng/ml.

Discussion

Exposure to progesterone even in males will cause the serum PIBF levels to exceed 100 ng/ml and frequently > 800 ng/ml using the new ELISA assay [28-30]. Though it is true that of the 11 women tested (six with cancer and five without) the highest levels (> 50ng/ml) were seen in the two women with cancer, clearly the levels do not compare close to what is seen with exposure to progesterone [28-30].

Possibly a larger series may show evidence that there is a significantly higher level of PIBF seen in some women with cancer. However, if PIBF is effective in suppressing an immune response against the cancer cells, it seems to be likely through a different mechanism than pregnancy where the serum PIBF levels are very high.

There is the possibility that mifepristone improves immunosurveillance through some other mechanism than PIBF. There is evidence that progesterone interacting in a non-genomic manner with progesterone receptor membrane 1 may suppress in an epigenetically manner T cell rejection of the fetal semi-allograft. Possibly this could apply to cancer cells [31].
Most of the data supporting the hypothesis suggesting that PIBF may be a way in which tumor cells, similar to the fetus, escapes immune surveillance, is based on the fact that most tumors in mice and humans will be suppressed by mifepristone whether the tumor is known to have progesterone receptors or not [18-22]. It is beyond the discussion of this manuscript to discuss the various ways that the progesterone receptor may be involved in factors needed for tumor growth but the possibility exist that progesterone receptor antagonist benefit has nothing to do with the immune system. For an excellent review of the way progesterone receptors may help in tumor growth, at least in tumors, e.g., breast cancer known to possess progesterone receptors, has been summarized by Daniel et al. [32].

The possibility exists that gynecologic cancers, as opposed to the others responding to mifepristone, just do not express PIBF and possibly these gynecologic tumors would not have responded to mifepristone therapy. There was only one gynecologic cancer, a leiomyosarcoma, in the study of mifepristone on providing palliative benefit to people with advanced cancer [22]. The woman with the leiomyosarcoma dramatically responded to mifepristone but her serum level of PIBF was not measured [22]. None of the gynecologic cancers in the present study were leiomyosarcoma.

One case does show that some cancers may dramatically respond to mifepristone even if no increase in serum PIBF is determined. A woman without elevated sera PIBF levels had either the acute phase of leukemia progression from her chronic lymphocytic leukemia or primary lung cancer. Death was considered imminent. All her lung lesions disappeared after six weeks of mifepristone. She remains well with good energy two years after therapy just continuing on 200 mg mifepristone daily [33]. This could suggest that the main benefit of mifepristone may be to inhibit the conversion in the tumor cell cytoplasm of the 90 kDa parent form of PIBF to the intracytoplasm 34-36 kDa isoform. It should be recalled that 29 of 29 human leukemia cell lines were found to have an enormous amount of mRNA devoted to the manufacturing of PIBF and yet no increase in serum PIBF was detected in this woman who responded so well to mifepristone [17].

A study is in progress to see if breast cancer that is progesterone receptor positive may show higher serum levels of PIBF than women with progesterone receptor negative breast cancer. If significantly higher levels of PIBF are found in the sera of tumors that are positive for progesterone receptors, perhaps these tumors may respond the best to progesterone receptor antagonists. Perhaps the high sera levels help protect the cancer from immune surveillance.

If more extensive testing of various cancers confirms a lack of significant elevation of serum PIBF levels, this test may not prove worthwhile to use as a method to determine who should be treated with progesterone receptor antagonists. If people with certain tumors can demonstrate slightly higher levels of PIBF than the sera of other patients, with other types of cancer, measurement of baseline levels before therapy, could be potentially useful as a marker for disease recurrence.

Thus this small pilot study does not support the importance of PIBF as a means of cancer cells escaping immune surveillance because in contrast to the pregnancy state no significant rise in serum PIBF was detected in the sera of women with gynecologic cancer. However, in view of the marked improvement seen in certain cancers treated by mifepristone, and the known increase in intracytoplasmic PIBF in all rapidly growing cells, attention should be placed on measuring intracytoplasmic PIBF in the tumor specimens directly. Possibly some tumors will have higher concentration than others and these may be the ones that best respond to progesterone receptor antagonists. Such a demonstration could at least generate interest in the oncologic group to consider progesterone receptor antagonists as a treatment even for cancers not known to be associated with progesterone receptors.

Once the PIBF assay is better refined, perhaps after studying a larger series of patients with cancer some discriminatory level may be detected, that could suggest that a malignancy is possibly present (even if not nearly as high as seen in people exposed to progesterone). It should be recalled that only two women in this study had PIBF levels > 50 ng/ml and both of these women had cancer. If mifepristone suppressed these levels perhaps these women would be found to have good palliation from therapy vs. those that failed to lower serum PIBF levels. Perhaps monitoring PIBF in women responding to progesterone receptor antagonists could alert the treating physician if a rise approaching baseline is occurring to influence the treating physician to either raise the dosage of the progesterone receptor antagonist or change to another progesterone receptor antagonist or add another type of chemotherapy or monoclonal antibody therapy.

References


Serum levels of the progesterone induced blocking factor do not precipitously rise in women with gynecologic cancer in contrast etc.


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Effect of taking a one time injection of one mg leuprolide acetate three days after embryo transfer on pregnancy outcome and level of first beta human chorionic gonadotropin (beta-hCG) level

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Summary
Purpose: To determine if the injection of a gonadotropin releasing hormone agonist (GnRHa) three days after embryo transfer will improve pregnancy and implantation rates. Materials and Methods: One mg s.c. of leuprolide acetate was randomly given based on patient decision three days after embryo transfer to some patients undergoing in vitro fertilization-embryo transfer (IVF-ET). Results: For women aged ≤ 43 the clinical pregnancy rate for those not taking the GnRHa was 39.5% (68/122) vs. 54.5% (42/77) for those taking leuprolide acetate (Chi-square, \( p = 0.0275 \)). The respective implantation rates were 22.6% vs. 30.2% (\( p = 0.0495 \)). There was no difference in first serum beta human chorionic gonadotropin (beta-hCG) levels according to whether leuprolide was used or not. Conclusions: Leuprolide acetate similar to other GnRH agonists can improve implantation rates following IVF-ET when injected once in mid-luteal phase. The beneficial effect may be on GnRH receptors in the endometrium rather than the embryo (which had been hypothesized to direct increased placental production of hCG).

Key words: Leuprolide acetate; Mid-luteal phase; Endometrial GnRH receptor; Improved implantation.

Introduction
In 2004 in an oocyte donation model, Tesarik et al. found an enhancement of embryo development potential by a single administration of a gonadotropin releasing hormone agonist (GnRHa) at the time of embryo implantation [1]. The benefit of luteal phase GnRHa in mid-luteal phase was confirmed by Pirard et al. [2]. Iwashita et al. found that GnRHa increased human chorionic gonadotropin (hCG) levels suggesting that this results from its action on placental GnRH receptors [3]. The higher levels of hCG suggested to Tesarik et al. that the GnRHa may have a direct beneficial effect on the embryo itself causing it to secrete more hCG which favors successful implantation [4]. The study by Lin et al. provided support for this theory by demonstrating expression of the human GnRHa receptor gene in the placenta and found a functional relationship to hCG secretion [5].

The objective of this study was to determine if the GnRHa leuprolide acetate at a one-mg dosage could improve implantation and live pregnancy rates after in vitro fertilization-embryo transfer (IVF-ET) similar to the other GnRHa, e.g., buserelin by Pirard et al and triptorelin by Tesarik et al. [2, 4]. Furthermore the study would determine if the use of a GnRH agonist in mid-luteal phase is more or less beneficial according to female age. Finally, the study would corroborate or refute the suggestion that the use of GnRHa in mid-luteal phase is associated with a higher first serum beta-hCG levels.

Materials and Methods
During a four month time period, all patients were given the opportunity of taking one mg leuprolide acetate in mid-luteal phase. They were advised of previous studies suggesting increased pregnancy rates [1-5]. However, they were explained that the present authors have no personal experience with this treatment. To ensure they received the medication, one requirement was that they would have to receive the injection at the present institution at the same time as their evaluation by pelvic sonography to determine if they reached a homogeneous hyperechogenic endometrial echo pattern [6]. Only cycles using controlled ovarian hyperstimulation with a gonadotropin releasing hormone antagonist (cetrorelix or ganirelix) were included followed by IVF-ET. There were no exclusions for previous failed IVF cycles or degree of ovarian reserve as evidenced by normal or increased day 3 serum FSH.

The women were stratified into age groups of ≤ 35, 36-39, 40-42, and ≥ 43. Clinical (ultrasound evidence of pregnancy at
Table 1. — Pregnancy and implantation rates and first serum beta-hCG level according to age and use or non-use of a single one-mg injection of leuprolide acetate in mid-luteal phase.

<table>
<thead>
<tr>
<th>Age</th>
<th>Without LA in the luteal phase</th>
<th>With leuprolide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤35</td>
<td>36-39</td>
</tr>
<tr>
<td># transfers</td>
<td>72</td>
<td>36</td>
</tr>
<tr>
<td># clinical pregnancy</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>% clinical/transfer</td>
<td>44.4</td>
<td>47.2</td>
</tr>
<tr>
<td>% delivered/ongoing</td>
<td>37.5</td>
<td>41.7</td>
</tr>
<tr>
<td>Avg. # embryos transfer</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>30.6</td>
<td>29.7</td>
</tr>
<tr>
<td>Avg. level 1st beta-hCG (mIU/ml)</td>
<td>280.8</td>
<td>336.4</td>
</tr>
</tbody>
</table>

Table 1 also shows the average first serum beta-hCG for those conceiving without and with leuprolide acetate supplementation. No increased level or trend was found for higher serum beta-hCG levels in those conceiving with vs. without leuprolide supplementation.

Conclusions

This study confirms the previous studies by Tesarik et al. and Pirard et al. that a single use of a GnRHa in mid-luteal phase can increase pregnancy rates following embryo transfer [1, 2, 4]. This is the first study showing that the GnRHa leuprolide acetate can improve implantation rates similar to buserelin and triptorelin. These data do not corroborate previous data suggesting that the mechanism for improved successful implantation following mid-luteal GnRHas may be by enabling the embryo to make more hCG. These data more suggest a possible direct effect of the GnRHa on the endometrium. GnRH receptors have been found in murine endometria [7]. The human uterus also has LH receptors [8]. Thus the GnRHa could stimulate an increased amount of endometrial LH to explain the beneficial effect of these agents on improving pregnancy rates.

References


Effect of taking a one time injection of one mg leuprolide acetate three days after embryo transfer on pregnancy outcome and level etc.


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The effect of conventional vs. mild ovarian hyperstimulation on the total number of live babies born from a given oocyte retrieval

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Summary

Purpose: To compare pregnancy outcome in women with normal oocyte reserve according to whether they received conventional or mild follicle stimulating hormone (FSH) controlled ovarian hyperstimulation (COH) further stratified by age. Materials and Methods: A ten-year retrospective study including all cycles (even multiple in given patients) was performed. Mild stimulation including all cycles initiated and continued with 150 IU FSH or less from early follicular phase. Everything else was considered conventional stimulation. Mild stimulation included natural cycles or those with just a boost of 75 IU FSH from the mid to late follicular phase. Only women with normal oocyte reserve were selected – serum FSH < 12 mIU/mL and serum E2 < 50 pg/mL. Live delivered pregnancy rates within three age groups (≤ 35, 36-39, and 40-42 years) were compared per embryo transfer and per embryo retrieval, i.e., the percentage of women having a live baby without proceeding to another IVF-ET cycle. Also compared were the average number of babies born from one retrieval. Results: For aged ≤ 35 there were no differences in pregnancy rates per transfer but a trend for higher pregnancy rates per retrieval with conventional stimulation. For all other age groups both pregnancy rates per transfer and retrieval were significantly higher with conventional stimulation. This was reflected with a higher average number of babies born per retrieval with conventional. Conclusions: For women with normal oocyte reserve in general, there is no advantage of mild vs. conventional COH other than cost saving. Of course there are exceptions, e.g., ovarian hyperstimulation with conventional COH.

Key words: Normal oocyte reserve; Controlled ovarian hyperstimulation; Mild FSH stimulation; Fresh and frozen embryo transfer; Pregnancy outcome.

Introduction

Some anecdotal case reports vividly demonstrate that in some instances the use of controlled ovarian hyperstimulation (COH) may create an adverse uterine environment preventing embryos from implanting [1, 2]. Nevertheless most IVF centers in the modern era are enjoying excellent live delivered pregnancy rates with fresh embryo transfer following conventional COH.

Baart et al. found that despite the creation of many embryos, there is an average of only 1.8 chromosomally normal embryos that advance to the blastocyst stage [3]. There may be a selective process where the best oocytes, i.e., those that are chromosomally normal, have better FSH receptors and are more likely to respond to lower FSH stimulation. Thus it is possible that mild FSH stimulation may create less embryos but the same number of chromosomally normal embryos. It is possible in some circumstances conventional COH will create an adverse uterine environment but not with mild stimulation. It is possible that both mild and conventional COH result in a similar frequency of adverse uterine environments and thus conventional COH has the advantage of more embryos cryopreserved for future embryo transfer. If one does develop more normal embryos with conventional vs. mild COH, the former may have the advantage of more children derived from one given oocyte retrieval.

The objective of the present study was to compare the number of babies born from a given oocyte retrieval considering whether a conventional or mild COH protocol was used in women with normal oocyte reserve.

Materials and Methods

A retrospective cohort comparison study over ten years was conducted. In vitro fertilization cycles were eliminated if day 3 serum FSH was >12 mIU/mL or serum E2 was > 50 pg/mL. The data were stratified according to three age groups: ≤ 35, 36-39, and 40-42 years. Data were stratified according to whether they had full vs. mild stimulation. Mild stimulation was considered if the woman started on no more than 150 IU FSH with an increase of 75 IU only when a GnRH antagonist was used. Significance was determined by Chi-square analysis.

The reason for choosing mild over conventional COH may have been related to: saving money not only on medication but the price
of the IVF-ET procedure is less when mild stimulation is used, previous failure to conceive with conventional stimulation (mild stimulation used to cover the possibility of high stimulation causing an adverse uterine environment), fear of the ovarian hyperstimulation syndrome, and desire not to have the dilemma of extra frozen embryos in case only one more child was desired.

Viable per transfer referred to live fetus at 12 weeks. If a fresh ET was not performed related to risk of OHSS or inadequate endometrial thickness, the category viable per transfer included that retrieval cycle but all transfers were frozen ETs. Thus this category states percentage viable in live delivered per fresh or frozen transfer. The category percentage delivered per retrieval includes all women having at least one baby born including the frozen transfer (if the fresh was not successful) and represents the numerator and the number of oocyte retrievals is the denominator. If a woman had an oocyte retrieval and she proceeded to another IVF-ET cycle without using her cryopreserved embryo, she was excluded from this study.

**Results**

The live delivery rate and the number of live babies born according to fresh vs. mild COH and age is seen in Table 1. The only comparison not showing a significantly higher live delivered pregnancy rate for full stimulation was in the live delivered pregnancy rates per transfer (fresh or frozen) in the younger (≤35 years) age group. But even in that younger group there was a significantly higher overall live delivery rate per retrieval (67% vs. 52.4%) (chi-square, \( p < 0.05 \)). Chi-square analysis found significantly higher rates with full stimulation in women aged 36-42 in both pregnancy rates per transfer and pregnancy rate per retrieval.

**Discussion**

The main disadvantage of a retrospective study is potentially inadvertent selection of better candidates in one treatment regimen vs. another. One advantage of a retrospective study is that it generally has more power in numbers than a prospective study which is generally funded. Another advantage of a large retrospective study is that, as in this one, all cycles during a specific time period were selected so there was no discrimination toward a more “ideal” group. A prospective study sometimes may not be representative of the majority of women seeking help with fertility.

For younger women, full stimulation resulted in about a 28% greater chance of having a live baby per retrieval and a 1.32 greater number of live babies produced. For age 36-39, full stimulation provided twice the chance of a live baby per retrieval and 1.83 as many live babies. For ages 40-42, full stimulation provided a 60% increased chance of a live baby per retrieval and 1.47 greater chance of producing live babies. Though implantation rates were slightly lower for mild stimulation vs. full stimulation for women ≥36-42, the main reason for higher live delivery rates per retrieval and more babies were related to more embryos formed.

**Conclusions**

These data show that though there may be a few cases where full COH adversely affects uterine environment; this must be in a small minority since implantation rates were slightly lower not higher with mild stimulation. Of course some bias of selection could account for a somewhat lower implantation rate since mild stimulation was frequently used for women failing to conceive in previous cycles with full stimulation.

**References**


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Introduction

There are some studies that suggest that when a woman has diminished oocyte reserve, as evidenced by a high day 3 serum follicle stimulating hormone (FSH) level, there are very poor pregnancy rates despite the transfer of normal appearing embryos [1-4]. There is evidence that the reason for the very poor in vitro fertilization-embryo transfer (IVF-ET) pregnancy rates with diminished oocyte reserve is related to a high rate of meiosis errors leading to aneuploidy [5]. This has led to the conclusion by many clinicians and researchers in the field of infertility that young women with diminished oocyte reserve have the same quality of oocytes as women of advanced reproductive age.

However, there are other studies that find pregnancy rates per embryo transfer in women with diminished oocyte reserve to be only slightly lower than their age peers with normal reserve [6, 7]. The main difference in methodology in those with very poor outcome vs. reasonably good outcome was the use of conventional to very high FSH dosage in the former vs. mild to minimal FSH stimulation in the latter [8, 9].

Considering the finding of Nasseri et al. of increased rate of aneuploidy in women having IVF-ET with diminished oocyte reserve who had conventional controlled ovarian hyperstimulation, it seems likely that the explanation for poor pregnancy rates demonstrated in some studies is an adverse effect of the high dosage FSH on the process of meiosis leading to non-disjunction of chromosomes [5, 10]. This may be related to the possibility that FSH acting on a specific FSH receptor causes the production of a key enzyme needed for chromosome separation that has been on the verge of being down-regulated by the chronic elevation of FSH. Adding higher levels of serum FSH through exogenous administration causes a critical higher level of serum FSH which now causes down-regulation of this specific FSH receptor, thus leading to a deficiency of this factor which protects against non-disjunction of chromosomes [11].

There are some data suggesting that even with normal oocyte reserve, with the creation of many embryos following conventional controlled ovarian hyperstimulation (COH), there may be just an average of 1.8 chromosomally normal embryos in the cohort that reaches blastocyst stage [12]. The possibility exists that women with normal oocyte reserve actually produce more normal embryos than those with diminished oocyte reserve but the reasons for similar pregnancy rates may be the dilution factor. For example, supposing the average number of normal embryos in women with diminished oocyte reserve is one embryo per retrieval vs. two in those with normal reserve. If only two total em
Table 1. — Pregnancy rates per embryo transfer in women aged 36-39 according to ovarian oocyte reserve.

<table>
<thead>
<tr>
<th>Reserve</th>
<th>Clinical pregnancy rate</th>
<th>Viable pregnancy rate</th>
<th>Live delivered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal reserve</td>
<td>37.3% (363/971)</td>
<td>32.5% (315/971)</td>
<td>30.7% (298/971)</td>
</tr>
<tr>
<td>Diminished</td>
<td>30.5% (150/492)</td>
<td>26.5%</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

The live delivered pregnancy rate per oocyte harvest was 47.2% (411/971) for normal reserve vs. 25.0% (123/492) for diminished reserve. Thus the pregnancy rate per oocyte harvest was almost 50% lower for the decreased oocyte reserve group.

Discussion

The pregnancy rate per oocyte harvest was twice as high for women with normal vs. diminished oocyte reserve yet the viable pregnancy rate was only 20% higher per fresh embryo transfer and live delivered only 40% higher. The explanation for the significantly higher pregnancy rate per oocyte harvest relates to higher pregnancy rates per frozen embryo transfer in this group coupled with more frozen embryo transfers in the normal vs. decreased reserve group. It also seems likely that part of the explanation could be related to a dilutional factor in the normal reserve group for fresh embryo transfer, i.e., more abnormal than normal embryos from a chromosome standpoint created and thus a better chance not to transfer one of the normal embryos since there would be a higher percentage of aneuploidy [12]. These data underscore the importance for each IVF center to have a good cryopreservation program if one is not able to be certain which embryos are chromosomally normal.

Despite the pregnancy per harvest being half as good in diminished vs. normal reserve, the pregnancy rate per fresh transfer was much more comparable. Thus this study confirms that when the specific principles of mild stimulation modified for diminished oocyte reserve are followed, reasonably good pregnancy rates per embryo transfer can be achieved in those women with diminished oocyte reserve.

Recently there has been a trend toward using mild stimulation even for women with normal reserve. When comparing pregnancy rates based on these data, they should also evaluate the data according to the pregnancy rate per oocyte harvest.

Similar data were presented at the 2012 American Society for Reproductive Medicine (ASRM) meeting in women aged ≤35. The live delivered pregnancy rate per oocyte harvest (age ≤35) were 74.9% (1226/1719) for women with normal reserve vs. 37.8% (144/380) for those with diminished oocyte reserve. However, the live delivered pregnancy rate per transfer was only 15% less for the group with diminished oocyte reserve.

Materials and Methods

A retrospective review of IVF-ET cycles over a ten-year period in women having the oocyte retrieval between the ages of 36-39 was performed. Two groups were compared – those with normal oocyte reserve with day 3 serum FSH ≤11 mIU/mL and those with diminished oocyte reserve (day 3 serum FSH ≥12 mIU/mL).

Conventional or mild FSH stimulation dosage may have been used for the normal reserve group but only mild stimulation for low reserve group. Only gonadotropin releasing hormone (GnRH) antagonist protocols were compared. Women with a day 3 serum estradiol >50 pg/mL were excluded. Women were eliminated from the study if they proceeded with another IVF-ET cycle before transferring all their cryopreserved embryos unless a live delivered pregnancy occurred.

Results

Table 1 presents the pregnancy rates per transfer according to ovarian oocyte status. The clinical and viable pregnancy rates were approximately 20% lower in the group with diminished oocyte reserve. The live delivered pregnancy rate was 40% lower for the diminished oocyte group.

The live delivered pregnancy rate per oocyte harvest includes the addition of a pregnancy by a subsequent frozen embryo transfer if the fresh only transfer did not result in a pregnancy or ended in a miscarriage as long as all embryos were derived from the given oocyte retrieval. The live delivered pregnancy rate per oocyte harvest was 47.2% (411/971) for normal reserve vs. 25.0% (123/492) for diminished reserve. Thus the pregnancy rate per oocyte harvest was almost 50% lower for the decreased oocyte reserve group.

References


The effect of oocyte reserve on pregnancy rates per oocyte harvest in women aged 36-39


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Assessment of follicular and serum VEGF and IGF-1 in ICSI patients: hMG vs rFSH

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Summary

Purpose: To investigate the effect of recombinant follicular stimulating hormone (r-FSH) and human menopausal gonadotropin (hMG) on follicular microenvironment via assessment of follicular and serum vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) levels in intracytoplasmic sperm injection (ICSI) cycles. Materials and Methods: Designed as a prospective cohort study. Twenty-five patients underwent controlled ovarian hyperstimulation (COH) with r-FSH and 20 patients underwent with hMG. Results: Both groups were comparable regarding the women's mean age and body mass index (BMI). The amount of VEGF (pg/ml) in serum and follicular fluid in the group I and II were comparable (275 ± 135.3 vs 300.7 ± 190.0; p > 0.05 and 2,081.1 ± 1095.1 vs 1,971.1 ± 975.6; p > 0.05, respectively). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 vs 204.1 ± 56.3, p > 0.05 and 176.1 ± 67.2 vs 185.8 ± 48.7, p > 0.05, respectively). Pregnancy rates were also comparable between groups. Conclusions: The hMG and r-FSH in COH produced comparable follicular microenvironment regarding follicular VEGF and IGF-1.

Key words: Recombinant FSH; hMG; Follicular fluid; VEGF; IGF-1.

Introduction

Vascular endothelial growth factor (VEGF) is released by the theca and granulosa cells and plays a basic and crucial function during the follicular growth and development via regulation of angiogenic processes in the ovary [1]. It has been shown that VEGF secretion, which is induced by gonadotropins, results in the formation of adequate vascular structures in the thecal cell layer of the follicle [2, 3]. Adequate follicular vascularity indicates good levels of intrafollicular oxygen and consecutively good oocyte quality [4].

Insulin-like growth factor (IGF-1) is also released by the theca and granulosa cells and it also plays an important role in follicular development, dominant follicular growth, steroidogenesis, inhibin secretion, oocyte maturation, and follicular atresia [5]. IGF-1 enhances impacts of gonadotropins and coordinates the functions of the theca and granulosa cells [6].

Gonadotropins are routinely used agents to stimulate follicles in the controlled ovarian hyperstimulation (COH) in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles. The human menopausal gonadotropin (hMG) and recently recombinant follicle-stimulating hormone (r-FSH) are widely used in COH. Several studies compared the performance of hMG with r-FSH regarding clinical pregnancy rates [7-9]. To the present authors' best knowledge, there is limited data about the comparing the effect of these agents on the follicle microenvironment.

Aim of this study was to compare the effects of the hMG and r-FSH on follicular microenvironment with assessing follicular and serum VEGF and IGF-1.

Materials and Methods

The study was designed as a prospective cohort study. It was conducted at Hacettepe University IVF Center. The patients who admitted to our IVF center for ICSI and embryo transfer (ET) during September 2008 and May 2009 were evaluated for eligibility to the study. Inclusion criteria were: [1] female age >18 and <40 years, [2] body mass index (BMI) between 18-29 kg/m² [3] patients having normal menstrual period [4], fresh cycles [5], ejaculatory sperm used for ICSI. Patients having any endocrine disease (hyperprolactinemia, hypothyroid and hyperthyroid disease, and Cushing disease), polycystic ovary syndrome (PCOS) and severe endometriosis, those with ovarian cyst or endometrioma, patients having hypergonadotropic hypogonadism, and those with a poor response in the previous IVF cycles were not included into the study.

Forty-five patients met the inclusion criteria and approved to participate in the study. All patients underwent controlled ovarian hyperstimulation consisting of luteal-long leuprolide acetate with or without oral contraceptive pre-treatment. When desensitization was achieved, as evidenced by plasma E2 levels of ≤ 50 pg/ml, the absence of ovarian follicles and endometrial thickness ≤ 6 mm on transvaginal ultrasound examination [10], the authors divided these patients into two groups according to the numbers which were randomly generated by software. Group I patients (n=25) underwent s.c. injection of gonadotropin (re-
combinant FSH (rFSH) preparation. Group II patients (n=20) underwent i.m. injection of gonadotropin hMG preparation. The starting dose of gonadotropins was determined based on the age of the female, antral follicle count at baseline transvaginal ultrasonography, BMI, and previous ovarian response, if available.

Ovarian response was monitored with frequent serum E2 measurements and transvaginal ultrasonography, as described previously [11]. The criterion for hCG administration was the presence of three or more follicles exceeding 17 mm in diameter.

Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound– guided puncture of follicles 36 hours after hCG administration. Following exposure to 20 IU/ml hyaluronidase in order to clean the cumulus cells, oocyte assessment was carried out. Blood serum was simultaneously collected from the patients who have follicular fluids without blood mixed and at least one morphologically normal oocyte. Follicular fluid and blood serum were centrifuged at 3,000 rpm for 15 minutes and kept at – 80°C until the analysis.

**VEGF and IGF-1 Assay**

The levels of VEGF and IGF-1 in serum and follicular fluid were studied at the same time in the clinical biochemistry laboratory. Amount of VEGF and IGF-1 in serum and follicular fluid were evaluated using enzyme immunoassay (ELISA) kits (human VEGF ELISA Kit and IGF-1 ELISA Kit). Minimum measurement sensitivity was > 5 pg/dl for VEGF and > 4.9 ng/dl for IGF-1.

Standard procedures were carried out for gamete-embryo handling and cleavage-stage ET was performed under abdominal ultrasonography guidance in all cases using a soft catheter. The luteal phase was supported by daily vaginal progesterone suppositories starting one day after oocyte pick-up.

Clinical pregnancy was defined as the presence of fetal heart tones and transvaginal ultrasonography, as described previously.

The statistical analyses were performed using Statistics Package for Social Sciences version 17.0 (SPSS). The chi-squared and Fisher exact tests were used to analyze nominal variables in the form of frequency tables. Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by Student t-test. Non-normally distributed metric variables were analyzed by Mann-Whitney U test. Pearson or Spearman correlation was used to investigate correlation. All p values of < 0.05 were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise. Institutional review board of our university approved the study protocol.

### Results

Both groups were comparable regarding the women’s mean age and BMI (Table 1). COH responses of the patients, embryologic and pregnancy outcomes and the amount of VEGF and IGF-1 in serum and follicular fluid are given in Tables 1-2.

No statistically significant difference was found between the groups in terms of the amount of gonadotropin used, the total number of the follicles ≥17 mm in size at the day of hCG administration, the number of oocyte cumulus complexes retrieved, the metaphase 2 oocyte number, the 2 pronuclear oocyte number, the number of grade 1 embryos existing at the third day, the total number of grade 2 embryos at the third day, the number of the transferred grade 1 embryos, the numbers of the transferred grades 2a and 2b embryos, and pregnancy rates (Table 2).

The amount of VEGF (pg/ml) in serum and follicular fluid in groups I and II were comparable (275 ± 135.3 vs 330.7 ± 190.0; p > 0.05 and 2081.1 ± 1095.1 vs 1971.1 ± 975.6; p > 0.05, respectively, Table 2). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 vs 204.1 ± 56.3; p > 0.05 and 176.1 ± 67.2 vs 185.8 ± 48.7; p > 0.05, respectively, Table 2).

Pregnant patients had lower follicular VEGF (pg/ml) levels when compared to non-pregnant patients (160.8 ± 747.8 vs 2669.1 ± 692.0, p < 0.05, Table 3). The serum VEGF levels were positively correlated with the number of oocyte retrieved, the number of M2 oocyte, and the number of 2PN oocyte (Table 4).
Table 3. — Ovarian responses, the VEGF, and IGF-1 levels in the serum and follicular fluid of the clinically pregnant and non-pregnant patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnant (n=19)</th>
<th>Non-pregnant (n=26)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>COH with r-FSH</td>
<td>40%</td>
<td>60%</td>
<td>NS</td>
</tr>
<tr>
<td>COH with hMG</td>
<td>45%</td>
<td>55%</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocyte-cumulus complexes</td>
<td>14.8 ± 5.7</td>
<td>12.3 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>No. of metaphase II oocytes</td>
<td>10.53 ± 3.7</td>
<td>7.27 ± 4.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.95 ± 0.8</td>
<td>2.62 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>353.4 ± 193.5</td>
<td>260.6 ± 125.1</td>
<td>NS</td>
</tr>
<tr>
<td>Follicular VEGF (pg/ml)</td>
<td>1160.8 ± 747.8</td>
<td>2669.1 ± 692.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum IGF-1 (ng/ml)</td>
<td>214.3 ± 64.0</td>
<td>217.0 ± 65.3</td>
<td>NS</td>
</tr>
<tr>
<td>Follicular IGF-1 (ng/ml)</td>
<td>195.8 ± 66.9</td>
<td>169.1 ± 66.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Non-significant; COH: Controlled ovarian hyperstimulation; VEGF: Vascular endothelial growth factor; IGF-1: Insulin like growth factor-1; r-FSH: Recombinant follicle stimulating hormone; hMG: Human menopausal gonadotropin.

Table 4. — Correlation of the VEGF and IGF-1 levels in the serum and follicular fluid with the ovary and embryologic responses (cross table).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum VEGF</th>
<th>FF VEGF</th>
<th>Serum IGF-1</th>
<th>FF IGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of follicles at the day of hCG</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocyte-cumulus complexes</td>
<td>r = 0.33 (&lt;0.05*)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No. of metaphase II oocytes</td>
<td>r = 0.38 (&lt;0.05*)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>No. of 2 pronuclear oocytes</td>
<td>r = 0.42 (&lt;0.05*)</td>
<td>NS</td>
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<tr>
<td>No. of day 3 embryos</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

r = Pearson correlation coefficient; *Correlation is significantly positive; FF: Follicular fluid; VEGF: Vascular endothelial growth factor; IGF-1: Insulin like growth factor-1; hCG: Human chorionic gonadotropin.

Discussion

In the present study, the authors noted that the amount of VEGF (pg/ml) in serum and follicular fluid in the group I and II were comparable (275 ± 135.3 vs 330.7 ± 190.0; p > 0.05 and 2,081.1 ± 1,095.1 vs 1,971.1 ± 975.6; p > 0.05, respectively, Table 2). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 vs 204.1 ± 56.3, p > 0.05 and 176.1 ± 67.2 vs 185.8 ± 48.7, p > 0.05, respectively, Table 2). In other words, hMG and r-FSH for COH in ICSI cycles did not produce different follicular and serum VEGF and IGF-1. The clinical pregnancy rates were comparable between hMG and r-FSH groups.

Monteleone et al. [4] investigated the correlation of the follicular VEGF with the grade of perifollicular vascularity. They noted that the follicular VEGF levels to be significantly correlated with grade of perifollicular vascularity. They also reported that the higher VEGF levels were associated with higher numbers of the grade 1 embryos and oocytes and pregnancy rates. It was hypothesized that increased VEGF level is an adaptation for the oocytes to create a better vascular structure and a better micro-environment to form. The authors pointed out that oocyte viability of VEGF might be a marker of the fertilization and embryo quality and it also might be an indicator of the follicular microenvironment due to formation of a perifollicular capillary network. The present study supported their findings. The present authors noted that there was a positive correlation between the numbers of oocyte retrieved, the number of metaphase 2 oocyte, the number of pronuclear oocyte, and serum levels of VEGF. However, this was in contradiction with the study by Manaul et al. [12]. In their study, no correlation was found between the amounts of VEGF in serum or in follicular fluid with the number of oocyte retrieved and the number of follicles at the day of hCG.

Tokuyama et al. [13] evaluated the follicular VEGF concentrations in three COH groups; Group 1: hMG cycles (n=19), Group 2: clomiphene citrate cycles (n=10), Group 3: natural cycles (n=9). They reported that the Group 1 showed lower VEGF concentrations in follicular fluid than Group 2 or Group 3. Excluding high responders from Group 1, no difference was found among these three groups. When they reclassified groups, they noted that the group of highest number of oocytes harvested showed lowest VEGF concentrations in follicular fluid.

Choi et al. [14] compared the effects of three different COH protocols (urinary FSH (u-FSH) only, r-FSH only and r-FSH + hMG) on the follicular IGF-1 and IGFBP-4 (insulin-like growth factor binding protein-4). They noted that follicular IGF-1 levels were comparable between groups (IGF-1 (ng/ml), 128 ± 12.6 vs 120 ± 8.9 vs 123 ± 13.8). Of interest, they reported that the follicular IGFBP-4 and pregnancy-associated plasma protein (PAPP-A) levels in r-FSH group were significantly higher than those of other groups (p < 0.05). Oosterhuis et al. [15] investigated the follicular IGF-1 concentrations in patients who underwent COH with hMG (n=23) and highly purified urinary FSH (n=47) for IVF. They noted that there was no significant difference between the groups received hMG and uFSH in terms of the rates of follicular IGF-1 (ng/ml), 116±92.7 vs 119±51.1, p > 0.05 and pregnancy.

When the present authors classified all patients in this study as pregnant and non-pregnant groups, the amount of follicular VEGF was found to be lower in the pregnant group than in the non-pregnant group (Table 3). However, the serum VEGF, follicular and serum IGF-1 levels were comparable between the pregnant and non-pregnant groups. No significant difference was observed between pregnant and non-pregnant groups in terms of the number and quality of the oocytes and embryos. Of interest, the number of metaphase 2 oocytes was to be higher in the non-pregnant group (p < 0.05). This was consistent with the literature. Numerous studies compared amount...
of VEGF in follicular fluid following the ovulation induction carried out with rFSH in the pregnant and non-pregnant groups. These studies reported amount of VEGF to be lower in the pregnant group. Whereas, no significant difference was reported in terms of the number and quality of the oocytes and number of embryo transfers [16, 17].

In the present study, the serum VEGF levels were positively correlated with the number of oocyte retrieved, the number of M2 oocyte, and the number of 2PN oocyte (Table 3). However, the authors failed to find an association between IGF-1 and the oocyte number, the metaphase 2 oocyte number, the pronuclear ooyct number, the follicle number, and the grade 1 embryo number. This result was consistent with the literature. Dorn et al. [18] did not establish a correlation between the amount of follicular IGF-1 and pregnancy rate.

There is also no consensus in the literature on the effect of suppression protocols (GnRH agonist vs GnRH antagonist) on the VEGF levels in follicles. Asimakopoulou et al. [19] conducted a study and they noted that the VEGF concentrations in supernatants from cultures with cetrorellix (2,315.1 ± 1,565.5 pg/ml) were moderately, but significantly lower than in controls (2,604.3 ± 1,907.1 pg/ml) or cultures with leuprolide acetate (2,558.8 ± 1,403.1 pg/ml). Therefore, in the present study, the authors preferred to use GnRH analog (leuprolide acetate) only.

It is known that the VEGF levels in preovulatory follicles are ten times higher than those in serum [20]. Several studies report similar results [16, 17]. The present study supported this finding (Table 2).

In conclusion, hMG and r-FSH produced comparable follicular microenvironment regarding VEGF and IGF-1 in patients undergoing ICSI cycles.

References


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Methamphetamine, smoking, and gestational hypertension affect norepinephrine levels in umbilical cord tissues

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Summary

Background: These studies were undertaken to determine methamphetamine (METH) and smoking effects on umbilical vascular dynamics and pregnancy outcomes. Materials and Methods: Umbilical cords (54) were collected prospectively at birth, washed of blood, and stored at -80°C. Cords were thawed and lysates prepared, then catecholamine levels quantified with enzyme-linked immunosorbent assay (ELISA). Results: Catecholamine levels in umbilical cords were not associated with maternal or gestational age, gravidity, parity, neonatal or placental weight. Neither smoking nor METH affected dopamine or epinephrine. However, smoking (two-fold) and METH (four-fold) decreased norepinephrine and together a 60-fold reduction occurred (p = 0.025). Cesarean section and hypertension were both associated with lower norepinephrine levels (p < 0.001) regardless of drug status. In normotensive pregnancies, smoking and METH significantly decreased norepinephrine levels (two-fold and 3.5-fold each, respectively) with a 40-fold decrease for METH/smoking together. Discussion: Depletion of norepinephrine by METH and smoking likely contributes to pregnancy complications, including the higher incidence of respiratory distress and postpartum hemorrhage in cesarean section.

Key words: Drugs of abuse; Epinephrine; Norepinephrine; Dopamine.

Introduction

When drugs of abuse are ingested during pregnancy they can pass from the maternal circulation, through the placenta, and to the fetus. Blood exiting the placenta is delivered directly to the fetal heart by the umbilical vein, pumped around the fetal circulation, then returned to the placenta by the umbilical arteries [1]. Therefore, the umbilical artery and vein not only play vital roles in the delivery of oxygen and essential nutrients and removal of wastes, but also provide a major route for chemicals to reach the fetus. In addition, any substance with vasoactive properties may interfere with efficient placental and umbilical function and pose a risk to the fetus [1].

Commonly, women do not recognize that they are pregnant until the second missed menstrual cycle and may accidentally continue to use recreational or medical drugs that they would otherwise avoid [2]. However, after knowingly becoming pregnant some mothers either do not or cannot stop their ingestion of drugs and this is a hallmark of addiction. Methamphetamine (METH) addiction is associated with pre-term birth, intra-uterine growth restriction, small-for-gestational-age babies and neonatal dependence [3].

The drug METH is an indirect sympathomimetic that causes central euphoric effects through excessive release of catecholamines agonizing adrenergic receptors [4]. Outside of the central nervous system, METH’s strongest effects occur in the cardiovascular system increasing systolic and diastolic blood pressure, and reflexive decreasing heart rate [4]. Eventually METH over-stimulation depletes catecholamines both centrally and peripherally. The cardiovascular sympathetic action is primarily mediated by norepinephrine at a- (vascular) and b- (heart and lung) adrenergic receptors, although dopamine effects on the renal system also contribute to blood pressure effects [5]. The umbilical cord vessels may provide a conduit for METH to directly affect fetal catecholamine signaling in the heart [6-8]. Additionally, maternal, and gestational tissues (placenta, uterus, and umbilical vein and arteries) also contain a- and b-receptors, hence the fact that METH may deplete catecholamines in the blood and interfere with sympathetic responses in these tissues is concerning [9, 10]. The primary effects of METH in the maternal-placental-fetal unit occur at a receptors in vascular smooth muscle and b-receptors in cardiac cells. Additionally, there is a distinct separation in dopamine receptor expression where umbilical arteries express D1 receptors [11], but D2, D3, and D4 receptors are only reported in the umbilical vein [12]. The functional consequences of dopamine signaling in the vessels in the umbilical cord, as compared to norepinephrine effects, are not well established and likely less critical.
The potential for METH to affect adrenergic signaling in the umbilical cord vessels, as well as the fetal cardiovascular system prompted the present authors to undertake this study. The hypothesis was that METH and smoking alter normal catecholamine signaling thereby affecting umbilical vascular dynamics, and perhaps in the fetal cardiovascular system. Here they present data supporting this, including that METH and smoking alter constitutive (from cesarean section without labor) and parturition-mediated norepinephrine levels in the umbilical cord. They also demonstrate that hypertension is associated with lower umbilical tissue norepinephrine. They subsequently propose a link between METH effects on noradrenergic signaling, vascular effects in the umbilicus, and adverse reproductive outcomes.

Materials and Methods

Tissue collection and processing

Cord samples were collected by the Hawaii Biorepository, which banks de-identified placental and blood samples from women at delivery, and links these samples with a database of prenatal, delivery, and infant outcome information derived from medical record data. Exempt approval was obtained from the University of Hawaii Committee for Human Studies for the subproject and from the Western Institutional Review Board (WIRB) for the HiBR. Each piece of cord was coded with a study number, washed thoroughly, then stored at -80 ºC until use. All cords were used within 12 months of collection. It has been demonstrated that when stored at -80 ºC, human norepinephrine and epinephrine are stable for at least six months and probably much longer when stored in tissue or whole blood and do not show the degradation observed in urine or buffers [13].

A sample of 54 cords were used for this pilot study. For processing to lysates, cord pieces were thawed, wet weight recorded, then cords homogenized mechanically with a homogenizer at 6,500 RPM for 30 seconds each in a 1:4 (w:v) Tris-HCl buffer containing five mM MgCl₂ and two mM PMSF (pH 7.4). Total lysates were normalized to two mg/ml protein using the Bicinchoninic acid method, then aliquoted and frozen at -80 °C until use [14].

Enzyme-linked immunosorbent assay (ELISA) for catecholamines

The levels of epinephrine, norepinephrine, and dopamine were determined with a commercial 3-CAT ELISA from as per the manufacturer’s instructions.

Data analysis and statistics

Quantification of catecholamines was performed blinded using only the study number for identification of samples. After ELISAs were completed and concentrations assigned, the study key was accessed and results were sorted into four groups: cords from non-smoking, non-METH patients (control), cords from METH using patients, cords from tobacco smoking patients, and cords from patients who both smoked tobacco and used METH.

Statistical analyses

Statistical analyses were performed using Prism 5.0 with significance set at $\alpha = 0.05$. Since these data are discrete, two-tailed student t-tests were used to assess differences between groups. Stratification and comparisons within groups were analyzed using multivariate analyses, ANOVA, and multiple regression for continuous data.

Results

Of the 54 samples tested, dopamine was detected in 38 cord tissues, norepinephrine in 48 cords, and epinephrine in 16 cords. None of the catecholamines correlated with maternal age, gestational age, neonatal weight, placenta weight, gravidity, or parity. Cesarean section was associated with significantly lower dopamine levels in umbilical cords. However, METH use by itself (in non-smokers) was associated with a four-fold decrease in umbilical norepinephrine levels,
Methamphetamine, smoking, and gestational hypertension affect norepinephrine levels in umbilical cord tissues

smoking was associated with a two-fold decrease in norepinephrine levels and in women who both abused METH and smoked almost 60-fold decreases in norepinephrine were observed ($p < 0.05$, Dunnett’s multiple comparison test, Figure 2B).

In the 38 samples where dopamine was detected, neurotransmitter levels were not associated with hypertension (Figure 3A). However, norepinephrine was significantly lower in umbilical cords from hypertensive pregnancies ($p < 0.001$, t-test, Figure 3B). Of the 16 cords where epinephrine was detected, no hypertensive cases were recorded.

While these drug effects are interesting, they must be further examined in light of the independent effects of delivery on dopamine and norepinephrine levels, as well as hypertension on norepinephrine levels. When stratified for delivery type and dopamine levels, drug ingestion has no effect on dopamine levels but cesarean section is consistently associated with higher dopamine levels in the umbilical cord vessels (Figure 4A). Vaginal birth is associated with higher levels of norepinephrine for all groups except smokers where there were no differences. Additionally, METH and METH + smoking were associated with successively lower norepinephrine levels compared to drug-free cords in both vaginal and cesarean births. These data demonstrate that both delivery type and drug ingestion independently alter norepinephrine levels in umbilical cords (Figure 4B). Finally, when the effects of hypertension and drug ingestion were stratified, two clear effects are identified. Firstly, in non-hypertensive cords, smoking and METH individually decrease norepinephrine levels. Control cord levels of norepinephrine were almost two-fold higher than in smokers and 3.5-fold higher than for METH users. Combined METH and smoking had a drastic effect on norepinephrine levels, lowering them 40-fold (Figure 4C). Secondly, in hypertensive pregnancies the levels of norepinephrine were drastically lowered in all samples tested with hypertensive cord norepinephrine levels being between 50 and 150-fold lower than drug free, non-hypertensive cords and with the norepinephrine levels within drug groups being between two- and 75-fold lower than the norepinephrine levels in the corresponding

**Figure 2.** — Differential effects of METH and smoking on umbilical cord catecholamines. (A) Smoking and METH do not significantly alter cord dopamine levels. (B) METH and METH + smoking combined significantly deplete cord noradrenaline levels. (C) No effects were observed on epinephrine. Bars are means ± SEM. * = $p < 0.01$ (Dunnet’s multiple post hoc comparison), capped bar at end of graph indicates ANOVA results. METH = methamphetamine. M+S = METH and smoking together, NR = none recorded.

**Figure 3.** — The effects of hypertension (clinically diagnosed, any grade) on umbilical cord tissue catecholamine levels. (A) Dopamine levels are not changed by hypertension in pregnancy. (B) Norepinephrine was significantly reduced by hypertension. (C) Epinephrine was undetectable in umbilical cords from hypertensive patients. *** = $p < 0.001$, t-test. NR = none recorded.
There were no apparent effects of drugs in depleting norepinephrine further over the effect of hypertension. These latter data imply that hypertension is more strongly effective at depleting norepinephrine in the umbilical cord vessels than METH and smoking (alone or together). However, despite the visually arresting effects in the graphs, these latter data should be interpreted with caution due to the paucity of samples in the hypertensive group. Additionally, concurrent drug administration to the mothers may have affected these results.

**Discussion**

This study demonstrates that hypertension and drug ingestion (smoking and METH) can deplete norepinephrine levels in the umbilical cord tissues. The effects of the drugs are synergistic in normotensive pregnancies, but hypertension has a greater norepinephrine depletion effect than the drugs. Additionally, the authors demonstrate that delivery method (cesarean vs. vaginal) affects catecholamine levels in the umbilical tissues, with lower levels of catecholamines present after cesarean section than normal birth.

Similar to the present findings that the tissues of the umbilical cord from cesarean section had significantly lower dopamine and norepinephrine levels compared to those from vaginal deliveries, other investigators have reported this phenomenon in total cord blood [15], umbilical artery blood [16], and umbilical vein blood [17]. Here, using washed cords, the authors showed the same results in umbilical cord tissues, where the catecholamines are presumably derived from the bloodstream and either bound to receptors on the surface of vessels smooth muscle, or present in the cytosol of the smooth muscle due to transport by the uptake 2 transporter [4].

The physiological reason(s) for the results showing lower norepinephrine in cesarean section deliveries compared to labor are likely straightforward. It has been demonstrated that elevated norepinephrine causes transient pulmonary hypertension in term human fetuses [18] and that high doses of norepinephrine cause vasoconstriction of the placental vascular bed [19]. Hence, increases in umbilical norepinephrine during parturition may be advantageous for activating the fetal lungs at birth and are also certainly useful for uterine contractions and preventing post-partum bleeding. If confirmed, this could explain the increased incidence of respiratory distress and post-partum hemorrhage in cesarean deliveries.

Similarly, with respect to transporters, studies have shown that in addition to depleting neuronal norephinephrine stores, METH is a potent inhibitor of both serotonin and norepinephrine transporters in reproductive tissues, with the norepinephrine transporter being more affected [20]. This provides a good explanation for our drug-related results.

Lower norepinephrine levels associated with METH use in umbilical vessel tissues may be caused by a combination of the classical mechanism for METH effects: depleted epi-nephrine release, less conversion to nor-epinephrine and lower circulating levels of the transmitter, but also by inhibition of norepinephrine uptake 2 transporters in reproductive tissues. Together, these mechanisms would combine to...
produce lower levels of norepinephrine detected in umbilical cords. An alternative hypothesis, is that since the majority of systemic catecholamines circulate in sulfo-conjugated form, METH and/or smoking affect sulfation of catecholamines. A recent study in rats demonstrated that several sulfotransferasess are upregulated by METH administration within 24 hours and this occurs in a time- and concentration-dependent manner [21], establishing in principle that these genes can be induced by METH. Since the placenta has high expression of sulfotransferases, lower levels of norepinephrine, and epinephrine in the tissues of the umbilical cord vessels due to increased sulfation across the placental vasculature is also a possibility.

The synergism reported herein for METH and smoking in lowering umbilical norepinephrine levels is of particular interest. A single report on this phenomenon has been previously published for humans where it was found that median epinephrine and norepinephrine concentrations in the cord blood of neonates were significantly lower in smokers compared with nonsmokers [22]. Based on the known physiological and pharmacological characteristics of both METH and smoking, it is sensible that their combined action would cause synergistically lower levels of catecholamines in circulating blood, as well as in other tissues including vascular smooth muscle.

Differences in umbilical tissue norepinephrine levels with hypertension are also intriguing. Hypertension in pregnancy is clinically diagnosed and can range from essential hypertension to preeclampsia – a disease characterized by abnormal vascular responses to placentation, increased systemic vascular resistance, and endothelial cell dysfunction [10]. The drastic hypertension-mediated depletion of norepinephrine levels observed in the umbilical cord (up to 150-fold), was stronger than METH-mediated norepinephrine depletion (up to 60-fold in normotensive pregnancies). Other authors have reported changes in umbilical cords with respect to hypertensive disorders in pregnancy, although these have primarily been ultrastructural [23] and/or involved alterations to blood flow [24, 25].

The authors acknowledge that this is a small study with only 54 samples and not all catecholamines were detected in all samples due to the limits of detection of the ELISA assay, given that largely intracellular levels of catecholamines were being detected, which are much lower than circulating levels in blood. Hence, with a small sample size of 54 individual cords, stratifying some of these data likely compromised statistical power. However, this is mitigated somewhat since non-detection of catecholamines in cords was not uniform. That is, no individual cords returned non-detection for all three catecholamines and no trends towards uniformly lower levels of any or all biogenic amines with collection, storage or processing were observed. Moreover, catecholamine levels were unlikely to be depleted due to storage conditions, since human norepinephrine and epinephrine are stable for at least six months (and probably much longer) when stored in tissue or whole blood, lacking the degradation observed in urine or buffers [13].

Furthermore, from a clinical perspective, the authors do not have data regarding medications used to treat maternal hypertension. In Hawaii, current practice encompasses the use of labetolol, hydralazine, and methyldopa (aldomet) for managing hypertension in pregnancy. Since labetolol acts directly at adrenergic receptors (mixed action) and hydralazine on smooth muscle (through a second messenger system), their effects would be expected to alter tissue responses to catecholamines [5]. Furthermore, the use of methyldopa is a potential confounder since it is a competitive inhibitor of DOPA decarboxylase which produces dopamine. Because dopamine is the precursor for norepinephrine and epinephrine, use of this drug would be expected to inhibit catecholamine production in peripheral tissues [5]. Despite this, the authors observed that although norepinephrine levels were lower in hypertension, dopamine levels were not, so confounding by methyldopa administration is not expected.

The authors believe that these results, particularly regarding the combined effects of METH and smoking on depleting norepinephrine in umbilical tissues, should be studied further. Rather than measuring blood levels of the catecholamines as a proxy, they measured tissue levels directly in washed umbilical cords. This means that although they only detected low levels of the molecules, these molecules are either directly bound to cell surfaces, or had been internalized into the cells of the umbilical vessels – representing the vasoactive fraction of the circulating transmitters. These studies also add to our understanding of the interplay between cesarean section and parturition signaling, since cesarean section was associated with much lower dopamine and norepinephrine levels than vaginal deliveries. This presents an avenue to investigate the mechanisms of well-known increases in respiratory distress and postpartum hemorrhage observed with cesarean deliveries. The authors have also provided a mechanistic direction that may elucidate how drugs of abuse ingested during pregnancy can affect pregnancy outcomes – namely through alterations to vasoactive signaling throughout pregnancy and at parturition. For mothers addicted to METH, future medical interventions to prevent adverse pregnancy outcomes could center on re-balancing appropriate catecholamine/vasoactive signaling in gestational tissues. For example, because beta blockers are contraindicated in pregnant METH users, determining the utility of other vasoactive drugs, such as the phosphodiesterase inhibitors, may prove useful. Although in its infancy, the authors hope that this research can assist to develop or repurpose existing pharmaceuticals and guide clinical practice to mitigate negative vascular effects of drug abuse and improve maternal and neonatal outcomes.
Acknowledgements

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Introduction

Ultrasonography has been approved as a useful diagnostic modality for identifying fetuses with chromosomal abnormalities [1]. Subcutaneous edema detected sonographically in the forms of nuchal edema, cystic hygroma (CH), or non-immune hydrops (NIH) may be a sign of intrauterine infection, fetal anemia, fetal heart disorders, and complicated twin pregnancies [2-4]. Whether or not these pathologies exist, there is a need for further investigation because of an increased risk of chromosomal abnormalities.

There are numerous studies of the incidence of chromosomal abnormalities in the presence of major fetal anomalies or various ultrasonographic soft markers in the literature. Over the limit value of nuchal translucency (NT), pathological chromosomal sets have been reported in more than 20% of cases [5-9]. According to previous studies, the chromosome abnormality rate varies from 3.4% to 39% when nuchal fold thickness is present [10, 11]. The risk of chromosomal abnormalities is reported to be even greater in cases with CH and ranges from 23% to 60% [12-15]. NIH is also frequently accompanied by aneuploidy and mostly results in the loss of the fetus [13].

The aim of this study was to describe the association between abnormal sonographic markers, such as nuchal edema in the first and second trimesters, as well as cystic hygroma, non-immune hydrops, and CH with non-immune hydrops, with chromosomal abnormalities, and to calculate the predictive value of these markers for chromosomal abnormalities in a retrospective study of 218 subjects.
Table 1. — Clinical characteristics of the study groups.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Nuchal edema in the first trimester (NT group) n = 56</th>
<th>Nuchal edema in the second trimester (NF group) n = 48</th>
<th>CH group n = 38</th>
<th>NIH group n = 41</th>
<th>CH associated with NIH (CH and NIH group) n = 35</th>
<th>Total n = 218</th>
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<td>48 (22)</td>
<td>38 (17.4)</td>
<td>41 (18.8)</td>
<td>35 (16.1)</td>
<td>218 (100)</td>
</tr>
<tr>
<td>Maternal age (y)**</td>
<td>28.3 ± 6.6</td>
<td>28.6 ± 6.4</td>
<td>27.6 ± 6.3</td>
<td>29.6 ± 5.3</td>
<td>26.6 ± 6.4</td>
<td>28.2 ± 6.2</td>
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<td>Gestational age at diagnosis (w)**</td>
<td>12.3 ± 0.8</td>
<td>19.6 ± 2.8</td>
<td>14.2 ± 2.8</td>
<td>19 ± 4.4</td>
<td>15.5 ± 3.1</td>
<td>16 ± 4.1</td>
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<td>Female to male ratio</td>
<td>29/27 (1.07)</td>
<td>27/21 (1.28)</td>
<td>21/17 (1.23)</td>
<td>21/20 (1.05)</td>
<td>27/8 (3.37)</td>
<td>125/93 (1.34)</td>
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<tr>
<td>Nuchal fold thickness (mm)**</td>
<td>4.2 ± 1.5</td>
<td>6.8 ± 0.9</td>
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<td>N/A</td>
<td>N/A</td>
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</tr>
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<td>Chromosomal abnormalities*</td>
<td>17 (30.4)</td>
<td>10 (5.4)</td>
<td>14 (36.8)</td>
<td>14 (34.1)</td>
<td>21 (60)</td>
<td>71 (32.6)</td>
</tr>
</tbody>
</table>

Values are expressed as *: n (%); **: mean ± standard deviation. 
CH = cystic hygroma; NIH = non-immune hydrops; N/A: not available; y = year; w = week; mm = millimeter

Maternal age (y)p = 0.132; female to male ratio p = 0.134; chromosomal abnormalities p ≤ 0.001

Table 2. — Distribution of the chromosome abnormalities among all fetuses.

<table>
<thead>
<tr>
<th>Chromosome abnormality type</th>
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<th>Nuchal edema in the second trimester (NF group)</th>
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<th>NIH group</th>
<th>CH associated with NIH (CH and NIH group)</th>
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<td>-</td>
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<td>3 (7.3)</td>
<td>13 (37.1)</td>
<td>26 (11.9)</td>
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<td>2 (5.7)</td>
<td>31 (14.2)</td>
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<td>Trisomy 18</td>
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<tr>
<td>Trisomy 13</td>
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<td>-</td>
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<td>Total</td>
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<td>5 (10.4)</td>
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</tr>
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<td>p values</td>
<td>0.008</td>
<td>-</td>
<td>0.030</td>
<td>0.092</td>
<td>0.005</td>
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</tr>
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</table>

All values are expressed as n (%); CH = cystic hygroma; NIH = non-immune hydrops.

Results

A total of 218 fetuses met the inclusion criteria. Mean maternal age of the study group was 28.2 years (range, 16–46). Mean gestational age at the time of initial diagnosis was 16 weeks (range, 10–25). Gestational ages of the NT, NF, CH, NIH, and CH with NIH groups were 12.3, 19.6, 14.2, 19, and 15.5 weeks, respectively. Gender was male in 93 (42.7%) and female in 125 (57.3%); with a female-to-male ratio of 1.34, which was statistically insignificant (p = 0.134). Table 1 summarizes the clinical characteristics of each study group.

Karyotype analysis was performed by AS in 127 (58.3%) patients, by cordocentesis in 23 (10.6%) patients, and by CVS in 68 (31.2%) patients. Of the 218 fetuses, there were 71 (32.6%) with abnormal karyotypes. The chromosomal abnormality rates in the NT, NF, CH, NIH, and CH with NIH groups were 17 of 56 fetuses (30.4%), five of 48 fetuses (10.4%), 14 of 38 fetuses (36.8%), 14 of 41 fetuses (34.1%), and 21 of 35 fetuses (60%), respectively. The chromosomal abnormality rate was statistically significantly higher in the CH with NIH group (p ≤ 0.001). Table 2 summarizes the distribution of chromosomal abnormalities in each group and among all fetuses.

The limit value of nuchal fold thickness for indication of karyotyping was accepted as ≥ five mm in the current study. In the NF group, six of the 48 fetuses’ NF thicknesses were ≥ five mm and < six mm, and all had normal karyotypes.
In 71 cases with detected chromosomal abnormalities, 37%, 44%, 15%, and 4% of the pathologic karyotypes were identified as monosomy X, trisomy 21, trisomy 18, and trisomy 13, respectively. Trisomy 21 was the most common pathological chromosome anomaly detected in the NT (21.4%) and NIH (22%) groups, with almost equal proportions. In the NF group, trisomy 21 was the only detected abnormal karyotype, with an incidence of 10.4%. Monosomy X was found to account for the highest proportion among the detected chromosomal abnormalities in the CH (23.7%) and CH with NIH (37.1%) groups. The incidences of trisomy 21 in NT group ($p = 0.008$), monosomy X in CH group ($p = 0.03$), and monosomy X in CH and NIH group ($p = 0.03$) were statistically significant.

**Discussion**

Genetic sonogram has been proven to be a useful tool for identifying fetuses with chromosomal abnormalities. The presence of various soft markers and major anomalies increases the patient’s risk for an anomaly and necessitates further investigation. Noia et al., in their study evaluated the natural history of cystic hygroma in the fetal and neonatal periods, suggested that ultrasonography on its own cannot diagnose a chromosomal anomaly; therefore, suspected lesions require confirmation by invasive investigations such as villus biopsy, AS, or cordocentesis [23].

During prenatal genetic counseling, the present authors’ aim to identify as many fetuses with abnormal karyotypes as possible while trying to avoid increasing the risk of abortion through unnecessary invasive interventions. An accurate prediction of the chromosomal abnormality rate is essential to satisfy parents and to protect the counselor. This study presents the genetic results for fetuses with subcutaneous edema in the forms of nuchal edema, CH, or NIH detected sonographically in the present clinic.

The results of the present study reveal that nuchal edema increases the risk of trisomy 21, trisomy 18, trisomy 13, and X-monosomy in the first trimester. In accordance with the literature, trisomy 21 was the most common of all chromosomal abnormalities in this group (NT group), with an incidence of 21.4%, and trisomy 18 was second most common, with an incidence of 5.4% [9]. Although NT alone has a good detection rate for trisomies, its sensitivity is better when combined with pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin [24]. Trisomy 10, triploidy, 47XXY, 47XYY, 47 XXX, 46,XX/47, and XX+12 are the other very rare chromosomal abnormalities detected in previous studies; none of these was identified in the present study groups [6, 25, 26].

Unlike in many previous publications, trisomy 21 was the only abnormal karyotype identified in the 10.4% of fetuses with second trimester nuchal edema (NF group) in the present study. Trisomy 18 and other chromosomal abnormalities were also detected in the literature, but trisomy 21 was the dominant abnormality [10, 11, 27].

Chromosomal anomalies were detected in 60% of cases in the CH with NIH group in the present study. Considering that the chromosomal abnormality rate was 36.8% in the CH group, the authors conclude that association with NIH results in a twofold increase in the rate of abnormal karyotype. Supporting the present findings, Beke et al. discovered chromosomal abnormalities in 20% of CH cases, while the chromosomal abnormality rate was 53.8% in cases of CH with NIH [7]. In a similar study by Nadel et al., chromosomal abnormalities were identified in 46.5% of CH cases, while CH and NIH occurring together was associated with pathological karyotypes in 83.78% [13].

In the present investigation, in the CH with NIH group, monosomy X accounted for 37.1% of karyotypes and was followed by trisomy 18 (11.4%), trisomy 21 (5.7%), and trisomy 13 (5.7%). The present results and the previous reports are contradictory in terms of the distribution in types of chromosomal abnormalities in fetuses with CH with NIH. Many researchers agree that monosomy X is the most common type and trisomy 21 is the second most common in this group; however, Beke et al. reported higher rates of trisomy 18 (38.46%) than monosomy X (15.38%) in their study [7].

In the CH group, the chromosomal abnormality rate of 36.8% was in agreement with the literature [14]. Monosomy X was the most common karyotype abnormality in the present study, with an incidence of 23.7%. The types of chromosomal abnormalities detected in this study were also concordant with previous reports [8, 14, 28]. However, some authors reported an incidence of trisomy 21 of 21.4–37.3%, with the highest proportion in this group [12, 15].

Hematologic, cardiovascular and metabolic diseases, infections, tumors, and many other conditions may give rise to NIH, including trisomy 21, trisomy 18, and monosomy X [7, 13]. In agreement with the literature, monosomy X and trisomy 21 were the most common types of aneuploidy in our investigation. Fritsch et al., in their study of 116 NIH patients, reported that in 22.4% of them, the etiology was chromosomal abnormalities [29]. Schwanitz et al. evaluated cases of NIH and found a pathological karyotype in 27.6%; the present data at 34.1% are consistent with these findings [30].

In this study, the authors were not able to rule out submicroscopic genomic alterations, single gene diseases, and other genetic syndromes; they consider this a limitation. Further studies are needed using other techniques, such as cytogenetic microarray analysis, which might indicate a different incidence of genetic abnormalities associated with fetal subcutaneous edema.

**Conclusion**

The present study confirms that subcutaneous edema detected sonographically, in the forms of nuchal edema, CH, or NIH, is a significant indicator of an abnormal karyotype.
and deserves further investigation. CH in association with NIH results in a twofold increase in the abnormal karyotype rate. By analyzing the distribution of chromosomal abnormalities, the authors established that nuchal edema and NIH mainly increase the risk of trisomy 21. CH with or without NIH is mainly associated with monosomy X.

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Asymptomatic bacteriuria screened by catheterized samples at pregnancy term in women undergoing cesarean delivery

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Summary

Objective: The objective of this study was to assess the frequency of urinary tract infection (UTI) with urine samples obtained via catheterization among women undergoing cesarean delivery at term pregnancy. Materials and Methods: A cross-sectional study involving 159 women in whom cesarean delivery was conducted at term pregnancy after a regular follow-up from first to third trimester. For screening and diagnosis of UTI during antenatal period, the authors used dipstick test and microscopic urinalysis, and urine culture was used in the presence of symptomatic UTI unresponsive to initial antibiotic therapy. A urine sample was obtained immediately after insertion of Foley catheter for urine dipstick test, microscopic urinalysis, and culture during cesarean delivery. Obstetric and UTI data were recorded. Results: Of 159 pregnant women, 95 (59.8%) did not develop UTI during antenatal care. There was no patient with symptomatic UTI at the admission for cesarean delivery. The authors found UTI with urine dipstick and microscopic urinalysis in 12 patients and of them, four patients had no history of UTI, and all the remaining eight patients had asymptomatic UTI during antenatal follow-up. UTI according to urine culture was encountered in three patients, two of them had one episode of UTI, and one had two episodes of UTI during antenatal follow-up. Conclusions: After regular antenatal follow-up screening with urine dipstick, microscopic urinalysis, and counseling of pregnant women regarding UTIs, the frequency of bacteriuria decreases considerably during cesarean delivery.

Key words: Asymptomatic bacteriuria; Pregnancy; Cesarean delivery.

Introduction

Detection of bacteria in the urine of a person without clinical findings of urinary tract infection (UTI) is defined as asymptomatic bacteriuria. Although the prevalence of asymptomatic bacteriuria (from 2% to 7%) in pregnant and non-pregnant women is comparable, this condition gains importance in pregnancy since it may increase the incidence of symptomatic bacteriuria and perinatal complications such as prematurity and intrauterine growth restriction. The causative organisms (generally E. coli) and their entry mechanisms are likely to be the same for both groups. Symptomatic bacteriuria is defined either as lower urinary tract (acute cystitis) or upper urinary tract (acute pyelonephritis) infection [1-6].

Anatomical and functional changes in the urinary tract in pregnancy can increase susceptibility to progression of the infection from asymptomatic bacteriuria to the stage of cystitis and even to acute pyelonephritis. The upper urinary tract shows dilatation as early as the first trimester of pregnancy and the bladder itself is displaced superiorly and anteriorly with the enlarging uterus [7]. Mechanical compression caused by enlarged uterus is the principle cause of hydroureter and hydronephrosis especially in the second and third trimesters; however, smooth muscle relaxation related to increased progesterone has also an important role. Smooth muscle relaxation decreases peristalsis of the ureters, increases bladder capacity, and causes urinary stasis. Changes in urine pH and osmolality and pregnancy-induced glycosuria and aminoaciduria are accepted as other factors facilitating bacterial growth leading to urinary infection [8].

During pregnancy, screening and treatment for asymptomatic bacteriuria are included in the standard obstetric care in many antenatal care centers and most antenatal guidelines require the routine screening for asymptomatic bacteriuria [1, 5, 9-12]. The main types of urine testing evaluated for the diagnosis of UTI were dipstick test and microscopic urinalysis. Dipstick tests have the advantage of providing an immediate result, and of being both cheap and easy to perform and interpret. Urine culture is generally considered to be the reference standard for UTI diagnosis; however, its duration of approximately 48 hours to give a result and higher cost are accepted as drawbacks [13]. A single urine specimen obtained from 12-16 weeks of pregnancy will identify most women with asymptomatic bacteriuria. According to suggestions of U.S. Preventive Services Task Force, all pregnant women should provide a clean-catch urine specimen for a screening culture at 12 to 16 weeks’ gestation or at the first prenatal visit, if later; however, the optimal frequency of subsequent urine testing during pregnancy is uncertain [14].

Although UTIs are accepted as important medical complications in pregnancy, several questions concerning this

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subject remain controversial and have become a motive for clinical studies [15-17]. In routine clinical practice, clean-catch midstream specimen is the best non-invasive method of urine collection. Catheterization or suprapubic bladder aspiration is rarely used because of their invasiveness. As with any type of laboratory specimen, there are certain criteria that need to be met for proper collection of urine that should be collected and processed with as little contamination as possible. Technically satisfactory collection of urine from women especially in the late stages of pregnancy can be difficult [18]. According to the authors’ knowledge, there is no study assessing the frequency of bacteriuria diagnosed with a urine specimen obtained via urinary catheterization before cesarean operation and evaluating its association with antenatal bacteriuria data, and investigating its contribution to infectious morbidity after cesarean delivery. The objectives of this study were to assess the frequency of asymptomatic bacteriuria with urine samples obtained via catheterization among women undergoing cesarean delivery at term pregnancy, after screening for bacteriuria during antenatal period, and to investigate its relationship with UTI during antenatal period and after cesarean delivery.

Materials and Methods

A cross-sectional study was carried out at the antenatal clinic of the present hospital involving 159 women in whom cesarean delivery was performed at term pregnancy between January and October in 2012. Pregnant women receiving all antenatal care beginning in the first trimester in the antenatal clinic according to our Protocol for Antenatal Care were included in this study. The patients were excluded if they had any signs or symptoms of UTI or if they had used antibiotics during the last two weeks on first antenatal visit. Women with underlying renal pathology, abnormality, or obstruction and with a history of chronic renal disease, recurrent urinary tract infection, renal transplant, diabetes, anemia, preterm labor, or taking immunosuppressive therapy were excluded. The authors also excluded women with long duration of surgery (>two hours) due to complications during cesarean delivery or women in whom bladder injury was developed. During study period, selected obstetric and UTI data of 159 pregnant women for their antenatal and postpartum periods were collected from patient records. The study was approved by the Human Ethical Research Committee of the authors’ University.

Screening and management of urinary tract infection

During antenatal follow-up, all the women underwent screening of UTI with analyses of urine at the first and subsequent antenatal visits. Patients were requested to give midstream urine samples in sterile containers after cleaning the urethral entrance after a standard instruction [18]. To minimize the risk of contamination, the women were counseled regarding the method of cleaning the urethral meatus, and then collecting about ten ml of urine as mid-stream (without decreasing the urine flow to start or stop the collection) into a sterile container.

For screening and diagnosis of UTI during antenatal period, the authors used dipstick test and microscopic urinalysis, and urine culture was used in the presence of symptomatic UTI unresponsive to initial antibiotic therapy. In case of asymptomatic and symptomatic UTI, fosfomycin (first-line) or a second-generation cephalosporin (second-line) were administered and dipstick test and microscopic urinalysis were repeated one week later after completion of treatment. Screening was continued for asymptomatic bacteriuria at each subsequent visit after completing antibiotic treatment.

Lower UTI was defined as the following criteria: symptomatic UTI as a clinical picture including frequent urination, pain in the bladder area, dysuria with positive urinary tests, asymptomatic UTI as positive urine tests without any symptoms, and signs of UTI. In all cesarean deliveries before beginning of surgery, the authors used a Foley catheter to empty the bladder for increased space in the pelvic cavity to protect the bladder during surgery and it was removed eight hours later after surgery. A urine sample was obtained immediately after insertion of Foley catheter for urine dipstick test, microscopic urinalysis, and culture.

Urinary analysis

All urine samples were immediately transferred and processed within one hour of collection in the laboratory. For urine dipstick test (SD Urocolor ten reagent strips for urinalysis), the stick was quickly dipped into the urine, waited for 60 seconds, and then read at the correct time interval as specified on the container. It was considered as positive if it was positive for nitrite, or both the dipstick leucocyte esterase and blood, or for all three. In microscopic urinalysis, samples were centrifuged at 2,000 rpm for five minutes, and sediments were examined microscopically to determine the percentage of leukocytes and bacteria. Microscopy was accepted as positive if there were > five leucocytes per high-power field or 15 bacteria per high-power field in centrifuged urine sediment.

During urine culture, samples were inoculated on blood agar and eosin-methylene blue agar in 30 minutes. Samples with 10^5 cfu/ml or more growth at 18–24 hours of incubation were examined macroscopically. Samples with no growth at this time point were re-incubated for another 24 hours. Identification and antimicrobial susceptibility testing was performed in accordance with M2-A9 standards and the Clinical and Laboratory Standards Institute Quality Manual [19]. Clinical data was abstracted from hospital records and presented as median (min-max) or percentage.

Results

During the study period, selected obstetric and UTI data of 159 patients were collected (Table 1). With the setting of this study in all women with cesarean birth at term pregnancy after regular follow-up from at the first to third trimester, there was no perinatal complication or outcome after regular antenatal care related to UTI.

Table 2 shows the results of laboratory work-up including urine dipstick and microscopic urinalysis during the antenatal follow-up of study population. Repeated UTIs were encountered in the first, second, and third trimesters in an increasing order (as second episode in first trimester (3.1%), as second episode in second trimester (6.9%), as second, and third episodes in third trimester (8.2% and 5.0%, respectively). Symptomatic UTI episodes were developed in 9 (5.7%) of the study population and in seven (4.4%) of them, urine culture was performed. Of 159 preg-
nant women, 95 (59.8%) did not develop UTI during antenatal care.

Table 3 presents results of laboratory work-up for asymptomatic UTI at cesarean delivery according to the results of screening for UTI. There was no patient with symptomatic UTI at the admission for cesarean delivery. The authors found UTI with urine dipstick and microscopic urinalysis in 12 patients and of them, four patients had no history of UTI during antenatal period, and all the remaining eight patients had asymptomatic UTI during antenatal follow-up. UTI according to urine culture was encountered in three patients, two of them had one episode of UTI, and one had two episodes of UTI during antenatal follow-up. It was not possible to draw conclusions with statistical analyses about correlation of results of urine dipstick and microscopic urinalysis with urine culture because of small number of patients. There were no postpartum UTI in any of the study subjects.

Discussion

Of 159 pregnant women, 95 (59.8%) did not develop UTI during antenatal care and 64 had asymptomatic or symptomatic UTI. Symptomatic UTI episodes developed in nine (5.7%) of the study population and in seven (4.4%) of them, urine culture was used. There were no patients with symptomatic UTI with the assessment of clinical and laboratory data related to UTI before and within three days after cesarean section. With urine dipstick and microscopic urinalysis, there were 12 patients with asymptomatic UTI and of these patients, four had no history of UTI during antenatal period, and all the remaining eight patients had asymptomatic UTI during antenatal follow-up. UTI was diagnosed with urine culture in three patients, two of them had one episode of UTI, and one had two episodes of UTI during antenatal follow-up. Because of the small number of patients with positive urine culture at cesarean delivery, clinical and UTI data were not statistically analyzed. There were no postpartum UTIs in any of the study subjects. Overall, with followed strategy for the diagnosis and management of UTI during antenatal period, the authors thought that the number of patients with asymptomatic UTI were reduced.
during antenatal period and at cesarean delivery. It was not possible to draw conclusions about correlation of results of urine dipstick and microscopic urinalysis with urine culture because of small number of patients.

After evaluation of perinatal outcome, the authors found no adverse result related to UTI in the antenatal period. Overall, they suggest that screening and management of UTI during antenatal follow up and patient information regarding UTI successfully decrease the rate of UTI before delivery and prevent complications resulting in bad perinatal outcomes. Generally, it is a goal to carry out surgical and anesthetic procedures to improve patient recovery and outcome. Stray-Pedersen et al. [20] assessed the status of postpartum bacteriuria in about 11,000 women by culture of voided midstream urine and detected UTI in about 8% of them. They performed the urine culture again with urine samples obtained by suprapubic aspiration. They could confirm UTI in about half of the patients in whom the first culture was found as positive. Overall, they concluded that confirmed bacteriuria was detected in 3.2% of puerperal women. They found that operative delivery as cesarean section, forceps and vacuum extractor delivery, epidermal anesthesia, and bladder catheterization could be considered risk factors for bacteriuria in the postpartum period.

A clean voided specimen with cleansing of the perineum and urethra is standard [21]. False-positive urine culture results are common due to contamination of the urine sample. The laboratory can suspect the possibility of contamination when there are many epithelial cells, multiple organisms, or bacteria but no leucocytes. However, a study of 100 adolescent pregnant women found perineal cleansing before midstream urine did not decrease bacterial contamination of the urine cultures [22]. During cesarean delivery, the present authors obtained the urine samples from urethral catheterization that was a routine procedure before surgery. Any form of catheterization can also potentially introduce micro-organisms and hence cause UTI in addition to being traumatic procedure. They carried out catheterization procedure in a standard manner to prevent introducing microorganisms to bladder. High number of patients with asymptomatic UTI at first visit may be related to difficulty of obtaining midstream urine samples by women; nevertheless, if the difficulties and complications of invasive urine sampling techniques were considered, midstream urine sampling could be a good option for evaluation of UTI.

The conventional and classical method for the detection of Asymptomatic bacteriuria (ASB) is urine testing with culture [23]. Although urine culture is the gold standard for ASB detection, it may not be applicable to all of the pregnant women when we consider that most of the deliveries occur in non-developed or developing regions of the globe. It is also time-consuming and requires an established microbiology laboratory [24]. Since simplicity and low cost is required for a test to be used as a universal screening tool, some other methods are investigated for the detection of ASB in pregnant women. Urine dipstick tests are easy to perform and give immediate result without a need of a laboratory. Although, the prediction value for ASB of this however has been reported to be conflicting [25], a recent meta-analysis that included studies of pregnant women concluded that negative results could rule out UTIs in asymptomatic pregnant women. On the other hand simple urine test solely is not recommended as a screening tool for ASB in pregnancy since it has low sensitivity [26, 27]. During enhanced urinalysis, uncentrifuged urine was tested with Gram-staining for bacteria and leukocyte counting with hemocytometer [28]. Kaçmaz et al. [29] compared the results of leukocyte esterase and nitrite urine dipstick tests with enhanced urinalysis and urine culture. They concluded that enhanced urinalysis did not provide additional advantage for detecting asymptomatic bacteriuria. In their study, leukocyte esterase, nitrite, and enhanced urinalysis had a sensitivity of 70%, 60%, and 50% with a specificity of more than 92%.

This study has some limitations. First, it was a single-site investigation and these results may not be applicable to other settings. Second, it had a small number of patients with UTI at cesarean delivery, not suitable for statistical analyses. Further studies may be helpful to assess the association of UTIs diagnosed at third trimester with UTIs diagnosed with urine specimens obtained by urine catheter at cesarean delivery. It may also be considered to carry out a further study to assess the frequency of UTIs with urine specimens obtained as mid-stream urine sample or urine sample with a catheter; this may help to understand the efficacy of protocols for urine sampling during pregnancy.

The first clinical implication of the present findings is that the follow-up of pregnant women with urine dipstick and microscopic urinalysis during antenatal period may provide an easy and effective approach to reduce the number of UTI episodes and perinatal morbidities related to UTIs. The addition of fosfomycin or a second-generation cephalosporin for the empirical treatment of UTIs may be considered as a considerably successful therapy protocol. This strategy may have a potential to reduce complications related to UTIs in women undergo cesarean delivery. Physicians need to keep in mind that the presence of bacteria in urine as a sole finding may not necessarily have a clinical value. It can be a result of colonization or contamination, as well as due to infection in the urinary tract. During antenatal care, provided that physicians conformed to a standard protocol for diagnosis and treatment of UTIs in pregnant women during regular follow-up, the episodes of asymptomatic or symptomatic UTIs and their complications may be decreased with an improvement in maternal and perinatal morbidities.

In conclusion, during antenatal follow-up, screening with urine dipstick and microscopic urinalysis and counseling of...
Asymptomatic bacteriuria screened by catheterized samples at pregnancy term in women undergoing cesarean delivery

References


Construction and measuring combination of KDR-targeted ultrasound contrast agent in vitro for evaluating endometrial receptivity

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Summary

Objective: To investigate the preparation of a new kind of targeted lipid ultrasound contrast agent with anti-KDR antibody based on biotin-avidin bridge (MB-BAB-KDR) which could combine specifically with KDR that increases during the time of embryo implantation. Then its binding capability in vitro was evaluated. Materials and Methods: The agitation of high-speed method was employed to prepare biotin-microbubbles (MB-B), and biotin–avidin mediated technique was used to produce MB-BAB-KDR. MB-BAB-KDR, MB-B, and biotin-microbubbles-KDR (MB-B-KDR) were incubated with human umbilical vein endothelial cell (HUVEC). Rosette formation rate was observed and calculated. Then, the parallel plate flow chamber technology was used to access attachment efficiency to KDR Fc. Results: The surface of bubbles could carry KDR antibody through “biotin-avidin” bridge. After incubated with second antibody, bright green fluorescence (II grade) could be observed in MB-BAB-KDR group, as compared with weak fluorescence in control groups of MB-B (0 grade) and MB-B-KDR (I grade). The surrounding rosette formation rate on HUVEC was 89.86% in MB-BAB-KDR group and that of control groups were 7.13% (MB-B-KDR) and 3.02% (MB-B) (p < 0.05). The number of MB-BAB-KDR bound to KDR Fc increased as the KDR Fc density increased (p < 0.05). Under the same concentration, the MB-BAB-KDR bound to KDR Fc increased as time extended. Conclusion: The successful preparation of MB-BAB-KDR with anti-KDR antibody which shows specially targeting binding capability with HUVEC and stability in shear stress may be served as a noninvasive detection of endometrial vascular KDR expression and provide an experimental foundation for evaluating endometrial receptivity in vivo.

Key words: Targeted contrast; KDR; HUVEC; Parallel plate flow chamber; Endometrial receptivity.

Introduction

The human endometrium undergoes a complex series of proliferative and secretory changes in each menstrual cycle and displays only a short period of receptivity, known as the “window of implantation”, necessary for the implantation of the blastocyst in uterus [1]. About 60% of failures in the process of in vitro fertilization-embryo transfer (IVF-ET) are due to defected endometrial receptivity [2]. Recent evidences show that the increasing vascular endothelial growth factor (VEGF) and its receptor-KDR, which can improve the vascular angiogenesis, remodeling, dilatation and permeability, as well as conduct the signal indication, are necessary in the successful embryo implantation [3-5]. However, detecting the expression level of VEGF and KDR to assess the endometrial receptivity, nowadays usually depends on the endometrial biopsy. Because of its invasiveness, it is suboptimal in clinical practice. In recent years, targeted contrast-enhanced ultrasound imaging, also named molecular ultrasound imaging has emerged as a promising new non-invasive imaging strategy for imaging biological processes at molecular level [6]. KDR is an ideal target for molecular imaging because of its overexpression and upregulation at the stage of the “window of implantation”. In this study, the authors prepared the perfluorocarbon-filled lipid target microbubbles-biotin-avidin-biotin-KDR (MB-BAB-KDR), based on the biotin-avidin bridge, which could generate multilevel biological amplification to increase the KDR consistency around bubbles. Then WE used human umbilical vein endothelial cell (HUVEC) and the parallel plate flow chamber system [7] to characterize microbubbles special attachment efficiency to KDR Fc.

Materials and Methods

Ordinary microbubbles-biotin (MB-B) preparation

DSPE-PEG2000-Biotin, DPPC, F68 and so on were mixed into distilled water and dissolved fully in the constant temperature (75°C) water bath and were shaken well with a determinate ratio [8]. The agitation of high-speed method was employed to prepare...
the perfluorocarbon-filled lipid microbubbles with biotin (MB-B) with piping into the whole fluorine propane (C3F8).

**Construction of microbubbles carrying KDR**

After being washed to remove excess free unincorporated lipid, streptavidin in a determining ratio were added to MB-B. Then they were washed to remove excess free unincorporated streptavidin and the biotin conjugated rat anti-mouse-KDR monoclonal antibodies were added to prepare MB-BAB-KDR. At last, the MB-BAB-KDR was washed to remove excess free unincorporated antibodies. Biotin-microbubbles-KDR (MB-B-KDR) was made only by adding KDR directly into MB-B without streptavidin. Prepared MB-B, MB-B-KDR and MB-BAB-KDR, they were stored in refrigerator at 4°C.

**Detection of property of microbubbles**

The three types of microbubbles were observed under light microscope. The mean diameter and density in all MB-B, MB-B-KDR, and MB-BAB-KDR were measured by Coulter particle counting instrument.

**Determination of biological activity of microbubbles**

Fluorescein-conjugated affiniPure goat anti-rat IgG (H+L) was considered as the second antibody. MB-BAB-KDR was incubated with the second antibody to assess linked condition. With the MB-B and MB-B-KDR as control groups, all the three microbubbles were observed under fluorescence microscope after washing excess free second antibody. Fluorescence degree was observed under a fluorescence microscope. A fluorescent-based classification method was made on fluorescence degree. The fluorescence intensity of very bright, little bright, and not bright were defined as II, I, and 0 grade, respectively.

**Detection of KDR expression of HUVEC**

HUVEC in logarithmic growth phase were incubated with 200 µl biotin-KDR monoclonal antibody for 30 minutes at 4°C. Then they were washed three times and incubated with fluorescein-conjugated affiniPure goat anti-rat IgG (H+L) for 30 minutes. After being washed three times and added with 500 µl distilled water, they were analyzed with a flow cytometer, with the same amount of suspended HUVEC as in the control group called NC group.

**Evaluation of specific binding capability of microbubbles**

MB-BAB-KDR, MB-B-KDR, and MB-B labeled with FITC were incubated with HUVEC which were labeled by DiI for ten minutes. The excess free unincorporated microbubbles were washed away and rosette formation rate was observed and calculated under a light microscope and fluorescence microscope with 20 power field.

**Flow-chamber adhesion**

Thirty-five-mm Petri dishes were air-dried after methanol rinsing and were incubated with 200 µl KDR Fc with different concentrations (10 ng/ml, 100 ng/ml, 200 ng/ml, 600 ng/ml, 1000 ng/ml) in PBS all night under the condition of 4°C. The blocked group and the 0 ng/ml KDR Fc group were control groups. After being washed six times with 0.05% Iwain 20, they were closed by 3% of TBS-calf serum albumin liquid. Petri dishes were mounted on a parallel plate flow chamber with controlled gasket thickness and channel width. The flow chamber was placed in an inverted position on a microscope with a high-resolution charge-coupled device camera for video recording. A suspension of MB-BAB-KDR (3.0×10^6/ml) was drawn through the flow chamber with an adjustable withdrawal pump. The number of microbubbles attached to KDR Fc was determined after six minutes of continuous flow at rates of 1.2 dyn/cm². Each flow chamber was imaged with a microscope. Sequences of images were sampled to collect the binding condition.

**Statistical analysis**

All the data were expressed as mean ± SD. K independent samples test was applied to compare the rosette formation rate among groups, ANOVA for repeated measures was applied to compare adhesion situation among groups, which were performed by the software SPSS 13.0. All $p < 0.05$ were considered as statistically significant.

**Results**

**Properties of microbubbles**

Microbubbles were observed under the microscope. Coulter particle counting instrument showed that the size of MB-B, MB-B-KDR, and MB-BAB-KDR, mainly 2.19±1.35 µm, 2.38±1.60 µm, and 2.42±1.71 µm. The density was about 9.8×10^9/ml, 9.6×10^9/ml, and 9.6×10^9/ml. There was no difference after the authors added biotin-avidin bridge and biotin-KDR antibody. All of three types of microbubbles were spherical and well-distributed.
Measurement of the biological combination

Bright green fluorescence (II grade) could be observed in MB-BAB-KDR incubated with second antibody, as compared with weak fluorescence in control groups (MB-B: 0 grade, MB-B-KDR: I grade) (Figure 1).

KDR expression

Control cell background fluorescence was NC group, the experimental group was HUVEC immune staining for anti-KDR, FITC HUVEC carrying rate was 99%, which expressed exoressin of KDR in HUVEC in the high status (Figure 2).

Specific binding capability

MB-BAB-KDR combined around or on the HUVEC and formed the wreath sample structure. The surrounding rosette formation rate on vascular endothelial cells was 89.86% (Figure 3), which were higher than the control groups: 7.13% (MB-B-KDR) and 3.02% (MB-B). There was a significant difference between MB-BAB-KDR group and the control groups, \( p < 0.05 \).

Adhesion studies

Shown in the flow shear stress of 1.2 dyn/cm², the MB-BAB-KDR could combine to KDR Fc with a concentration of 1000 ng/ml, 600 ng/ml, 200 ng/ml, 100 ng/ml, and ten ng/ml groups. The recorded video was disposed with image pro-plus (IPP) software (Figure 4). Black hollows were stabilized adhesion MB-BAB-KDR and the trajectory of rolling were the mobile bubbles. MB-BAB-KDR combined with different concentration KDR Fc package and showed that adherence of MB-BAB-KDR increased with increasing KDR Fc density (\( F = 571.926, p < 0.01 \)). Observed within six minutes the number of MB-BAB-KDR bound to KDR Fc increased as the time going. MB-BAB-KDR hardly combined with the blocked control microbubble group and the 0 ng/ml group. Throughout the six-minute period, there were only three to five totally combined on the flow chamber Petri dish and in different places and different time points, namely the combination of random and could not resist the shear force (Figure 5).
Discussion

Targeted ultrasound imaging is an emerging research field of the burgeoning in recent years, and gradually becomes a hot topic in the world. Targeted contrast agent of microbubble carries the specific antibody or ligand which can combine with antigen or receptor on cell surface specially [9]. Ultrasonic testing targeted contrast agent, which distributes in tissues and organs, can obtain the molecular characteristics, so it is referred to as “ultrasonic molecular imaging” [10]. Along with the in-depth study of vascular endothelial cell function, recent studies have shown that ultrasonic molecular imaging research mainly focuses on the specific molecule expressed in the endothelial cells. Local echo is strengthened consecutively and succeeds in targeting imaging through the combination or aggregation of the ligand-carrying contrast agents with the specific molecular targets on the surface of endothelium. It shows great prospects in the research fields of cancer, atherosclerosis, thrombus, inflammation, and so on [11-14]. Some researchers have achieved good ultrasonic imaging on the new-born vascular region of tumors on mice by targeting KDR, the receptor of VEGF [11, 12].

Studies have found that the combination of VEGF and KDR exerts many biological functions on embryo implantation, such as vascular endothelial cells proliferation, angiogenesis, vascular permeability, signal transduction, changes of gene expression in endothelial cells, regulation of endometrial development, and so on [15-19], especially allocated in epithelial cell, stroma cell, myometrium, and vascular endothelial cells [20, 21]. Experiments showed that there was a significantly decrease in the pregnancy rate of the Rhesus monkey after administered VEGF antagonists five to ten days after ovulation [22]. At present, detecting the VEGF and KDR level in the endometrium depends on biopsy, which is not practical in the IVF-ET cycle because of its invasiveness.

It will be an simple and non-invasive way to access the endometrial receptivity relative factors-VEGF and KDR by using the targeted ultrasonic imaging technology, which takes the KDR of the endometrial vascular epithelial cell as targets. If quantitative analysis of the expression of KDR in the endometrium during the IVF-ET cycle is successful, it will avoid blind embryo transfers and increase the successful implantation rate. Meanwhile, it will reduce the economic and spiritual pressure of the patients.

In this study, biotin–avidin-mediated technique was used for the attachment of anti-mouse KDR monoclonal antibody to produce MB-BAB-KDR successfully. There was little effect on the consistency of microbubbles after the authors added biotin-avidin bridge and biotin-KDR antibody. Coulter Particle Counting instrument showed that the size and the density of MB-BAB-KDR were well-distributed; about 94.1% were smaller than five μm in diameter. After incubation with second antibody, the fluorescence intensity of MB-BAB-KDR group was brighter than MB-B-KDR and MB-B groups, which signified that biotin-avidin bridge mode not only could build a targeted microbubble successfully, but also could maintain the higher biological activity. In the in vitro experiment which tested the ability of targeting combination, MB-BAB-KDR could specifically combine with the HUVEC which with positive KDR expression and form the typical abundant garlands samples. That indicated again that MB-BAB-KDR can remain the activity and has the character of specific combination. Parallel Plate flow chamber is one of the important techniques to study the cellular mechanical behavior (especially the cell adhesion) in vitro, and its use of mimicking the blood flow situation can almost takes the place of in vivo experiments. This study applied the fluid mechanics calculation method to test the stability of the cell adhesion, under the condition of same velocity, which means in the same shear stress in Parallel Plate flow chamber. The human body microvessels and capillary blood flow shear stress is very small, generally less than 1.0 dyn/cm². The present experiment adopted the shear stress as 1.2 dyn/cm² and MB-BAB-
KDR stable adhesion was observed on the KDR Fc. With the increase of the concentration of antigen, targeted adhesion showed increasing trend, which makes it possible for microcirculation targeted molecular imaging of endometrium.

The present study constructed the targeted liposome microbubble MB-BAB-KDR successfully, tested the basic physical properties, proved its biological activity, targeting specific binding ability, and stability of adhesion in vitro. The study laid a foundation for a noninvasive and half-quantitative detection of KDR of endometrial endothelial cells and made an effect on promoting the targeted molecular imaging of other endometrial receptivity relating cytokines. All these achievements provided a technical support to further evaluate the targeted therapy and its effect, based on ultrasound mediating drug or gene which would improve the endometrial receptivity.

Acknowledgement

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References

Assessment of quantitative and qualitative changes of proteoglycans and glycosaminoglycans in normal breast tissue during the follicular and luteal phases of the menstrual cycle

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Summary
The effect of sex hormones on extracellular matrix compounds, such as proteoglycans (PGs) and glycosaminoglycans (GAGs), in mammary tissue remains poorly understood. The elucidation of extracellular matrix component functions could clarify pathophysiological conditions, such as cyclical mastalgia (breast pain). The authors examined the quantitative and qualitative changes of PGs and GAGs in normal breast tissue during the follicular and luteal phases of the menstrual cycle. Twenty-eight eumenorrheic patients with benign breast nodules were divided into groups: Group A included 15 follicular patients and Group B included 13 luteal phase patients. Breast tissue adjacent to the nodules was biochemically analyzed to evaluate the types and concentrations of PGs and GAGs. The distribution of proteoglycans during the menstrual cycle was analyzed with immunofluorescence. PG concentrations were elevated ($p < 0.01$) during the luteal phase compared with the follicular phase, whereas the concentrations of GAGs did not differ significantly. Immunofluorescence revealed that decorin was mainly found in the intralobular stroma. PG concentrations were elevated during the luteal phase, likely due to the influence of sex hormones on macromolecular synthesis. The PG decorin was observed in normal breast tissue in the intralobular stroma. Although the concentration of GAGs, including dermatan and heparan sulfate, varied cyclically, the differences were not significant.

Key words: Breast Tissue; Proteoglycans; Glycosaminoglycans; Menstrual Cycle.

Introduction
During the menstrual cycle, both the breast epithelium and stroma undergo morphologic, biochemical, and functional changes under the influence of sex hormones. Both tissues are more active during the luteal phase of the menstrual cycle [1-6]. Interlobular edema and ductal and acinar proliferation in this phase of the cycle are responsible for the increase in breast volume related to cyclical mastalgia (breast pain). The cyclical changes at the cellular level in the breast are associated with hormonal changes during the follicular and luteal phases of the menstrual cycle and are largely the result of changes in the extracellular matrix [7].

The extracellular matrix is a complex structure that surrounds and supports the cells to maintain an organized tissue structure. The extracellular matrix is closely related to cell growth, movement, and differentiation, and it controls the shape and function of tissues through receptors in cell surface [8]. Among several components of the extracellular matrix, proteoglycans (PGs), and glycosaminoglycans (GAGs) form a hydrophilic semi-fluid gel that allows the circulation of nutrients, hormones, and several chemical messengers [9]. Proteoglycans are macromolecules composed of a protein axis to which one or more GAG chains are covalently bound. Due to the presence of its sulfate and carboxyl groups, GAGs possess many negative charges that determine their functional properties, dictating the selective permeability of the basal membrane and the hydration of the extracellular matrix [10,11]. The objective of the study was to assess the PGs and GAGs in the extracellular matrix of breast tissue in different phases of the menstrual cycle.

Materials and Methods
Selection of patients, tissue samples, metabolic labeling of PGs with $^{35}$S-sulfate, and extraction of PGs and GAGs
Thirty-one eumenorrheic women between the ages of 15 and 35 years were prospectively selected for the study. The participants had not used any hormonal contraceptives during the past six months and exhibited benign breast nodules confirmed by fine needle aspiration. Patients with endocrinopathies and those who were pregnant were excluded from the study.

During the surgical procedure to remove the nodules, fragments of adjacent breast tissue were removed for the study. The regularity of both the breast tissue and nodules were confirmed by histopathological testing. Two patients were excluded due to histopathological changes, and one patient was excluded due to difficulty in characterizing the menstrual cycle phase of the patient. Therefore, 28 patients were divided into two groups according their menstrual cycle phase. Dates of the previous period,
subsequent period, and serum dosage of progesterone determined the cycle phase. Levels of serum progesterone equal or higher than three ng/ml defined the luteal phase [12]. Group A, which had 15 patients, represented samples collected during the follicular phase, and group B, with 13 patients, represented samples collected during the luteal phase.

Samples of regular breast tissue from follicular and luteal phases of the menstrual cycle obtained immediately after surgery were washed with five ml of PBS containing gentamicin (four mg/ml) and subsequently placed in culture bottles containing ten ml of F12 culture medium with no fetal bovine serum in the presence of 35S-sulfate (50 µCi/ml of medium), 100 µl of penicillin (10,000 U) and streptomycin (100 mg) antibiotic solution. The tissues were maintained in primary culture for 24 hours at 37°C in a CO₂ incubator. After this period, the medium was removed, and two volumes of methanol were added under agitation and maintained for 18 hours at -20°C. The resulting precipitate was collected by centrifugation (1,300 X g, 15 minutes), dried, and resuspended in one ml of distilled water for further analysis. Proteoglycans and glycosaminoglycans were extracted from two pieces of removed tissue.

Characterization of PGs and GAGs

The extracted PGs and GAGs (labeled or not with 35S-sulfate) were characterized by a combination of agarose gel electrophoresis, polyacrylamide gel electrophoresis, immunoblotting, and degradation with specific mucopolysaccharidases.

PGs and GAGs were analyzed by agarose gel electrophoresis in a 1,3-diaminopropane acetate buffer 0.05 M, pH 9.0 (PDA), developed by Jaques et al. [13] and modified by Dietrich and Dietrich [14]. Enzyme degradation with specific mucopolysaccharidases (condroitinases AC, B) [15, 16] and heparitinase II [17] from Flavobacterium heparinum were performed according to established methods [18, 19].

Quantification of 35S-sulfate labeled PGs and GAGs

Quantification of the radioactive compounds was performed by radioactivity count with scintillation cocktail. The electrophoresis gel bands in agarose or the radioactive chromatograms, identified by radioautogram, were removed, immersed in the scintillation cocktail, and radioactivity was quantified in the scintillation counter. Radioactivity in each sample was expressed as counts per minute (cpm).

Immunofluorescence

To understand tissue architectural changes, it is necessary to understand the location, distribution, and interaction with other extracellular matrix components. Thus, to analyze the distribution of PGs, the authors used immunofluorescence with anti-decorin and anti-versican monoclonal antibodies.

Statistical analysis

Student’s t-test (software JMP 8 Statistical Discovery) was used to analyze the variables, with a level of significance of 5% (p < 0.05).

Results

Patients were between 16 and 35 years of age. The average age of patients in group A (first phase of the cycle) was 22.27 ± 1.35 years of age, and the average age in group B (second phase of the cycle) was 24.69 ± 1.63 years of age, demonstrating homogeneity between the groups. To evaluate PGs and GAGs in normal breast tissue, PGs and GAGs were extracted at greater than 90% yield, which was possible in only 12 cases due to the lack of material obtained for analysis without use of metabolic labeling. The quantification of PGs and GAGs was performed by slide densitometry. The average PG concentration in the follicular phase was 27.2 (± 3.6) µg/g of dry tissue and 43.6 (± 3.7) µg/g of dry tissue in the luteal phase. The average concentration of GAG in the first phase of the cycle was 1.2 (± 0.3) µg/g of dry tissue and 3.2 (± 0.9) µg/g of dry tissue in the second phase of the menstrual cycle. Student’s t-test demonstrated that the differences observed between concentrations of PGs in normal breast tissue in follicular and luteal phases were statistically significant (p < 0.01). PG concentrations exhibited a mild initial increase during the proliferative phase, a decrease around the 14th day, a new increase during the luteal phase, and a decrease at the end of the menstrual cycle (Figure 1).

The GAGs identified by agarose gel electrophoresis included dermatan sulfate and heparan sulfate. Immunoblotting identified the PG decorin. Figure 2 shows the immunolocalization of decorin (green) and versican (red) in normal breast tissue during the follicular and luteal phases. The cells nuclei are marked in blue. Only decorin staining was positive in both phases of the menstrual cycle, with higher concentrations in the breast lobes. Specifically, concentrations were highest in the intralobular stroma, where there is higher concentration of cells, and concentrations were lowest in the interlobular stroma.

Discussion

Several studies in literature consistently describe parallel changes in the menstrual cycle and breast lobular kinetics, reporting relative inactivity during the follicular phase and more intense cell proliferation during the luteal
Figure 2. — Immunofluorescence microscopy of the PGs decorin and versican from normal breast tissue in the proliferative and luteal phases of the menstrual cycle. (A) DAPI (nucleus); (B) decorin; (C) versican; (D) image superposition. Scale bar: five μm.

Figure 3. — Proposed explanatory model for the origination of cyclical mastalgia (breast pain).
phase. Proliferative activity reaches its peak around the 25th day of the cycle, simultaneous with the progesterone peak and the second increase in estrogen [1-4]. Thus, changes in the proliferative activity of the breast lobe correspond perfectly to the hormonal curve of estradiol and progesterone.

Few studies in the literature describe the effect of sex hormones on the extracellular matrix of female breast tissue, and the understanding and importance of extracellular matrix changes has only recently been appreciated.

Some animal studies report a possible synergistic effect between sex hormones and GAG synthesis [20]. Further, the breast stroma rich in extracellular matrix expresses hormonal receptors. These findings have motivated the present authors to study the effect of female sex hormones on the synthesis of PGs and GAGs in female breast tissue. Moreover, the pathophysiology of mastalgia, a frequent complaint in the clinical practice, is likely affected by its relationship with hormonal changes during the menstrual cycle, although the relationship remains to be fully elucidated.

Data obtained from the present study demonstrate a significant increase in the concentration of the PG decorin in the intralobular stroma during the luteal phase. The GAGs dermatan and heparan sulfate were also observed and tended to be elevated during the second phase of the cycle. Thus, sex hormones may influence PGs and GAGs, given that their concentrations and rate of synthesis change during the menstrual cycle.

The GAGs present in PGs exhibit a large anionic charge due to their sulfate and carboxyl groups. Therefore, these molecules attract a cloud of cations, mainly sodium. By osmosis, water is brought into the extracellular matrix, forming a gel. This mechanism could explain the common breast edema in the pre-menstrual phase and the consequent breast pain during this period.

The prevalence of breast pain is variable, according to different studies, and affects approximately 70% of women [21]. In less severe cases, the initial clinical approach involves verbal instructions to differentiate this pain from that of breast cancer, and the problem is resolved in approximately 85% of cases [22].

Drug treatment is only used in severe and incapacitating cases of breast pain. The literature reports a large number of drug treatment options, and placebo treatment itself exhibits a response rate of 19% in clinical trials [23]. Tamoxifen and danazol are highly effective, but their numerous side effects lead to low treatment compliance.

The present study proposes an explanatory model for cyclical mastalgia pathophysiology, as shown in the algorithm in Figure 3. Furthermore, these results may initiate a line of research to identify effective drugs to treat mastalgia, including dinoprostone (prostaglandin E2) and misoprostol (synthetic prostaglandin E1 analog), which exhibit proven activity on the extracellular matrix of other sites, such as the cervix [24-30].

References


Assessment of quantitative and qualitative changes of proteoglycans and glycosaminoglycans in normal breast tissue during the follicular etc.


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Can we predict postpartum depression in pregnant women?

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Summary

Objective: The authors aimed to determine risk factors for postpartum depression (PPD) in this prospective study. Materials and Methods: The study included 285 pregnant women. The first assessment was conducted during pregnancy and a second time at 24 weeks after delivery. The participants were asked to fill out a series of questionnaires, which included psychosocial variables and socio-demographic characteristics, the Beck Depression Inventory (BDI), and Edinburgh Postnatal Depression Scale (EPDS). After delivery, 276 mothers participated again in the study and filled out a similar series of questionnaires. Results: A significant difference was not found between the socio-demographic and obstetric factors of mothers. A significant relation was found between the BDI score, which is used in antepartum depression evaluation, and EPDS score, which is used on postpartum depression evaluation (rho: 0.433 to 0.645, p < 0.0001). In cases in which BDI score was more than 6 were selected, phenomena in which could develop PPD had 90.3% sensitivity and 45.3% specificity. Conclusions: The authors found that there was a correlation between EPDS score and BDI. The rate of PPD was found to be significantly higher in women, who had a depression history. Patients who have a potential risk of PPD should be evaluated during the postpartum period in terms of depression.

Key words: Postpartum depression; Pregnancy; Beck Depression Inventory; Edinburgh Postnatal Depression Scale.

Introduction

Postpartum depression (PPD) is a significant public health issue, which is considered to be affecting about 10-15% of women across the world [1-5]. Findings related to PPD can be observed two weeks after childbirth or within a year; most medical staff do not recognize this condition or relevant symptoms do not suggest PPD [6-8].

PPD’s rate of incidence ranges between 3.5% and 40% according to geographical and cultural characteristics of the region, where the study was conducted [9-16]. Symptoms of PPD can be mistaken for maternity blues, which affects 60-70% of mothers and it can be quite difficult to distinguish such symptoms. On the other hand, symptoms of PPD can be somatic complaints, extreme fatigue, weeping, lack of appetite, feeling of guilt, hopelessness, and under more serious conditions, self-rejection and suicidal ideation and attempts. This condition affects family, particularly and significantly the relationship between mother and child [17-19].

It is suggested that various factors play a role in the development of PPD. One of these is hormonal changes. Social, psychological and physical changes also play a role in the development of PPD [20-22].

Pregnancy and postpartum period are the most important and happiest periods of a woman’s life; however, since responsibility of being a mother on the one hand, and emotions related to this role on the other, may disrupt the woman’s mental balance, depression is a condition, which must be taken into account during pregnancy monitoring and postpartum controls. Therefore, the authors aimed to conduct this study in order to determine the risk factors, which might play a role in the frequency and development of PPD.

Materials and Methods

The study groups were conducted in 285 pregnant women attending Bezmialem Medical Faculty Gynecology and Obstetrics Clinic. Three forms were used in order to collect data required for the study. The first form is a document prepared for determining socio-demographic characteristics of mothers who were included to the study, the second form was the Beck Depression Inventory (BDI), which is developed by Beck et al. and adapted to Turkish, the third form was the Edinburgh Postnatal Depression Scale (EPDS), a postpartum depression risk determining document, developed by Cox et al., and adapted to Turkish by Ergin-deniz et al. [23-25]. After the women signed informed consent forms submitted to them by the researcher, the survey was filled in by researchers and the BDI and EPDS forms were filled in by the participants.

First, BDI was implemented in 285 pregnant women, who applied to Bezmialem Medical Faculty Gynecology and Obstetrics Clinic. Nine pregnant women, whose Turkish cut-off score was over 17, were considered to be depressive, excluded from the study, and directed to Psychiatry Polyclinic. Remaining 276 pregnant women were given demographic survey and an interview was planned for the 24th week after childbirth. Ten women, who developed complications during and after the birth, were excluded from the study. Researchers could not reach 96 of 266 women, who were included in the study or they did not accept
to attend these meetings; 170 women in the postpartum period, who were invited to controls in the 24th week, filled in the EPDS forms. According to the score valid for studies conducted in Turkey, those whose score were 13 and over were considered to be depressive. After the evaluation of forms, mothers, who were considered to be at risk (those achieving 13 and over from EPDS), were informed about their condition, and directed to apply to psychiatry department.

Mothers were divided into two, namely those who had the possibility of being diagnosed with depression, and those who did not, according to EPDS score. Chi square was used for categorical variables in the comparison between two groups. Student-t test was used for numeric variables. Pearson’s correlation analysis was used to determine independent factors related to PPD. Estimated value for PPD of Beck score was calculated by using ROC curve.

Results

The study was conducted in 170 cases, who applied to Bezmialem Medical Faculty’s Outpatient Polyclinics of Gynecology and Obstetrics Clinics. Socio-demographic data of mothers, who participated in the study is shown in Table 1. A significant difference was not found between the socio-demographic characteristics of mothers, who participated in the study (age, duration of marriage, working status, number of living children, economic status, parity, abortion history, education status, delivery method, and smoking). When EPDS cut-off score was assumed as ≥ 13, while postpartum depression was not observed in 81.7% of mothers (n = 139) in the 24th week; incidence rate of depression was 18.3% (n = 31) (Table 2). However, rate of PPD was found to be significantly higher in women, who had a history of depression.

A significant relation was found between the BDI score, which was used in antepartum depression evaluation, and EPDS score, which was used on postpartum depression evaluation (rho: 0.433 to 0.645, \( p < 0.0001 \)) (Figure 1). When cases in which Beck score was more than 6 were selected phenomena, which could develop PPD had a 90.3% sensitivity and 45.3% specificity (Figure 2).

Discussion

PPD is risky not only for the mother, but also for the child, and it can be ignored even by healthcare staff [26]. Although pregnancy and childbirth are normal physiologic phenomena, they may negatively effect maternal health [27]. Postpartum period is a sensitive period, in which risks of psychiatric diseases increase for women [28]. While many women can adapt themselves to developing physiologic, psychological, and social changes related to pregnancy and childbirth, some women may develop mental disorders at different levels. In this study, pregnant women were evaluated during their postpartum periods and incidence rate of PPD was found to be 18.3%. According to various studies conducted, this rate is higher compared to the rate of PPD for women who are living in Western countries, and similar to the rate of postpartum depression for women living in Latin America countries. [29, 30]

When the literature is analyzed, it is observed that clinic depression can develop in 13% of women after delivery. [11]. This rate increases to 26% for adolescent mothers, to 38.2% for women with low income, who give birth for the first time. [31, 32] However, a relationship was not found between parity, economic status, and PPD in some studies. [33]. In the present study, a relationship between level of income and parity was found.

When PPD’s relation with the education status of mothers was analyzed, studies with different findings are available. In some studies, it was concluded that a low level of education can be one of the significant risk factors [34, 35]. On the other hand, there are also studies, which could not

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Table 1. — Socio-demographic data of mothers.

<table>
<thead>
<tr>
<th>Maternal age (mean ± SD)</th>
<th>28.8 ± 5.6 (min=17, max=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of marriage</td>
<td></td>
</tr>
<tr>
<td>Shorter than 1 year</td>
<td>27 (15.8%)</td>
</tr>
<tr>
<td>Between 1-5 years</td>
<td>53 (31.2%)</td>
</tr>
<tr>
<td>Between 5-10 years</td>
<td>45 (26.5%)</td>
</tr>
<tr>
<td>Longer than 10 years</td>
<td>45 (26.5%)</td>
</tr>
<tr>
<td>Working status</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (15.3%)</td>
</tr>
<tr>
<td>No</td>
<td>144 (84.7%)</td>
</tr>
<tr>
<td>Number of living children (mean ± SD)</td>
<td>0.9 ± 0.9 (min=0, max=5)</td>
</tr>
<tr>
<td>Economic status</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>110 (67.7%)</td>
</tr>
<tr>
<td>Medium</td>
<td>58 (34.1%)</td>
</tr>
<tr>
<td>High</td>
<td>2 (1.2%)</td>
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<tr>
<td>Parity</td>
<td></td>
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<tr>
<td>Primiparous</td>
<td>59 (34.7%)</td>
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<td>Multiparous</td>
<td>111 (65.3%)</td>
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<td>Planned pregnancy</td>
<td>156 (91.7%)</td>
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<tr>
<td>Unplanned pregnancy</td>
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<td>Education status</td>
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<td>Primary school</td>
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<td>High school</td>
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<tr>
<td>University</td>
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<td>88 (51.8%)</td>
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<tr>
<td>Operative delivery</td>
<td>82 (48.2%)</td>
</tr>
<tr>
<td>Smoking</td>
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</tr>
<tr>
<td>Yes</td>
<td>22 (12.9%)</td>
</tr>
<tr>
<td>No</td>
<td>148 (87.1%)</td>
</tr>
<tr>
<td>Depression history</td>
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<tr>
<td>Yes</td>
<td>15 (8.8%)</td>
</tr>
<tr>
<td>No</td>
<td>155 (91.2%)</td>
</tr>
<tr>
<td>Residence</td>
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<tr>
<td>Rent</td>
<td>77 (45.3%)</td>
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<td>Own property</td>
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<tr>
<td>Domestic violence</td>
<td>0</td>
</tr>
<tr>
<td>Hb level</td>
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<tr>
<td>Antepartum</td>
<td>11.3±1.2 (min=9.1, max=14.1)</td>
</tr>
<tr>
<td>Postpartum</td>
<td>11.8±1.1 (min=9, max=14.5)</td>
</tr>
</tbody>
</table>
Can we predict postpartum depression in pregnant women?

Table 2. — Comparison of the data of the groups with or without postpartum depression.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>EPDS ≥ 13 - n (%)</th>
<th>EPDS &lt; 13 - n (%)</th>
<th>Chi square</th>
<th>Odds ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD)</td>
<td>28.6 ± 5.8 (19-40)</td>
<td>29.2 ± 5.2 (17-40)</td>
<td></td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td>Duration of marriage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shorter than 1 year</td>
<td>4 (12.9%)</td>
<td>22 (16%)</td>
<td></td>
<td>OR: 0.78</td>
<td>0.87</td>
</tr>
<tr>
<td>Between 1-5 years</td>
<td>9 (29.1%)</td>
<td>44 (32%)</td>
<td>p = 0.064</td>
<td>OR: 0.68</td>
<td>0.91</td>
</tr>
<tr>
<td>Between 5-10 years</td>
<td>10 (32.2%)</td>
<td>35 (25%)</td>
<td></td>
<td>p &lt; 1 year/other</td>
<td>0.55</td>
</tr>
<tr>
<td>Longer than 10 years</td>
<td>8 (25.8%)</td>
<td>38 (27%)</td>
<td></td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td>Working status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (13%)</td>
<td>22 (15.8%)</td>
<td>OR: 1.26</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27 (87%)</td>
<td>117 (84.2%)</td>
<td>p = 0.68</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Number of living children (mean ± SD)</td>
<td>1.06 ± 1.06</td>
<td>0.94 ± 0.87</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>21 (67.7%)</td>
<td>89 (64%)</td>
<td>OR: 1.17</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>9 (29%)</td>
<td>49 (35.2%)</td>
<td>p = 0.42</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1 (3.3%)</td>
<td>1 (0.8%)</td>
<td>Low / medium + high</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>9 (29%)</td>
<td>50 (36%)</td>
<td>OR: 0.72</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>22 (70.1%)</td>
<td>89 (64%)</td>
<td>p = 0.46</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (19%)</td>
<td>35 (25.5%)</td>
<td>OR: 0.71</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25 (80.5%)</td>
<td>104 (74.6%)</td>
<td>p = 0.49</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Planned pregnancy</td>
<td>26 (83.7%)</td>
<td>130 (93.5%)</td>
<td>OR: 0.36</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Unplanned pregnancy</td>
<td>5 (16.2%)</td>
<td>9 (6.5%)</td>
<td>p = 0.08</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Education status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>16 (51.6%)</td>
<td>61 (43.8%)</td>
<td>OR: 1.36</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>6 (19%)</td>
<td>40 (28.8%)</td>
<td>p = 0.43</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>6 (19%)</td>
<td>29 (20.9%)</td>
<td>Primary school/other</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>3 (9.8%)</td>
<td>9 (6.5%)</td>
<td></td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Delivery method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal delivery</td>
<td>16 (51.6%)</td>
<td>81 (58.3%)</td>
<td>OR: 0.76</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Operative delivery</td>
<td>15 (48.4%)</td>
<td>58 (41.7%)</td>
<td>p = 0.49</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (6.5%)</td>
<td>20 (14.4%)</td>
<td>OR: 0.41</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29 (93.5%)</td>
<td>119 (85.6%)</td>
<td>p = 0.24</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Depression history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (19.4%)</td>
<td>9 (6.5%)</td>
<td>OR: 3.4</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25 (80.6%)</td>
<td>130 (93.5%)</td>
<td>p = 0.029</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rent</td>
<td>11 (35.5%)</td>
<td>66 (47.5%)</td>
<td>OR: 0.6</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Own property</td>
<td>20 (64.5%)</td>
<td>73 (52.5%)</td>
<td>p = 0.22</td>
<td>0.31</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. — A significant relation was found between the Beck Postpartum analysis and EPDS score in postpartum depression (rho: 0.433 to 0.645, p < 0.0001).

Figure 2. — In cases in which Beck score is more than 6 were selected phenomena, which could develop PPD had a 90.3% sensitivity and 45.3% specificity.
determine a correlation between mother’s educational level and EPDS scores [36, 37]. In the present study, a statistically significant relation was not found between mothers’ education status and rate of PPD.

When rate of PPD was compared with mother’s occupation, or whether the mother’s was working, it was determined in some studies that mothers’ professional status did not significantly affect level of PPD [38–40]. In the present study, a statistically significant relation was not found between mothers’ occupation status and rate of PPD, which is in line with the literature.

While a correlation with delivery method was found in some studies, a difference was not found in the present study, similar to some other studies [32, 41, 42].

In many studies, the fact that the pregnant woman has a depression history was evaluated as a significant risk factor for depression [8, 11]. In the present study, it was determined that 19.4% of the cases had a depression history (OR: 3.4, p = 0.029). When it was evaluated accordingly, the present authors can conclude that depression history is the most significant risk factor for postpartum depression.

In the present study group, the authors found that there was a correlation between EPDS score and BDI. Incidence rate of depression for women, whose BDI score was determined as 6 and more in the antenatal period, was found to be significantly higher. As healthcare personnel, we must identify patients who have a potential risk of postpartum, and evaluate them during the postpartum period in terms of depression.

References


Can we predict postpartum depression in pregnant women?


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Maternal hemodynamic influence on uteroplacental oxygen distribution during cesarean section

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⁴ College of Dental Science, RAKMHSU, Rak (UAE)

Summary
This study investigated maternal hemodynamic influence on uteroplacental oxygen distribution and neonatal outcome during cesarean section (CS). CS was performed on 80 parturients using two anaesthetic techniques: spinal anaesthesia (SA) and general balanced anaesthesia (GBA). Indications for CS were exclusively obstetric related. Monitored maternal parameters were: ECG, heart rate (HR), non-invasive blood pressure (NIBP), saturation (SaO₂). Gas parameters in umbilical artery, vein, and neonatal capillary blood were sampled. Vitality was assessed by the Apgar scoring, first breath-taking time and the first breastfeeding attempt. Hypotension was the most common finding after SA induction. GBA group presented changes such as QT inversion (12.5%), tachycardia (55%), and bradycardia (2.5%). SA group experienced higher rates of sinus tachycardia (45%) and ventricular dysrhythmias (2.5%). Neonatal oxygenation was significantly higher in SA group. Higher quality of early neonatal adaptation in the SA group confirms it as the technique with the least neonatal risk during CS.

Key words: Cesarean section; Early neonatal adaptation; Transplacental oxygenation.

Introduction
The increasing number of cesarean sections (CS) worldwide over recent years has given rise to a need for research into the influences of maternal central hemodynamic changes in anaesthesia on newborns. Neonatal outcomes following CS are directly influenced by maternal health and respiratory and hemodynamic stability during anaesthetic induction. Inadequate uterine blood flow may result in impaired fetal oxygen uptake [1]. Uterine blood flow is not autoregulated and is a major determinant of oxygen delivery across the placenta. Factors that may obstruct uteroplacental perfusion and reduce the oxygen supply of the neonate are: maternal hypo- or hypertension, ECG rhythm disturbances that might result in impaired fetal oxygen uptake, maternal hypoventilation, and reduced concentration of maternal arterial blood oxygen. Decreased maternal values of oxygen can also be a direct cause of fetal asphyxia. Uteroplacental perfusion is proportional to blood pressure and therefore reduction to below a certain threshold results in inadequate fetal oxygenation. Because of fetal reserve and the different compensatory mechanisms, healthy fetuses can tolerate a decrease of 40-50% oxygen delivery without any untoward effect [2]. Acute respiratory acidosis of neonates can be caused by an accumulation of CO₂ because of a decrease in either uterine or umbilical flow. Maternal hypocapnia (< 25 mmHg) will cause uterine and umbilical vessel vasoconstriction [3]. Mechanical hyperventilation will increase thoracic pressure and reduce venous return as well as cardiac output and thus reduce uteroplacental blood flow [4]. Maternal alkalosis will shift the oxygen-hemoglobin dissociation curve to the left, and thus will have difficulty to extract oxygen [5].

Materials and Methods
The study was approved by Ethics Committee of the Medical faculty of the University of Belgrade (250/I-5). The present investigation comprised 80 American Society of Anaesthesiologists (ASA) I pregnant women (28 years, range: 20-38) in the 37th to the 42nd week of pregnancy, who were randomly assigned to be treated using one of two techniques of anaesthesia during CS. The indications for CS were exclusively obstetric related indications. In the first group (40 women) the anaesthetic technique used was spinal anaesthesia (SA) and in the second group (40 women) general balanced anaesthesia (GBA) was used. All patients were visited on the morning of the surgery by the investigator and informed consent was obtained from all patients. The protocol for the assignment of anaesthetic technique was randomised using the STATA commercial statistical analysis program. The randomisation was performed immediately before the initiation of anaesthetic procedures during CS.

During the preoperative period, the authors continuously monitored maternal non-invasive blood pressure (NIBP), heart rate...
Results

Two groups of pregnant women (n = 40) were recruited for elective CS under either SA (group 1) or GBA (group 2). In the period between completed induction of anaesthesia and neonatal extraction (7-18 min), the authors found significantly different values of maternal systolic arterial pressure (p = 0.001) and diastolic arterial pressure (p = 0.01) between the groups with increased incidence of hypotension in the SA group (Table 1). Hypotension was gradually treated with ephedrine bolus doses or by continuous infusion (6–30 mg) causing a rapid recovery in blood pressure values.

During the same period, HR values differed significantly between the two groups (p = 0.001) with a noticeable increase in frequency in group 2. Decrease in HR was also observed only in group 2, with an incidence of 2.5%. Ventricular extrasystolic dysrhythmia was observed in only one patient (2.5%) in group 1. Mild Q-T wave inversion in the standard D3 segment.

Table 1. — Main values of maternal systolic and diastolic non-invasive blood pressure during the time between anaesthetic induction and extraction of the neonate.

<table>
<thead>
<tr>
<th>Non-invasive blood pressure</th>
<th>Anaesthetic technique</th>
<th>Number of patients (N)</th>
<th>Main value of blood Pressure (mm Hg)</th>
<th>Standard deviation (SD)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>Spinal</td>
<td>40</td>
<td>109.45</td>
<td>14.757</td>
<td>0.001</td>
</tr>
<tr>
<td>General</td>
<td>40</td>
<td>129.85</td>
<td>15.734</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>Spinal</td>
<td>40</td>
<td>73.72</td>
<td>12.816</td>
<td>0.013</td>
</tr>
<tr>
<td>General</td>
<td>40</td>
<td>80.55</td>
<td>11.038</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(HR), five-lead ECG dynamics, and pulse oximetry. They analysed maternal arterial blood gas (ABG) samples during the extraction of the neonate. Umbilical artery, vein and neonatal capillary samples were taken after extraction and used for the measurement of partial pressure of oxygen (PaO₂), carbon-dioxide (PaCO₂) and pH values. Neonatal Apgar score variables were monitored at the first (Apgar 1) and fifth minute (Apgar 2) after the extraction of the newborn. The time of the first breath was recorded and the effectiveness of the first attempt to breastfeed was noted.

Preoperative administration of antacids, H2 receptor agonists, and metoclopramide was performed due to aspiration prophylaxis. GBA was induced using propofol (two mg/kg), succinylcholine (one mg/kg), and the maintenance of anaesthesia was performed using 0.6%–1% sevoflurane combined with a mixture of 50% nitrous oxide and oxygen. Opiates were not administered before the delivery of the neonate. Before SA patients were pre-loaded with 1,000 ml crystalloid and/or colloid (Ringer lactate or Hartmann’s solution and/or Hydroxyethyl-starch). SA was performed in a sitting position using a 26-7 gauge Sprotte needle inserted preferably at L2-L3 or L3-L4 level. Patients received 2.4(± 0.3) ml of spinal 0.5% bupivacaine with 10–20 mcg fentanyl. Nasal oxygen supply was maintained (3-5 l/min) during the whole operation. Hypotension was randomly prevented by using bolus doses of 5-10 mg ephedrine (in case of less than 80% BP baseline values), repeated in one to two minutes until BP returned to normal values.

After the estimation of the primary adaptation scores, every neonate from SA group was put to the mother’s breast for the first breastfeeding attempt. The same procedure was accomplished to the GBA parturient after complete recovery from the GBA.

Differences in outcome measures between matched pairs were assessed using universal analysis. Chi-square tests or Fisher’s exact tests (for independent samples) were used to detect significant differences (p-values) between the groups in terms of outcome variables. Continuous variables were compared using the non-parametric Mann-Whitney U test. A p-value of < 0.05 was considered to be significant.

Table 2. — Incidence of ECG disturbances in both groups in the period before neonatal extraction during cesarean section.

<table>
<thead>
<tr>
<th>ECG</th>
<th>Anaesthetic Technique</th>
<th>General</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus rhythm</td>
<td>Spinal</td>
<td>21 (52.5%)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>Presence of Q wave</td>
<td>General</td>
<td>22 (55%)</td>
<td>40 (50%)</td>
</tr>
<tr>
<td>Ventricular extrasystolic dysrhythm (VES)</td>
<td>Spinal</td>
<td>1 (2.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>General</td>
<td>1 (1.3%)</td>
<td>1 (1.3%)</td>
</tr>
</tbody>
</table>

Table 3. — Saturation values (SpO₂) in the umbilical artery and vein, neonatal capillary samples, and maternal artery soon after the extraction of the neonate.

<table>
<thead>
<tr>
<th>SpO₂</th>
<th>Anaesthetic technique</th>
<th>Mean value (%)</th>
<th>Standard deviation (SD)</th>
<th>Standard error (SE)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical artery</td>
<td>Spinal</td>
<td>27.11</td>
<td>13.08</td>
<td>2.01</td>
<td>0.53</td>
</tr>
<tr>
<td>General</td>
<td>25.61</td>
<td>7.61</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical vein</td>
<td>Spinal</td>
<td>54.44</td>
<td>14.67</td>
<td>2.26</td>
<td>0.39</td>
</tr>
<tr>
<td>General</td>
<td>51.90</td>
<td>11.90</td>
<td>1.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal capillary</td>
<td>Spinal</td>
<td>71.27</td>
<td>12.08</td>
<td>1.86</td>
<td>0.02</td>
</tr>
<tr>
<td>General</td>
<td>65.42</td>
<td>11.27</td>
<td>1.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal artery</td>
<td>Spinal</td>
<td>98.99</td>
<td>1.66</td>
<td>1.06</td>
<td>0.06</td>
</tr>
<tr>
<td>General</td>
<td>98.82</td>
<td>1.09</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — Anaesthetic induction and extraction of the neonate.

<table>
<thead>
<tr>
<th>ECG</th>
<th>Anaesthetic technique</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus rhythm</td>
<td>Spinal</td>
<td>33 (41.2%)</td>
</tr>
<tr>
<td>Presence of Q wave</td>
<td>General</td>
<td>40 (50%)</td>
</tr>
<tr>
<td>Ventricular extrasystolic dysrhythm (VES)</td>
<td>Spinal</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>General</td>
<td>1 (1.3%)</td>
</tr>
</tbody>
</table>

Table 4. — Anaesthetic induction and extraction of the neonate.

<table>
<thead>
<tr>
<th>ECG</th>
<th>Anaesthetic technique</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus rhythm</td>
<td>Spinal</td>
<td>33 (41.2%)</td>
</tr>
<tr>
<td>Presence of Q wave</td>
<td>General</td>
<td>40 (50%)</td>
</tr>
<tr>
<td>Ventricular extrasystolic dysrhythm (VES)</td>
<td>Spinal</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>General</td>
<td>1 (1.3%)</td>
</tr>
</tbody>
</table>
fifth min (9.0) of neonatal adaptation compared with the GBA group (Table 5). Average neonatal first breath-taking time was shorter in the SA group (3 ± 2 s) compared with the GBA group (4 ± 3 s). After primary adaptation scores were estimated, each neonate in the SA group was put to its mother’s breast for its first attempt at breastfeeding. The same procedure was done in the GBA group following complete recovery from anaesthesia. The release time of SA group newborns (three to five days) from the maternity ward was significantly shorter than the release time of GBA newborns (four to seven days) ($p < 0.05$).

### Discussion

In light of the increasing number of CSs over recent years, the present study analysed the number of functional maternal influences that may disturb regular neonatal adaptation after extraction [6]. The most common haemodynamic findings were hypotension, compensatory tachycardia and, rarely, bradycardia [7]. The authors found statistically significant differences between the two groups in haemodynamic parameters during the period of induction of anaesthesia and soon after. Hartmann et al. [8] evaluated 3,315 pregnant women that underwent CS using SA and found hypotension in 30% of the cases. Carpenter et al. [9-11] found a 33% incidence of hypotension during CS, defining hypotension as systolic values of less than 90 mmHg or 10% less than original blood pressure values. Tarkikila and Isola [12] defined hypotension as a 30% decrease in pre-anaesthetic systolic pressure or its decrease to less than 85 mmHg. Maayan-Metzger et al. [13] found no negative effects of time-related delivery intervals on vital fetal parameters.

In the present study, increased heart rate soon after induction was present in 45% of cases in the SA group, and in 55% of cases in the GBA group. The authors also noticed a 2.5% decrease in maternal heart rate in the GBR group in the same period. Brenck et al. [14], in a study of 1,154 births, found a 12.7% rate of post-inductional bradycardia. Other studies have reported increases in heart rate from 0.3% [15] to 24% [16]. As a result of changes in autonomic control, pregnant women at term usually have heart rates of between 90 and 95 beats/min [17].

Maternal ECG analyses from the present study showed a 2.5% incidence of ventricular skipped beats and a 12.5% incidence of ischemic QT wave in the GBA group. Zhu et al. [18] confirmed the protective and nutritional effect of sevoflurane on the myocardium, its perfusion, and the metabolism of oxygen during GBA.

PaO₂ artery values of parturient were significantly higher in the SA group compared with those in the GBA group. In this group, umbilical artery PaO₂ was 3.85 ± 1.28 kPa compared with general group values of 2.68 ± 1.01 kPa. Umbilical vein main PaO₂ values in the SA group were 5.65 ± 1.67 kPa, compared with 4.59±1.40 kPa in the GBA group. Lindblad et al. [19] confirmed slower umbilical perfusion and hypooxygenation of the neonate in cases of spinal sympathectomy. Ngan Kee et al. [20] reported their findings of PaO₂ values evaluating 60 neonates. Their values performing general anaesthesia with 50% FiO₂ and sevoflurane 1 vol% were very close to the present results (umbilical vein PaO₂ 4.7 kPa and umbilical artery blood PaO₂ 2.9 kPa). Lawes et al. [21] found a main value of neonatal umbilical vein PaO₂ of 3.9 kPa. In the present study, the main value of umbilical artery PaCO₂ was 6.55 ± 1.10 kPa in the SA group, compared with 6.16 ± 1.25 kPa in the GBA group.
group ($p > 0.05$). Umbilical vein PaCO₂ in the SA group differed significantly to the of the GBA group, with values of 5.12 ± 0.92 kPa in the SA group and 5.63 ± 0.99 kPa in the GBA group. Westgate et al. [22], in their study of 1,942 neonates at delivery, presented results of PaCO₂ values in the umbilical vein of 5.4 kPa and of 7.1 kPa in the umbilical artery.

Mokarami et al. [23] in a study of 58 newborns immediately after extraction confirmed similar pH (7.305) and PaCO₂ values (7.30 kPa) and only significantly different lactate concentration between general and spinal group of ($p = 0.03$). Kotaska et al. [24] reported in the study of 189 neonates delivered by CS reference values of arterial cord blood: pH (7.05–7.39), pCO₂ (5.01–10.60 kPa), pO₂ (1.17–9.36 kPa), that are in the range of the present study’s neonate blood gas values.

Conclusion

This study demonstrated that both the spinal and general anaesthetic techniques used in CS can cause a number of maternal haemodynamic disturbances and decreased perfusion in umbilical circulation. Considering the significantly higher values of transplacental oxygenation, capillary neonatal oxygen and increased quality of neonatal adaptation soon after extraction in the SA group, this technique should be considered the technique of first choice for CS.

References


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Introduction

Measurement of uterine artery dynamics like pulsatility index (PI) is routinely performed in the follow of both singleton and multiple pregnancies to predict pre-eclampsia [1-6]. At all, it is the failure of trophoblastic invasion changing uterine artery blood flow pattern and PI [7]. Therefore, in the need of normative values of PI measurements discriminating ones with risk, reference ranges for singletons and twin pregnancies have been previously determined [1, 6, 8].

In the literature, the possible effect of assisted reproductive techniques where hormonal manipulations are done in the women on PI measurement in twin pregnancies has not been studied before. Thus, the authors aimed to compare the uterine artery PI measurements between spontaneous and IVF twins in the second trimester.

Materials and Methods

All medical records of twin pregnancies, whose fetal screening was done between May 1999 and December 2013, were evaluated retrospectively. All twin pregnancies without detected/suspicious anatomical or genetic fetal anomalies, systemic diseases, biochemical abnormalities, and familial genetic diseases were included in the data analyses. Fetuses with no information on spontaneous or IVF conception and fetuses with undetermined uterine artery impedance of second trimester were excluded from the data analyses. Results: A total of 151 twin pregnancies were evaluated in the analyses. The percentages of spontaneous and IVF twins were 24.5 % and 75.5 %, respectively. Mean gestational age was 19.95 ± 2.25 weeks in IVF twin group and 20.10 ± 2.19 weeks in spontaneous twin group. The difference of the gestational age between groups was not statistically significant. Mean uterine artery impedance was found as 0.78 ± 0.22 in IVF twins and 0.96 ± 0.31 in spontaneous twins respectively). Mean values were significantly lower in the IVF twins (p = 0.09).

Discussion

In the pathophysiology of pre-eclampsia the failure of trophoblastic invasion in early pregnancy causes hemodynamic changes in the uterine arteries [7]. Measurements of uterine...
artery dynamics like PI have been shown to be sensitive to predict pre-eclampsia risk during pregnancy and thus it is routinely performed in the following of both singleton and multiple pregnancies to [1-6]. Previously, in the literature, to discriminate pregnancies with risk of pre-eclampsia, reference ranges of uterine artery PI for singletons and twin pregnancies have been determined before [1, 6, 8].

In twin pregnancies, some possible confounding factors like placental location, parity, and chorionicity related PI measurements have studied [8-12]. In this present study the authors determined for the first time the effect of possible effect of IVF pregnancy which includes hormonal manipulations, on PI measurements in twin pregnancies compared to spontaneous twin pregnancies.

The effect of chorionicity (monochorionic vs dichorionic twins) on PI measurements has been assessed in two studies [9, 10]. In one of studies which screened 423 twin pregnancies (27% monochorionic) for uterine artery PI at 20-22 gestational weeks, the median of the lowest, mean, highest PI of uterine arteries was found as 0.69, 0.79, and 0.87 in twins [9]. Measurements of PI above the 95th percentile were determined as significant risk factors for pre-eclampsia and adverse pregnancy outcome with the sensitivity of 35% but no statistical significant difference was present between uterine artery PI measurements of monochorionic and dichorionic twin pregnancies [9]. In the second study, 360 twin pregnancies (10% monochorionic) were examined for uterine artery PI at 22–24 gestational weeks [10].

In a prospective study in which effect of placental location on uterine artery PI was studied and compared between singleton and twin pregnancies, ipsilateral artery PI measurements were consistently lower than contralateral artery PI measurements in singleton and twin pregnancies [11]. Patterns of PI change throughout the pregnancy were different between singleton and twin pregnancies. A steady decrease toward term was present in singleton pregnancies whereas a steady decrease until 27 weeks of gestation and no change thereafter was present in twin pregnancies. Also, the magnitude of PI measurements of both side uterine arteries of twin pregnancies was lower than that of singleton pregnancies at any gestational week [11].

In another study performed to compare effect of parity on PI measurement in singleton and twin pregnancy included in 16 nulliparous and 16 parous women with non-preeclamptic singleton gestations and 16 nulliparous and 16 parous women with dichorionic twin gestations [12]. Measurements were performed at 17-18 and 26-27 weeks of gestation. Average PI in nulliparous women was significantly higher than that in parous women at 17-18 weeks of gestation in both singleton and twin pregnancies. However, there were no significant differences in PI at 26-27 weeks of gestation between nulliparous and parous women of singleton or twin pregnancies. It was concluded that parity has a significant effect on uterine artery blood flow in the early second-trimester of non-preeclamptic singleton and twin pregnancies [12].

In addition to parameters related to PI measurements discussed above, the present study showed the effect of IVF
twin pregnancy on PI measurements in second trimester. The uterine artery PI measurements in the second trimester are significantly lower in IVF twin pregnancies compared to the spontaneous twin pregnancies. In this present study, the possible reason for this significant difference has not been investigated. Possibly, hormonal manipulations done in IVF might be responsible; it is to be further investigated in another clinical study.

Conclusions

The uterine artery PI measurements in the second trimester are significantly lower in IVF twin pregnancies compared to the spontaneous twin pregnancies.

References


Pregnancy-associated plasma protein A levels are decreased in obstetric cholestasis

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Summary
Objective: Obstetric cholestasis is a cholestatic disease usually commencing in the third trimester of pregnancy and characterized by pruritus, elevation of liver enzymes, and increase in bile acids. The objective of this study was to compare the first trimester serum indicators of obstetric cholestasis with normal pregnancies. Materials and Methods: Thirty-five patients diagnosed with obstetric cholestasis in a three-year period with first trimester biochemical assessment available were included in the study. Seventy patients with concordant pregnancy weeks, matched-age normal pregnancies were included as the control group. Pregnancy-associated plasma protein A (PAPP-A) and free beta-human choriionic gonadotropin (beta-hCG) levels were analyzed. Results: No difference was observed between the two groups in terms of age and week of pregnancy. While the mean PAPP-A level was 0.76 ± 0.31 multiples of the medians (MoM) in the obstetric cholestasis group, it was determined to be 1.5 ± 0.84 in the control group (p = 0.0001). Among the two groups, the hCG levels were found to be higher in the obstetric cholestasis group (1.2 ± 0.79 MoM vs. 0.98 ± 0.53, p = 0.041). Conclusion: In this study, the first trimester PAPP-A levels in the obstetric cholestasis cases were found to be significantly lower than the control group. Low PAPP-A levels should be a warning for obstetric cholestasis.

Key words: Obstetric cholestasis; Pregnancy-associated plasma protein A.
Table 1. — Demographic and laboratory characteristics of the two groups*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obstetric cholestasis (n=35)</th>
<th>Control group (n=70)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.8 ± 4.5</td>
<td>27.8 ± 5.7</td>
<td>0.074</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>68.2 ± 10.4</td>
<td>62.3 ± 10.3</td>
<td>0.081</td>
</tr>
<tr>
<td>PAPP-A (MoM)</td>
<td>0.76 ± 0.31</td>
<td>1.50 ± 0.84</td>
<td>0.0001</td>
</tr>
<tr>
<td>hCG (MoM)</td>
<td>1.2 ± 0.79</td>
<td>0.98 ± 0.53</td>
<td>0.041</td>
</tr>
<tr>
<td>NT (MoM)</td>
<td>0.94 ± 0.41</td>
<td>0.87 ± 0.27</td>
<td>0.32</td>
</tr>
<tr>
<td>CRL</td>
<td>60.4 ± 11.0</td>
<td>56.2 ± 11.0</td>
<td>0.074</td>
</tr>
</tbody>
</table>

Abbreviations: PAPP-A: pregnancy-associated plasma protein A; hCG: human chorionic gonadotropin; MoM: Multiples of the medians; NT: Nuchal translucency. CRL: Crown rump length. *: Values are mean ± SD.

While the average PAPP-A level was 0.76 ± 0.31 MoM in the obstetric cholestasis group, it was determined to be 1.50 ± 0.84 in the control group (p = 0.0001). Among the two groups, the hCG levels were found to be higher in the obstetric cholestasis group (1.2 ± 0.79 MoM vs. 0.98 ± 0.53, p = 0.041).

Discussion

Some recently published studies indicate that low maternal serum PAPP-A levels could be effective in foreseeing different obstetric complications [5-8]. It has been suggested that PAPP-A levels below 0.04 MoM could be an indicator for disrupted placental functions [8].

Obstetric cholestasis is especially more frequent in the third trimester when the serum estrogen concentration has peaked and in multiple pregnancies where the hormone levels are higher [9]. The increased estrogen and progesterone, with addition of environmental factors, causes a cholestasis manifestation in pregnant women with convenient genetic background. In the present study, the first trimester PAPP-A levels in the obstetric cholestasis cases have been found to be significantly lower than the control group. There are numerous studies on first trimester low PAPP-A levels and associated developing pregnancy complications. A study by Carbone et al. in 2011 [10] demonstrated that low levels of PAPP-A in the screening test conducted during the first trimester have a relation with SGA at delivery. Kirkegaard et al. [11] have published a study in 2011, indicating that low PAPP-A levels are associated with SGA. Spencer et al. [12] conducted a study in 2010 on the association of low PAPP-A levels in the first trimester with future development of preeclampsia. Goetzinger et al. [13] published a study in 2010, significantly associating the increased preterm labour risk with low PAPP-A levels in the first trimester.

As far as the present authors are aware, there is only one study [14] determining PAPP-A levels in obstetric cholestasis. In contrast to the present results, they found increased serum levels of PAPP-A compared to controls but they had only 15 patients with obstetric cholestasis.

In the present study, PAPP-A levels were found to be significantly low in the obstetric cholestasis cases compared to the control group. Low PAPP-A levels should be a warning for obstetric cholestasis.

References


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Incidence and factors associated with nosocomial infections in a neonatal intensive care unit (NICU) of an urban children’s hospital in China

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Summary

Objective: The study’s aim was to assess incidence and epidemiologic profile of nosocomial infection (NI) in a NICU of China, and to identify main risk factors of NIs. Materials and Methods: Chi square test for discrete variables and independent t-test for continuous variables to examine the association with NI. Univariate regression model was applied to the variables to predict the NI status. Finally the multivariate model was utilized with stepwise methods included all variables in the univariate model to extrapolate the independent variables to NI. Results: Infection rate in NICU during the study period was 6.2 episodes per 100 patients. Infection density was 4.2 episodes per 1,000 patient-days. Infection rate of ventilation-related pneumonia was 3.4 episodes per 1,000 mechanical ventilation (MV) days. Central line-associated bloodstream infection rate was 5.4 episodes per 1,000 central line days. Gestational age < 32 weeks, with congenital malformation, twins or triplets, gastric tube feeding, operation, duration of prophylaxis antibiotic use, duration of probiotic use, duration of parenteral nutrition were the risk factors associated with NI in NICU. We discussed the association between intrinsic factors of infants and health care procedures with NI. Conclusion: This study provided information for prevention strategies of NI, that will ultimately improve the healthcare service level.

Key words: Nosocomial infection; Neonatal intensive care unit; Risk factors; Incidence.

Introduction

Nosocomial infection (NI) refers to healthcare associated infection that was recognized over one century ago. Obstetrics and Gynecology doctors, Ignac Philipp Semmel-Weis observed that the mortality rate of women delivering and of the newborns could be highly reduced through hand disinfection of midwives [1]. Nowadays healthcare-associated infection has become a severe problem worldwide, which takes a high death toll in human lives and affects hundreds of millions of patients each year [2].

Neonatal intensive care unit (NICU) patients, including preterm babies and infants who require surgeries are unique and highly vulnerable group of people. The research indicated that the NI rate was on the rise in the past decade, due to the increasing survival rate of early preterm infants and the extensive use of invasive healthcare procedures [3]. The incidence of NI in NICU varied from 6%-40%, which depended on the proportion of preterm infants and the proportion of surgery in NICU, which was much higher than the incidence rate of normal neonatal wards, which varied from 0.5 - 1.7% [4].

Another key factor which is influencing the prevalence of NI in NICU is socio-economic level of the nation. The situation is much worse in developing countries than in developed countries such as USA and European countries. A recent review from WHO found that prevalence of healthcare-associated infection which was 15.5 per 100 patients in developing countries, which was much higher than the prevalence reported from Europe and the USA. Pooled overall healthcare-associated infection density in adult intensive-care units was 47.9 per 1,000 patient-days (95% CI 36·7~59·1), at least three times as high as incidences reported in the USA [5].

The outcome associated with the NI was the increasing mortality and morbidity, as well as the prolonged hospital stay. In the USA, the extension of hospital stay and the additional treatment for healthcare-associated infection cost the government 17 to 29 billion dollars [3]. The degree of prematurity, birth weight, applied mechanical ventilation (MV) and exposure to central venous catheter were the risk factors reported in previous articles [6].

There was increasing trends in the past decades of NI in China. According to the published articles, the infection rate of NICU in China varied from 7% - 11.6% [7,8]. However, the risk factors, such as length of hospitalization, applied gastric tube, and applied ventilation associated with the NI in NICU in China are rarely reviewed and analyzed [9]; most of the studies only discuss the epidemiology profile of NI in NICU [8, 10, 11]. Since China’s healthcare system’s regulation procedure, efficiency, and socio-economic situa-
tions are unique, it is absolutely essential to conduct the research on the NI infection incidence and risk factors in order to control and minimize the influence. This study aimed to assess the incidence and epidemiologic profile of NI in NICU of China, and to identify the main risk factors of NIs.

Materials and Methods

Setting

The study was conducted in the NICU of Guangzhou Women and Children’s Medical Center. There were 30 cots in NICU, with three m² surface area for each cot and one m distance between two cots. The ratio of nurse-to-cot was 1:4. The entire area was separated into two parts, one for the non-infectious patients and one for infectious patients. The NICU was equipped with air filters. There are about 800 patients admitted in the NICU per year. Annual patient days are around 11,000. There are about 16% of the patients admitted for surgery and 60% preterm birth among all admitted patients during the study period. The present authors accepted patients aged mainly within 28 days, but if the infants were preterm and the weight was not heavier than 4,000 g, this rule was not applied. All the NI cases were informed by the attending physicians and confirmed by the associate chief doctor or chief doctor and the experienced infection control staff.

Inclusion and exclusion criteria

This study was a retrospective cohort survey with a nested case control study. All 1,653 Chinese infants admitted in the NICU during the study period from December, 2009 to May, 2012 were included as the subjects. All Chinese infants admitted and diagnosed with at least one episode of NI in the NICU of Guangzhou Women and Children’s Medical Center from December of 2009 to May of 2012 were included as the cases. There were 81 infants with 103 episodes in total. Meanwhile 300 controls were randomly sampled based on the computerized records of all infants admitted and stayed in NICU for more than 48 hours without infection in the hospital in the same period. The infants who died (n=8) or were discharged or transferred to other department within 48 hours (n=23) after being admitted in NICU were excluded. The subjects with missing data in more than two variables (n=0, there were two subjects only that each had one variable missing) were also excluded.

Ethical approval

This study was approved by the Medical Ethic Committee of Guangzhou Women and Children’s Medical Center. All the data already existed in the computerized medical recording system, so no informed consent was needed.

Data collection procedures

All data were collected in the database of medical records in the Guangzhou Women and Children’s Medical Center during June to July 2012, by two trained attending physicians independently with the standard data extraction sheets [9]. All the NI cases were identified by the associate chief doctor or chief doctor and the experienced infection control staff. Two postgraduate medical students were responsible for the data entry to the computers separately and double checked the data to ensure correct entry.

Measurements

The measurement and the variables of demographic and family characteristics, the clinical, and the healthcare procedures in the study were categorized and defined as follows:

The measurements of NI in NICU

- Incidence rate of NI: the new cases of NIs occurred in a given time period (year)/all the persons at risk in that given time (year) *100
- Incidence density: the new episodes of NIs occurred in a given time period /the number of patients-days in a given time*1,000
- Ventilator-associated pneumonia infection rate: new episodes of ventilator-associated pneumonia in a given time/ the number of ventilator-days *1,000
- Central line-associated bloodstream infection rate: new episodes of central line-associated bloodstream infections / the number of central line-days*1,000

Demographic characteristics of the infants

- Sex: male as the reference group.
- Age: days as the units were recorded.
- Gestational age: The number of weeks that passed from the first day of the last menstrual period (LMP) to the birth day. It was categorized into two groups: early preterm birth: born before 32 weeks of pregnancy and non–early preterm birth born after 32 weeks of pregnancy which was the reference group.
- Birth weight: very-low–birth-weight (VLBW) ≤ 1,500 g and non-VLBW > 1,500 g (reference group).
- Congenital malformation: defined as the infants with congenital heart diseases, congenital chromosomal abnormalities, tumor and congenital abnormal body structure which required medical, surgical or cosmetic treatments. Infants without congenital malformation were the reference group.
- Singletons: categorized into two groups: singleton (reference group); twins or triplets.

The family characteristics

- Maternal age: the age of the mother at the time of delivery.
- Advanced maternal age: defined as the women older than 35 years when she gave birth to her first child.
- Pregnancy complications: defined as the symptoms and problems which were associated with the pregnancy for the present infants. Diagnosis was based on “The International Classification of Diseases, 10th Revision, Clinical Modification” (ICD-10-CM) [12]. These mainly included diabetes mellitus in pregnancy (024.901) and severe pre-eclampsia (014.101).

Healthcare procedures associated variables

All the variables related to healthcare procedures, such as receipt of pulmonary surfactant and umbilical vein catheterization (UVC), the peripherally inserted central catheters (PICC) or arterial catheter (or both) and urinary catheter, intubation and ventilation, receipt of nasal continuous positive airway pressure (NCPAP), use of probiotics and antibiotics, and receipt of operation were counted from admission until the onset of NI occurring in case groups or until discharge of non-infection control group.

The duration of ventilation and the duration of using probiotics were recorded from the day of receipt of the procedures or probiotics until the procedures were completed or until the NI, whichever was earlier.

Data analysis

The authors described the NI rate, infection density and device-related infection rate, seasonal distribution, the type of infection, and the pathogens identified to reveal the profile of NI in NICU.

The authors compared the general characteristics, the family characteristics, and healthcare related procedure characteristics of
cases when they had the first episodes of NI after admission with the control group using Chi square test for discrete variables and independent t-test for continuous variables to examine the association with NI. For patients with more than one episode of NI, the authors only considered the first episode in the case control analysis.

First, the authors applied each of the variables to fit the binary regression model, then they conducted a multivariate model with variables which showed a statistically significant association with NI in univariate analysis (p value less than 0.05) to predict the outcome. As some variables in the multivariate model (adjusted OR) showed inconsistent results with univariate model (crude OR), hence the authors check the multicollinearity within the model by correlation matrix. At last, they applied the multivariate model with stepwise methods with all the variables in the previous model to get the independent variables to NI.

Results

Incidence rate of NI

During the study period, there were 1,653 infants admitted to NICU. The total number of episodes of NIs was 103. The calculation indicated that the NI rate was 6.2 episodes per 100 patients (95% CI, 6.0 - 6.4 episodes per 100 patients).

Incidence density

The total patient-days were 24,547, in which resulted 4.2 episodes per 1,000 patient-days (95% CI, 4.0-5.8 episodes per 1,000 patient-days) in the infection density.

Ventilation associated pneumonia infection rate

There were 24 episodes of ventilation-associated pneumonia during the study period, plus 7,069 MV days. The infection rate of ventilation-related pneumonia was 3.4 episodes per 1,000 MV days (95% CI, 3.2-3.5 cases per MV patient-days)

Central line-associated bloodstream infection rate

1,482 central line days, there were eight infected episodes; therefore the infection rate was 5.4 episodes per 1,000 central line days. (95% CI, 5.1 - 5.6 episodes per central line days)

Crude death rate

There were seven deaths in the NI cases and 75 deaths (including the death in the NI cases) among all patients admitted in NICU during the study period, resulting in a death rate of 8.6% and 4.5%, respectively.

There were 16 (20.0%) patients out of 81 infants had two NIs, while six (7.4%) out of 81 infants had three or more NIs. Among 103 episodes of NI in the study, pneumonia accounted for 34% of all the episodes (Table 1). The major agent of NI was Klebsiella pneumonia, which was most commonly cultivated in airway secretions, followed by Pseudomonas aeruginosa, which was more commonly cultivated in blood and airway secretions (Table 2).

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>First episodes (N=81)</th>
<th>Second episodes (N=16)</th>
<th>Third episodes or more (N=6)</th>
<th>Total (n=103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>25(31%)</td>
<td>5(31%)</td>
<td>5(83%)</td>
<td>35(34%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>21(26%)</td>
<td>3(19%)</td>
<td>1(17%)</td>
<td>24(23%)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>11(14%)</td>
<td>3(19%)</td>
<td>0</td>
<td>14(14%)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3(4%)</td>
<td>3(19%)</td>
<td>0</td>
<td>6(6%)</td>
</tr>
<tr>
<td>Thrush</td>
<td>7(9%)</td>
<td>1(6%)</td>
<td>0</td>
<td>7(7%)</td>
</tr>
<tr>
<td>Surgery wound infection</td>
<td>4(5%)</td>
<td>1(6%)</td>
<td>0</td>
<td>5(5%)</td>
</tr>
<tr>
<td>Other</td>
<td>12(15%)</td>
<td>0</td>
<td>0</td>
<td>12(12%)</td>
</tr>
</tbody>
</table>

Table 1. — Distribution of 103 episodes of NI in NICU.

<table>
<thead>
<tr>
<th>Genus, species</th>
<th>First episodes (N=40)</th>
<th>Second episodes (N=5)</th>
<th>Third episodes or more (N=4)</th>
<th>Total (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumonia</td>
<td>12(30%)</td>
<td>1(20%)</td>
<td>1(25%)</td>
<td>14(29%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6(15%)</td>
<td>1(20%)</td>
<td>1(25%)</td>
<td>8(16%)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>5(13%)</td>
<td>0</td>
<td>0</td>
<td>5(10%)</td>
</tr>
<tr>
<td>Pathogenic escherichia coli</td>
<td>3(8%)</td>
<td>0</td>
<td>0</td>
<td>3(6%)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3(8%)</td>
<td>0</td>
<td>0</td>
<td>3(6%)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>2(5%)</td>
<td>0</td>
<td>0</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>2(5%)</td>
<td>0</td>
<td>0</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1(3%)</td>
<td>0</td>
<td>1(25%)</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>2(5%)</td>
<td>0</td>
<td>0</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1(3%)</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>0</td>
<td>2(40%)</td>
<td>0</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Others</td>
<td>4(10%)</td>
<td>1(20%)</td>
<td>1(25%)</td>
<td>6(12%)</td>
</tr>
</tbody>
</table>

Table 2. — Distribution of 49 pathogens of NI in NICU No. (%) of pathogens identified.

Characteristics of study subjects related to NI (related to second outcome)

For 381 enrolled infants in the study, there were 35.8% females in the NI cases group and 36.3% females in the control group respectively; thus, there was no significant sex difference between groups. The mean age of NI cases group and control group were also similar. However, the number of early preterm birth in the NI cases group was 43.2%, which was much greater than the percentage in the control group (18%). Moreover, there was 9.9% VLBW infants in the NI cases group, which was much greater than in the control group (2.0%; p < 0.001). The proportion of infants with congenital malformation in NI cases group was 30%, which was much higher than the proportion in the control group (10%; p < 0.001). There were also significantly more twins/triples in NI cases group than in control group (35.8% vs. 17.6%; p < 0.001). Overall, there were significantly smaller gestational ages and birth weight, more congenital malformations, more twins or triplets in the NI cases group than in the control group (Table 3).

The mean age of mothers in two groups was similar, which was around 29 years. There were significantly more advanced maternal age mothers in the NI cases group
than in the control group. Only 2% of the mothers were older than 40 years in both groups. The main complications of pregnancy in the mothers were diabetes mellitus and hypertension, which were approximately 6% and 5%, respectively, with no significant differences in both groups (Table 4).

Mean length of NICU stay was 53.1 days in NI cases group, which was much longer than those in non-infected control group (16.6 days); 92% of the infants’ NICU stay in NI cases group were lasted for six to 109 days, and only two infants’ NICU stay were far from the mean. After excluding two outliers (more than 454 days), the difference of NICU stay length was still significant \( p < 0.001 \). There were more infants in NI cases group requiring exogenous pulmonary surfactants, UVC, PICC and urinary catheter (UC), intubation, ventilation, operations, which indicated that the NI cases group had more severe medical conditions than the non-infected control group (Table 5).

Due to the more medical procedures that were performed in NI cases group, the percentage of patients who had intravenous prophylactic antibiotics was greater. Furthermore, the authors found that more infants in the non-infected control group were given probiotics, and had longer duration than the infected case group.

Because of the incomplete data of the entire 12 months in 2009 and 2012, the authors compared all the episodes of four seasons in 2010 and 2011 (51 episodes in 2010 and 47 episodes in 2011). The finding is that there was no difference in the seasonal pattern of NI (Table 6). A better comparison should be based on incidence or incidence density, as the number of patients might vary in different periods. It is unfortunately that data were not available; therefore the authors could only compare the number of episodes.

Potential factors related to NIs

In order to predict whether or not the NI would occur, the authors applied the binary logistic regression analysis to background factors, which related to the NIs. Gestational age older than 32 weeks and females showed pro-

Table 3: Background characteristics of infected infants and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nosocomial infected cases (%) (N=81)</th>
<th>Non-infected controls (%) (N=300)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female)</td>
<td>29(35.8%)</td>
<td>109(36.3%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Age (days)</td>
<td>5.7±13.4</td>
<td>6.1±10.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early preterm birtha</td>
<td>35(43.2%)</td>
<td>53(17.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate and mild preterm birthb</td>
<td>28(34.6%)</td>
<td>121(40.5%)</td>
<td></td>
</tr>
<tr>
<td>Term infantsc</td>
<td>18(22.2%)</td>
<td>125(41.8%)</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1,500g</td>
<td>31(38.3%)</td>
<td>47(15.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt; 1,500g</td>
<td>50(61.7%)</td>
<td>253(84.3%)</td>
<td></td>
</tr>
<tr>
<td>Congenital malformation</td>
<td>24(29.6%)</td>
<td>30(10%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Early preterm birth: gestational age ≤ 32 weeks
b Moderate and mild preterm birth: gestational age > 32 and < 37 weeks
c Term infants: gestational age ≥ 37 weeks

Table 4: Mother’s characteristics of NI neonates and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nosocomial infected cases (%) (N=81)</th>
<th>Non-infected controls (%) (N=300)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (yrs)</td>
<td>29.6±5.1</td>
<td>29.0±4.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Advanced maternal age (yrs)</td>
<td>22(21.4%)</td>
<td>39(13.0%)</td>
<td>0.041</td>
</tr>
<tr>
<td>Older than 40 years</td>
<td>2(1.9%)</td>
<td>6(1.9%)</td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>53(17.7%)</td>
<td>22(14.4%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6(5.9%)</td>
<td>16(5.3%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Severe pre-eclampsia</td>
<td>6(4.9%)</td>
<td>8(2.7%)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table 5: Healthcare procedures associated characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nosocomial infected cases (%) (N=81)</th>
<th>Non-infected controls (%) (N=300)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receipt of pulmonary surfactant</td>
<td>28(34.6%)</td>
<td>40(13.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of UVCa</td>
<td>35(43.2%)</td>
<td>39(13%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of UVC (days)</td>
<td>3.6±4.5</td>
<td>1.1±3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of PICC / arterial catheter</td>
<td>28(34.6%)</td>
<td>34(11.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of PICC / arterial catheter</td>
<td>5.4±11.8</td>
<td>2.5±9.7</td>
<td>0.044</td>
</tr>
<tr>
<td>Receipt of urinary catheter</td>
<td>20(24.7%)</td>
<td>46(15.3%)</td>
<td>0.013</td>
</tr>
<tr>
<td>Receipt of gastric tube</td>
<td>55(67.9%)</td>
<td>99(33.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of Intubation 1 times</td>
<td>29(50.0%)</td>
<td>77(76.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>2 times</td>
<td>18(31.0%)</td>
<td>20(19.8%)</td>
<td></td>
</tr>
<tr>
<td>3 times</td>
<td>4(6.9%)</td>
<td>3(3.0%)</td>
<td></td>
</tr>
<tr>
<td>4 times or more</td>
<td>7(12.1%)</td>
<td>1(1.0%)</td>
<td></td>
</tr>
<tr>
<td>Receipt of ventilation</td>
<td>52(64.2%)</td>
<td>103(34.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of ventilations (days)</td>
<td>4.0±5.9</td>
<td>1.6±4.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Receipt of NCPAP</td>
<td>21(25.9%)</td>
<td>41(13.7%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Prophylactic antibiotics</td>
<td>73(90.1%)</td>
<td>212(70.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of using prophylactic antibiotics</td>
<td>8.7±6.9</td>
<td>4.5±4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Use of probiotics</td>
<td>19(23.5%)</td>
<td>108(36.0%)</td>
<td>0.034</td>
</tr>
<tr>
<td>Duration of using probiotics</td>
<td>2.3±5.4</td>
<td>3.7±6.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Use of parenteral nutrition</td>
<td>62(76.6%)</td>
<td>205(63.8%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Duration of using parenteral nutrition</td>
<td>8.6±9.0</td>
<td>6.7±8.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Receipt of operation</td>
<td>29(35.8%)</td>
<td>36(12%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td>53.1±69.7</td>
<td>16.6±16.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Umbilical vein catheterization (UVC);

b Peripherally inserted central catheters (PICC).
Table 6. — NI episodes in four seasons of 2010 and 2011.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>2010</th>
<th>2011</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring (Mar - May)</td>
<td>14(27.5%)</td>
<td>10(21.3%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Summer (Jun - Aug)</td>
<td>14(27.5%)</td>
<td>6(12.8%)</td>
<td></td>
</tr>
<tr>
<td>Autumn (Sep - Nov)</td>
<td>17(33.3%)</td>
<td>21(44.7%)</td>
<td></td>
</tr>
<tr>
<td>Winter (Dec - Feb)</td>
<td>6(11.8%)</td>
<td>10(21.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. — Crude and adjusted OR for background characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Crude OR (95% CI)</th>
<th>p value</th>
<th>Adjusted OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female)</td>
<td>0.98 (0.59-1.63)</td>
<td>0.31</td>
<td>0.65 (0.31-1.34)</td>
<td>0.24</td>
</tr>
<tr>
<td>Birth weight &lt;2,000 g</td>
<td>2.84 (1.70-4.74)</td>
<td>&lt;0.001</td>
<td>1.63 (0.61-4.30)</td>
<td>0.33</td>
</tr>
<tr>
<td>Gestational age &gt;32 weeks</td>
<td>0.28 (0.17-0.48)</td>
<td>&lt;0.001</td>
<td>0.53 (0.21-1.38)</td>
<td>0.2</td>
</tr>
<tr>
<td>Congenital malformations</td>
<td>3.79 (2.06-6.96)</td>
<td>&lt;0.001</td>
<td>4.37 (1.53-12.50)</td>
<td>0.006</td>
</tr>
<tr>
<td>Twins or triplets</td>
<td>2.6 (1.51-4.47)</td>
<td>0.001</td>
<td>3.88 (1.69-8.92)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*OR adjusted by sex, birth weight <2000g, gestational age >32 w, malformations, twins, intubation, vein or arterial catheter, ventilation, duration of ventilation, prophylactic antibiotic, duration of probiotics, operation, parenteral nutrition, duration of parenteral nutrition, gastric tube feeding.

Table 8. — Crude and adjusted OR for healthcare procedures.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Crude OR (95% CI)</th>
<th>p value</th>
<th>Adjusted OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receipt of intubation</td>
<td>4.97 (2.90-8.52)</td>
<td>&lt;0.001</td>
<td>2.51 (0.92-6.81)</td>
<td>0.07</td>
</tr>
<tr>
<td>Receipt of vein or arterial catheter</td>
<td>5.77 (3.41-9.76)</td>
<td>&lt;0.001</td>
<td>3.92 (1.76-8.70)</td>
<td>0.001</td>
</tr>
<tr>
<td>Receipt of ventilation</td>
<td>3.43 (2.05-5.73)</td>
<td>&lt;0.001</td>
<td>0.52 (0.18-1.49)</td>
<td>0.22</td>
</tr>
<tr>
<td>Duration of ventilation</td>
<td>1.1 (1.05-1.16)</td>
<td>0.071</td>
<td>1.02 (0.95-1.10)</td>
<td>0.62</td>
</tr>
<tr>
<td>Using prophylactic antibiotics</td>
<td>3.79 (1.75-8.19)</td>
<td>&lt;0.001</td>
<td>1.17 (0.38-3.64)</td>
<td>0.78</td>
</tr>
<tr>
<td>Duration of using antibiotic</td>
<td>1.14 (1.09-1.19)</td>
<td>&lt;0.001</td>
<td>1.13 (1.05-1.21)</td>
<td>0.001</td>
</tr>
<tr>
<td>Using probiotics</td>
<td>0.55 (0.31-0.96)</td>
<td>0.04</td>
<td>2.95 (0.92-9.48)</td>
<td>0.07</td>
</tr>
<tr>
<td>Duration of probiotics</td>
<td>0.96 (0.92-1.01)</td>
<td>0.09</td>
<td>0.88 (0.80-0.96)</td>
<td>0.004</td>
</tr>
<tr>
<td>Receipt of operation</td>
<td>4.09 (2.31-7.25)</td>
<td>&lt;0.001</td>
<td>6.17 (2.47-15.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of parenteral nutrition</td>
<td>1.51 (0.86-2.67)</td>
<td>0.15</td>
<td>0.98 (0.38-2.52)</td>
<td>0.97</td>
</tr>
<tr>
<td>Duration of parenteral nutrition</td>
<td>1.02 (0.997-1.06)</td>
<td>0.08</td>
<td>0.94 (0.90-0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Receipt of gastric tube feeding</td>
<td>4.3 (2.54-7.26)</td>
<td>&lt;0.001</td>
<td>3.27 (1.51-7.09)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*OR adjusted by sex, birth weight <2000g, gestational age >32 w, malformations, twins, intubation, vein or arterial catheter, ventilation, duration of ventilation, prophylactic antibiotic, duration of probiotics, operation, parenteral nutrition, duration of parenteral nutrition, gastric tube feeding.

Table 9. — Correlation matrix
tection to NI. Gestational age older than 32 weeks group was 72% less likely to get NI [OR 0.28 and \( p < 0.001 \)]. However, after adjusted by all the significant background factors in the univariate regression model and all healthcare procedure related factors, the association was not significant. Females were 2% less likely to get NI than males, but it was not striking [OR 0.98, \( p = 0.24 \)]. Infant with congenital malformation had significant harmful effect on the NI in both univariate and multivariate models. Congenital malformation infant was about four times more likely to get NI than the infant who was free from the disease. Birth weight less than 2,000 g and multiple births also showed harmful to NI. Only the multiple births founded significant association after adjustment (Table 7).

Compared to the infants without operations, those infants who underwent surgery were five times more likely to get NI. The harmful effects were showed in both univariate model and multivariate model. The infants with gastric tubes and received vein or arterial catheters also were significantly more likely to get NI than the controls after adjusted by all the other factors [OR 3.27, \( p = 0.003 \)] (Table 8).

Duration of using probiotics showed protection to NI. Infants who used probiotics longer were less likely to get NI. The association was significant after adjustment [OR 0.88, \( p = 0.004 \)]. Moreover, for the infants who received intubation, the length of ventilation and the use of prophylactic antibiotics showed to be harmful to NI, however after adjusted the effects were not significant.

Nevertheless, some factors such as parenteral nutrition and ventilation were harmful to NI in the univariate model, then showed protection in multivariate model. Just like those who had probiotics, the factor evened and then went to the opposite direction after adjustment.

As some factors (e.g. using probiotics and receipt of ventilation) showed inconsistent results with the univariate model, the authors investigated the multi-collinearity within the model by correlation table (Table 9). The score of using probiotics and duration of using probiotics was -0.743, meanwhile the score of intubation and ventilation was -0.568. Due to the absolute score of some factors in the correlation matrix that was greater than 0.7, the authors applied the forward conditional methods of the regression model.

**Independent factor related to NIs**

Receipt of operation [OR 5.32, \( p < 0.001 \)], with vein or arterial catheter [OR 4.76, \( p < 0.001 \)] and gastric tube feeding [OR 3.88, \( p < 0.001 \)] with congenital malformation [OR 4.43, \( p = 0.002 \)] and congenital malformation infants [OR 3.24, \( p = 0.003 \)] had a higher risk to have NI. Gestational age >32 weeks [OR 0.35, \( p = 0.01 \)], the length of using probiotics [OR 0.88, \( p = 0.005 \)], and length of using parenteral nutrition [OR 0.94, \( p = 0.008 \)] were the protection factors of NI (Table 10).

### Table 10. — Independent factors for NI.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age &gt; 32 weeks</td>
<td>0.35</td>
<td>0.16-0.80</td>
<td>0.01</td>
</tr>
<tr>
<td>Congenital malformation</td>
<td>4.43</td>
<td>1.72-11.39</td>
<td>0.002</td>
</tr>
<tr>
<td>Twins or triplets</td>
<td>3.24</td>
<td>1.51-6.95</td>
<td>0.003</td>
</tr>
<tr>
<td>Duration of use prophylactic antibiotics</td>
<td>1.13</td>
<td>1.06-1.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of parenteral nutrition</td>
<td>0.94</td>
<td>0.90-0.99</td>
<td>0.008</td>
</tr>
<tr>
<td>Receipt of vein or arterial catheter</td>
<td>4.76</td>
<td>2.27-9.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of operation</td>
<td>5.32</td>
<td>2.30-12.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Receipt of gastric tube feeding</td>
<td>3.88</td>
<td>1.87-8.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of probiotics</td>
<td>0.88</td>
<td>0.81-0.96</td>
<td>0.005</td>
</tr>
</tbody>
</table>

### Discussion

The essential measure to NI control is to implement close monitoring and to promote full awareness of the risk factors of infection. To accurately measure the extent of NI is the first step of controlling. There are many confounders which influence the infection rate, such as different medical conditions and the number of admissions. Effective confounder control, more accurate measurement, and timely identification of the patients at risk, as well as comparison with other institutes are needed. Therefore in recent years, more and more studies were done not only on incidence rate of infection, but also on incidence density and device-associated healthcare-associated infection rate (DA-HAI rate) to measure NI. In the present study, the authors used all of these ways to measure the extent of NI.

According to the present results, the NI rate of our NICU was 6.2 episodes per 100 patients (95% CI, 6.0 - 6.4 episodes per 100 patients). These were similar to the data from NICU of other cities in China which infection rate varied from 7% - 11.6% [7-9,13,14], and American and European countries which varied from 6% - 40% [4, 15-17]. The present incidence density was 4.2 episodes per 1,000 patient-days (95% CI, 3.4-5.0 episodes per 1,000 patient-days) which was lower than that from Asian countries such as Korea, in which the incidence density was on average 15.1 infections per 1,000 patient days [18]. The wide range of infection rate was due to the different kinds of patients in NICU, which could be the percentage of preterm infants and the infants who required surgery among all patients admitted, because preterm infants and post-surgical infants were more vulnerable. There were about 10% - 16% of the patients admitted for surgery and 50% - 60% were preterm births yearly in the present NICU, hence the infection rate was relatively low. In the meantime, the central line-associated bloodstream infection rate and the ventilation associated pneumonia infection rate were also lower than the data from International Infection Control Consortium (INICC), which were 5.4 episodes per 1,000 central line days and 3.4 episodes per 1,000 MV days versus 12.2 episodes per central line days and 9.0 episodes per 1,000 MV days, respectively. The probable reasons were
the low utilization of central line and ventilation. The present central line utilization ratio (number of central line-days/number of patient-days) [19] was about 11% and ventilation utilization ratio (number of ventilator-days/number of patient-days) [19] was about 10%, which were much lower than the data from INICC surveillance reports from 2003 to 2008, which were 25% and 13%, respectively [20]. Meanwhile, the present central line-associated bloodstream infection rate and the ventilation-associated pneumonia infection rate were also lower than the data from INICC, which were 5.4 episodes per 1,000 central line days and 3.4 episodes per 1,000 MV days versus 12.2 episodes per central line days and 9.0 episodes per 1,000 MV days.

In the present study, pneumonia was the most common type of infection, which accounted for 34% of all types of infections. The result was consistent with a study from Beijing and a study from Guangzhou [7, 21]. However, many studies from other countries found that sepsis was the most common type of infections [15, 22, 23]. One of the reasons of the high incidence rate of pneumonia may be that its diagnosis was easy to confirm by the doctor. Pneumonia can be diagnosed by tachypnea of the infants and the characteristic manifestation of chest X ray, which were easily observed and available. However, it was relatively difficult to diagnose sepsis. Positive blood culture is still the golden standard of sepsis diagnosis. Prophylactic antibiotics are commonly used in neonates, especially preterm infants, and the small blood sample increases false negative rate of the blood culture, leading to missed diagnosis of sepsis. Some studies showed high incidence of urinary tract infection [20, 24], but this could not be confirmed by the present study. This could be due to the low utility ratio of urinary catheter.

The first three pathogens of NI in the present study were Klebsiella pneumonia, Pseudomonas aeruginosa, and Candida albicans, and these three pathogens represented 55% of all episodes of NIs. Some studies found that gram-positive cocci caused the largest proportion of infection, including Methicillin-resistant Staphylococcus aureus (MRSA), and vancomycin-resistant enterococci [25]. A Japanese NI study also showed that their pathogens were dominated by MRSA [26]. However, the study from Washington University showed that the trend was changing over the past five years, and the dominant bacteria in NI was gram-negative bacillus [27]. The study was consistent with the present findings. The bacterial type varied in different areas. However, the common feature was the development of multi-resistant bacteria. Broad-spectrum antibiotics, use of dexamethasone, and prolonged hospitalization were the risk factors of infection [28,29]. The present study did not include the information of drug sensitive tests. The present authors need to further explore on this in the future. Nosocomial fungal infection is a great threat to infants, especially to the preterm infants with devastating consequences [30]. After regular prophylactic use of antifungal drugs to the VLBW infants with broad-spectrum antibiotics, the incident rate is decreasing [31].

Few studies reported seasonal changes of NI in NICU. The present study found no association between seasons and NI. The possible reason may be the setting of the present NICU is an isolation ward. Anyone that enters it must change their clothes and shoes, and wear a cap and mask. Visitors and parents are not allowed to go into the ward. Most of the patients were admitted at their first day of life. NICU is equipped with central air purification. Meanwhile, all the infants in NICU were under incubation, which provided a stable temperature and humidity environment. Hence there was less chance to be infected from outside of the ward. A study from the UK reported the seasonality of invasive Candida infection in preterm low birth weight infants. They collected in total 52 data over eight years and 73% of all cases developed infection during September to February [32]. Another study in NICU of Guangzhou, China showed nosocomial invasive fungal infection occurred in the Spring which was statistically significantly higher than that in other seasons (30). One possible reason of the seasonal trend may be that not all the NICU wards are isolated and the fungal conidia outside could have an influence on the inside fungal conidia level [33].

The present study showed that the birth weight and gestational age were inversely associated with NI. Many studies found the same association with birth weight and gestational age and the rate of NI. Lower birth weight and earlier gestational age increased the risk of NI [4,34,35]. The survey of Vermont-Oxford neonatal network on 36 NICU in the USA found that the incidence of nosocomial sepsis in NICU was 26% in the infants with birth weight 501 g to 750 g; 22% in infants birth weighing from 751 g to 1,000 g; 15% in infants weighing 1,001 g to 1,250 g, and 8% in infants weighing from 1,251 g to 1,500 g [36]. A report of INICC surveillance in 2002 showed the central line-associated bloodstream infection rate in birth weight less than 1,000 g preterm infants was 10.8 / 1,000 central line days, the ventilation associated pneumonia infection rate was 3.3 / 1,000 MV days; the central line-associated bloodstream infection rate in infants with a birth weight from 1,001 g to 1,500 g was 6.4 / 1,000 central line days, the ventilation associated pneumonia infection rate was 2.5 / 1,000 MV days; the central line-associated bloodstream infection rate in infants with birth weight from 1,501 g to 2,500 g was 4.1 / 1000 central line days, ventilation associated pneumonia infection rate was 2.1 / 1,000 MV days; the central line-associated bloodstream infection rate in preterm infants with birth weight heavier than 2,500 g was 3.7 / 1,000 central line days, ventilation associated pneumonia infection rate was 1.4 / 1,000 MV days. Low birth weight and early gestational age were indicators of the severity of disease. However, the research also showed, in multivariate analysis, that early gestational age was the independent risk factor, rather than birth weight [37] and the present study had the same conclusion.

Congenital malformation included digestive tract malformations, congenital heart diseases, and trauma in the present
study, all of which accounted for circa 30% infants in the infected group, and in the non-infected control group it only accounted for 10% infants. The difference was significant. After adjusting for all of the other variables, congenital malformation had around a five-fold risk of NI as compared to the infants without. Congenital malformation contributed to the morbidity and mortality of neonates and infants. A study revealed that gastrointestinal malformations were associated with a mortality rate of 60.9% as compared with 21.1% in those with central nervous system malformations [38]. Digestive tract malformation accounted for around half of malformation in the present study and most of them required surgery. This may partly explain why congenital malformation infants had much higher risk to NI.

A study found perinatal mortality in twins that was much higher than singletons, which was five times more than singletons. The incidence of malformation in twins was three times as compared to former singletons [39]. Birth weight of multiple birth babies was smaller than the singletons. The need to share the nutrition in the uterus, makes them weaker than singletons. The present result showed that twins or triplets had a three-fold risk of NI compared to singletons, even after adjusting for birth weight and gestational age.

In order to provide nutrition and medicine to severely ill infants, UVC or PICC are often needed. However, intravenous interventions were associated with blood stream infection [37]. Central line catheter and the length of catheter were the main risks of NI for neonates, especially for VLBW infants [10, 40]. The present study results contributed more evidence. A study showed that more than five days’ UVC increased the risk of sepsis at least 21 times [40]. Another study found that when duration of PICC in situ was more than nine days, the risk of PICC infection increased three times [41]. Many central line associated infections were caused by Staphylococcus or other pathogens colonized in the skin around catheter. The colonized pathogens could migrate into the blood stream and cause sepsis [10]. A study found that in infants with birth weight less than 1,000 g, because of bacterial colonization in the catheter, taking a blood sample from the central line was one of the risk factors of central line related infection. On the other hand, using heparin and catheter disinfection were protecting factors [42]. The present study result was consistent with this. The present infants with central line catheter had circa a four-fold risk to get infection compared to those without the catheter.

Some studies showed that parenteral nutrition was a risk factor of nosocomial sepsis in preterm infants [40,43]. However in the present study, parenteral nutrition showed minor protective effect on NI (OR=0.94, p = 0.008). Around 45% of patients only received lipid free parenteral nutrition in the present study. Some studies only focused on patients received lipid containing parenteral nutrition [43]. Research has shown that intralipid increased myocardia and bacteremia caused by malassezia species and coagulase negative staphylococci [23]. So the possible reason may be due to the different components of the parenteral nutrition. On the other hand, parenteral nutrition provided the energy and protein to the infants who were severely ill or preterm as they had insufficient intakes. Sufficient nutritional support was important to improve perinatal and long-term outcomes [44]. According to the present study, parenteral nutrition showed some increased risk of NI in the univariate model and this may be due to the close correction of low birth weight and small gestational age. After adjusting for these factors in multivariate model, parenteral nutrition became protective.

The present authors observed that receipt of gastric tube was an independent risk factor of NI in NICU (OR 3.88, p < 0.001), which concurred with previous studies [45]. Using gastric tube might directly damage the gastric mucosa, which was a barrier against infection [46]. Continuous tube feedings also weaken the acid barrier function of the stomach by increasing gastric acidity levels [9]. Repeating insertion was very common in NICU, due to difficulty of fixing the orogastric tube. Research found that there were some conditional pathogenic bacteria, such as staphylococcus epidermidis colonized in the nasopharynx and skin of 78% of infants after two weeks’ admission. Transmission of the bacteria in NICU also occurred by the hands of healthcare staff. A study showed a significant relationship between acquisition of bacteria by patients and oropharyngeal and healthcare instrumentation, including the use of suctioning, and use of gastric feeding tubes [46].

Bacterial colonization is a precursor to NI. The bacterial colonization rate indicates the risk of occurrence of bacterial the infection of the bacterial in NICU. Severe diseases of the newborn and the widespread use of antibiotics in NICU result in conditional pathogenic bacteria colonization in the body. Lactic acid bacteria and bifidobacteria are the normal colonization bacteria, which inhibit the growth of aerobic and facultative anaerobic bacteria, and ultimately achieve the balance of the intestinal flora. There was evidence showing the benefit of enteral probiotics to severe necrotizing enterocolitis (NEC) in VLBW infants [47]. A meta-analysis studied on the prophylactic use of enteral probiotics in prevent antibiotic-associated diarrhea showed the prophylactic use of probiotics reduced the risk of antibiotic-associated diarrhea in neonates (OR=0.28, 95% CI=0.20-0.38). The present study also found that the duration of using prophylactic enteral probiotics was associated with a reduced risk of NI (OR= 0.88, p = 0.005). The longer duration of using prophylactic enteral probiotics, the infants in NICU were less likely to develop NI. Future studies should also focus on the duration of use to guide clinical applications.

According to the present study, the proportion of prophylactic use of antibiotic in NICU infants (90%) were more than that of non-infected infants (70%), and the mean length of using antibiotics (8.7 ± 6.9 days) in infected infants was longer than non-infected infants (4.5 ± 4.8 days), and the difference was significant. After adjusting for the background factors and healthcare procedures related factors, prophy-
lactic use of antibiotics showed a slightly harmful effect of NI (OR 1.13, p < 0.001). The prevalence of prophylactic of antibiotics use was relatively high in the present NICU compared to a general hospital in US, the use of antibiotics in premature infants was much higher, which was 60% in suburban hospital and 43% in the inner city hospital [48]. Such comparison may not be so fair, because of the different composition of patients and healthcare policy. The present hospital included infants requiring surgery and the proportion of preterm infants was relatively high. Although there was biological justification to apply prophylactic antibiotics to preterm infants and infants with invasive intervention, there was no evidence that it could improve the overall mortality of preterm infants at present [49]. A study found that prior use of third-generation cephalosporin was a risk factor for ESBL-producing E. coli or K. pneumonia infection [50]. The present study showed the duration of use prophylactic antibiotics had some harm effect on NI. More researches are needed to evaluate the duration and timing of prophylactic antibiotic use.

The present authors observed that the infants undergoing surgery were five times as likely to get NI in NICU compared to the infants who did not need it. Surgery destroys the integrity of the skin, and foreign bodies, such as drainage, can let pathogens invade into the body more easily. Because of the defects in inherent immunity, and neutrophil activation, preterm infants were more susceptible than older infants. Infants after surgery may have decreased feeding, which worse the situation. The infants who required surgery were often in serious condition and needed to spend more time in hospital. Hence they had more chance to have invasive intervention. The site of surgery, the duration, and the environment of the operating room had an influence on the risk of infection. A retrospective study showed that skin preparation, urinary catheter, and procedure duration were risk factors of surgical site infection (SSI) [51].

**Conclusion**

NI rate of NICU has been increasing in the past decade, which is related to higher morbidity and mortality, prolonged hospital stay and increased hospitalization expenses [3]. A multicenter prospective cohort study found that the mortality of neonates with NI was five-fold as neonates without infection [22]. As the present study showed the NIs in NICU are associated not only with intrinsic factors but also to factors related to the healthcare procedures. Hence, to reduce the NI rate depends on enhancing the healthcare level. The present study is one of the few studies comprehensively assessing the risk factors associated with NIs of NICU in China. The findings of the study could provide a better understanding in the epidemiology of NI in NICU in China, and the relevant evidence for prevention strategies.

The present study identified the infection rate of NI in a NICU of Guangzhou, China. Gestational age < 32 weeks, congenital malformation, twins or triplets, gastric tube feeding, surgical operation, duration of prophylaxis antibiotic use, duration of probiotic use, and duration of parenteral nutrition were the risk or protective factors associated with NI in NICU. Multicenter prospective study may be conducted in the future to investigate specific risk factors on different birth weight or gestational age of China. The present study also provided information for the prevention strategies of NIs and may improve the healthcare service level.

**References**


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Ultrastructural analysis of granulosa cells of IVF patients

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Summary
Objective: To compare the percentage and ultrastructure of normal and abnormal granulosa cells and their effect on fertilization and pregnancy rate between gonadotropin releasing hormone (GnRH) agonist and antagonist treatment. Materials and Methods: In this study, granulosa cells obtained from 22 women undergoing in vitro fertilization (IVF) treatment due to unexplained infertility with either with GnRH agonist (n=11) or GnRH antagonist (n=11) were evaluated by light and electron microscopy. Results: GnRH agonist and antagonist therapy was found to have no effect in terms of abnormal granulosa cell percentage (0.0679 ± 0.08977 vs 0.0481 ± 0.05164; p > 0.05), fertilization [85 (45-90) vs 75 (64-93)] and pregnancy rate (37% vs 46%). Light microscopic observations showed similar features of normal cells of agonist and antagonist-treated cells. Ultrastructural evaluation also revealed that there was no difference between cells of two treatment groups. Conclusion: Both GnRH agonist and antagonist treatment for ovarian stimulation may have similar effects on granulosa cells at the morphological and ultrastructural level, as well as on fertilization and pregnancy rates.

Key words: Granulosa cell; Ultrastructure; In vitro fertilization; Fertilization rate; Pregnancy rate.

Introduction
Controlled ovarian hyperstimulation (COH) protocols are widely used to obtain multiple oocytes in vitro fertilization (IVF) programmes. Suppression of gonadotropin secretion can be achieved with either gonadotropin releasing hormone (GnRH) agonists or antagonists. Both groups of drugs are routinely used for ovarian stimulation to prevent luteinizing hormone (LH) surge. However, the use of GnRH antagonists has been shown to result in lower follicular fluid and serum estradiol concentration than GnRH agonist treatment. It has also been known that application of GnRH antagonist protocol decreases the duration of ovarian stimulation and the incidence of ovarian hyperstimulation syndrome [1-3].

In this study, the authors aimed to compare ultrastructural differences and percentage of normal and abnormal isolated human granulosa cells from follicular aspirates of women undergoing IVF treatment with agonist or antagonist protocol.

Materials and Methods
Patients
Granulosa cells were obtained from 22 women undergoing assisted reproduction due to unexplained infertility and treated either with GnRH agonist (n=11) or the GnRH antagonist (n=11). Patient characteristics are shown in Table I.

Stimulation protocols
The 11 patients underwent controlled ovarian hyperstimulation consisting of luteal long leuprolide acetate and recombinant follicle stimulating hormone (FSH) using the step-down protocol. When desensitization was achieved, as evidenced by plasma E2 levels of ≤ 50 pg/ml, the absence of ovarian follicles and endometrial thickness ≤ six mm on transvaginal ultrasound examination [4], daily s.c. injection of recombinant FSH was commenced. The starting dose of gonadotropin was determined based on the age of the female, antral follicle count at baseline transvaginal ultrasonography, day 3 FSH and E2 levels, body mass index (BMI), and previous ovarian response, if available.

The 11 patients in the GnRH antagonist group underwent COH consisting of cetrorelix and recombinant FSH, using the step-down protocol. When desensitization was achieved, as evidenced by plasma E2 levels of 50 pg/ml or less, the absence of ovarian follicles and endometrial thickness ≤ six mm on transvaginal ultrasound examination, [5] a daily s.c. injection of recombinant FSH (Gonal-F) was commenced. The starting dose of gonadotropin was determined based on female age, antral follicle count at baseline transvaginal ultrasonography, day-3 FSH and estradiol (E2) levels, BMI, and previous ovarian response, if available. Flexible GnRH antagonist protocol was used. If serum E2 level was more than 600 pg/ml and/or if leading follicle exceeding 14 mm in diameter were present, cetrorelix 0.25 mg was initiated as daily injections up to the day of oocyte pick-up. Ovarian response was monitored with frequent serum E2 measurements and transvaginal ultrasonography, as described previously [6].

The criterion for human chorionic gonadotropin (hCG) administration was the presence of three or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration.

Standard procedures were carried out for gamete-embryo handling and cleavage-stage ET, or blastocyst transfer was performed under abdominal ultrasonography guidance in all cases using a soft catheter. The luteal phase was supported by daily vaginal progesterone suppositories starting at one day after oocyte pick-up. Clin-
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Gestational pregnancy was defined as the presence of an intrauterine gestational sac by transvaginal ultrasonography. The ethical review board of our university approved the study protocol.

**Granulosa cell isolation**

Granulosa cells collected from follicular fluid after oocyte retrieval procedures was pooled in a tube and centrifuged at 500 g for ten minutes. After centrifuging, supernatant was removed, cumulus cells were then fixed in 2.5% glutaraldchyde dissolved in 0.1M phosphate buffer for two hours at room temperature for electron microscopic evaluation. After washing three times for ten minutes in 0.1M phosphate buffer (pH 7.4), cells were post-fixed with 1% osmium tetroxide. The cumulus cells were subsequently infiltrated and embedded in araldite (Epon812, EMS) after dehydrating in an ethanol gradient at room temperature. Semi-thin (one-µm thick) sections for light microscopy were stained with toluidine blue. Abnormal cells had smaller and denser nuclei than normal ones. Cytoplasm of abnormal cells were also stained darker than normal cell’s cytoplasm. Lipid droplets were observed both in normal and abnormal cells (Figure 1).

**Statistical analysis**

Shapiro-Wilk test was used to test the assumption of normality of groups. Mann-Whitney U test was used as appropriate statistical comparison because of the groups were not distributed normally (p < 0.05). Fisher’s exact test was used to compare categorical variables.

Data are expressed as median (interquartile range). Differences were considered significant when p < 0.05. Box plots were achieved with MS Excel 2010 software.

**Results**

**Light microscopy observations**

Observations were carried out at x100 magnification on three fields randomly chosen by means of a DM 6000B microscope and photographed using a digital microscope camera. Both GnRH agonist and antagonist-treated groups showed the presence of two morphologically different cell populations: normal and abnormal cells. Toluidine blue, x100.

**Ultrastructural observations**

Normal and abnormal cell populations were also characterized detailed with transmission electron microscopy. Cells displaying round, euchromatic nuclei with normal perinuclear cisternae, were identified as normal. These cells had well-developed endoplasmic reticulum and mitochondria with tubular cristae as well as number of lipid droplets in their cytoplasm (Figure 2).
Abnormal cells were characterized as shrunken cells with heterochromatic nuclei. Although the perinuclear cisternae were normal, the contour of the nuclei were irregular. The cytoplasm of these cells were examined condensed and poor in organelles. The ratio of cytoplasm and nucleus were changed. The volume of cytoplasm was decreased (Figure 3).

Both in normal and abnormal cells in agonist and antagonist group were similar in ultrastructural level. In agonist and antagonist group, normal granulosa cells had euchromatic nucleus with normal cytoplasmic organelles. Also abnormal granulosa cells of both groups had shrunk cytoplasm with poor organelles and heterochromatic nuclei with irregular contour. In some of the normal and abnormal aspirated granulosa cells of both agonist and antagonist groups had still intercellular junctions.

**Statistical results**

When comparing the two groups of women under investigation, the mean percentage of abnormal cells was similar between them (0.0679 ± 0.08977 vs 0.0481 ± 0.05164; p > 0.05). The mean percentage of normal cells was also similar between agonist and antagonist group (0.9321 ± 0.08977 vs 0.9519 ± 0.05164; p > 0.05) (Figure 4).

**Therapeutic outcome parameters**

Patient and cycle characteristics, fertilization rates, embryo data and clinical outcomes of the two groups under investigation are shown in Table 1. All parameters investigated were similar between the two stimulation protocols (p > 0.05).

GnRH agonist and antagonist therapy was found to have no effect in terms of abnormal granulosa cell percentage (0.0679 ± 0.08977 vs 0.0481 ± 0.05164; p > 0.05), fertilization [85 (45-90) vs 75 (64-93)] and pregnancy rate (37% vs 46%).

**Discussion**

Granulosa cells surrounding the oocytes support oocyte development in several ways. They provide nutrients for the growing oocyte and they produce hormones. They also control both nuclear and cytoplasmic maturation of the oocyte selected for ovulation. It is also known that numerous gap junctions have been observed among the granulosa cells [8] and between the oocyte and the granulosa cells [9]. These gap junctions enable the passage of nucleotides, amino acids and sugars from granulosa cells to the oocyte for its growth and development [10]. Cellular changes in granulosa cells could influence oocyte quality during follicular development and also after the ovulation [11].
In the present study, the authors aimed to compare the percentage of normal and the abnormal granulosa cells and their effect on fertilization and pregnancy rate between GnRH agonist and antagonist treatment. They also detailed the changes in granulosa cell morphology and ultrastructure by light and electron microscopy.

There are studies that report the morphological and ultrastructural properties of granulosa cells, relation between the granulosa cell apoptosis and assisted reproductive technology outcome. Most of the studies focused on apoptosis as cellular injury. In some them, the overall cellular changes in granulosa cells were investigated. Giampietro et al. [8] found that granulosa cells apoptosis were comparable between GnRH agonist and antagonist therapy [12]. Another study revealed that fewer apoptotic granulosa cells in women who had an ongoing pregnancy after IVF treatment than in women who did not conceive [13]. Nakahara et al. have shown that lower incidence of apoptotic bodies in individual follicles is associated with better outcomes for oocytes [14]. Another study from Nakahara group also revealed that the incidence of apoptotic bodies was significantly higher in mural granulosa cell masses than in cumulus cell masses.

They concluded that the incidence of apoptotic bodies in mural granulosa cell masses could be used as an indicator of IVF success [15]. A study by Rotmensch et al. described two different granulosa cell morphology in IVF patients. Cells associated with non-fertilizable oocytes had significantly smaller cell areas, tended to be tightly packed, and exhibited abundant intercellular gap junctions and adherence junctions, whereas cells associated with fertilized oocytes tended to be widely dispersed, frequently contained interiorized gap junctional elements and showed morphological correlates of high steroidogenic activity [16]. Ultrastructural properties of human granulosa cells were also studied by Krajci et al. in 1989. In this study, early and late preluteinized granulosa cells were characterized by the presence of high amount of pale smooth endoplasmic reticulum (SER), a number of osmiophilic granules of various size and electron density, as well as of polymorphic mitochondria with tubular cristae in large/pale cells. They also described small/dark cell ultrastructure as shrunken cell with an indented heterochromatic nucleus, numerous free ribosomes, scarce SER, and only a few mitochondria exhibiting condensed cristae [18].

The present authors studied overall morphological and ultrastructural changes in granulosa cells under two different stimulation protocols in assisted reproductive technology patients similar with Centurione et al.’s study [18]. However the present results were not consistent with this study. The present findings suggest that there was no difference in terms of the percentage of normal and abnormal granulosa cells between GnRH agonist and antagonist treated groups. Morphological and ultrastructural findings were also similar for normal cells of agonist and antagonist treated groups, as well as abnormal cells. In conclusion, both GnRH agonist and antagonist treatments for ovarian stimulation may have similar effects on granulosa cells at morphological and ultrastructural level, as well as on fertilization and pregnancy rates.

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Ultrastructural analysis of granulosa cells of IVF patients


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Pregnancy-induced hypertension (PIH) is a multifactorial disease manifested due to a complex combination of environmental factors and several predisposing genes including factors in the renin angiotensin (RA) system [1]. It is estimated to affect 6% to 8% of US pregnancies [2]. A recent report indicated women with PIH were at increased risk of preeclampsia, cesarean delivery, renal dysfunction, and placental abruption; associated risks to the fetus include intrauterine growth restriction, preterm delivery, low birth weight, and neonatal intensive care unit (NICU) admission [3]. Nonetheless, the pathogenesis of the disease still remains enigmatic.

It has been known that the RA system plays a key role in blood pressure regulation [4]. Therefore, many investigators have postulated and proved that alterations in the RA system play a significant role in the pathophysiology of PIH [5, 6]. As one of the main components of the RA system, human angiotensin II type 1 receptor (AT1R) has been recently cloned and localized to chromosome 3q [7]. Moreover, it might be a plausible candidate for susceptibility to PIH in a previous study [8]. In addition, a polymorphism of the AT1R gene which was an adenine/cytosine (A/C) base substitution at position 1166 was identified, and an increased prevalence of the C allele in hypertensive disorders was found [9]. Szombathy et al. also confirmed that the A1166C variant of AT1R significantly increased the risk of PIH [10]. However, Schmidt et al. [11] and Takami et al. [12] were against the association that AT1R A1166C polymorphism could increase risk of PIH.

It is clearly observed that an inconsistent result of previous studies existed on the association between the AT1R A1166C polymorphism and the risk of PIH. This may be resulted from the small sample size of the researched patients and/or the different ethnicity of the patients. To provide the current best evidence, the present authors conducted a meta-analysis of case control studies with the aim to show the relationship of AT1R A1166C polymorphism and the risk of PIH explicitly.

### Materials and Methods

#### Search strategy

The authors performed the pre-established search strategies and retrieved literatures in a systematic way from the PubMed, MEDLINE, Springer, China National Knowledge Infrastructure (CNKI) and Wanfang database with the retrieval deadline of January 24th, 2014. The keywords used for all searches were in three aspects: 1) “pregnancy-induced hypertension”, “gestational hypertensive disorders”; 2) “AT1R”, “Angiotensin-II type 1 receptor”, “AGTR1”; AND 3) “polymorphism”, “genetic”, “variant”. There were no language restrictions of the retrieved literatures. In addition, a manual search of print documents and the citations from relevant original studies and review articles was retrieved for any additional studies.

### Summary

**Purpose:** The purpose of this study was to perform a quantitative review of previous case control studies examining the association between AT1R A1166C polymorphism and pregnancy-induced hypertension (PIH). **Materials and Methods:** Odds ratio (OR) and 95% confidence intervals (CI) were used as measures of effect sizes. Overall effect sizes were derived using a random-effects model or fixed-effects model when appreciated, and stratified by ethnicity. Funnel plots and Egger’s regression asymmetry tests were utilized for publication bias detection. **Results:** A total of ten articles (including 920 PIH cases and 1408 controls) were included in this meta-analysis. The overall effect sizes (OR = 2.14, 95% CI: 1.54 - 2.98, p < 0.00001) of additive model indicated PIH patients had a significant higher frequency of allele C. Meanwhile, the OR of the dominant model was 2.22 (95% CI: 1.51 - 3.26, p < 0.00001) which signified that PIH patients also had a significant higher frequency of AC+CC genotypes. The subgroup analyses were in line with the overall outcomes except the Caucasians PIH patients had a non-significant CA+CC genotypes (OR = 1.37, 95% CI: 0.95 - 1.98, p > 0.05). The Egger’s test of additive model (p = 0.451) and dominant model (p = 0.623) revealed no statistical significance for publication bias. **Conclusion:** The meta-analysis suggested that the AT1R A1166C polymorphism was significantly associated with the PIH, especially in Asian subjects.

**Key words:** AT1R; A1166C polymorphism; Meta-analysis; Pregnancy-induced hypertension; Previous case control studies.
Inclusion and exclusion criteria

Studies included in the present meta-analysis should meet the following criteria: 1) research design was case-control study; 2) study object was human beings; 3) the case group were pregnant women with PIH, and the control group were healthy pregnant women or with other kinds of hypertension; 4) the articles were studies of the association between AT1R A1166C polymorphism and PIH; 5) the genotype of AT1R A1166C should be provided or could be calculated out from the data of the studies, and the data should conform to the Hardy-Weinberg equilibrium (HWE). Besides, references would be excluded when the following conditions appeared: 1) the case and the control were family members or close relatives; 2) article was non-original literature such as review, letters, and comments; 3) paper was re-publication or the literature was used with same population data.

Data abstraction and quality evaluation

Articles were reviewed and filtered out independently by two investigators according to the prior criteria. Then the data were extracted independently in duplicate using a standardized form to assess the eligibility for inclusion. In brief, information were tabulated according to article’s first author’s name, year of publication, research conducted region, age and gestation of the pregnant women, the sample size of the case, and control group of each study. When completed, the information tables were exchanged and checked. Any discrepancies were resolved by discussion and by referencing to the original publication.

The quality of the articles was evaluated according to the standard of Clark’s study [13]. There were ten terms in the standard, and each item was recorded for one score. In this scoring system, the article was regarded high quality literature if the evaluation score was above 5; otherwise, the literature was poor quality and was not suitable for comprehensive evaluation in the meta-analysis [14].

Statistical analysis

This study aimed at investigating whether there were associations between the AT1R A1166C polymorphism and risk of PIH of the pregnant women. Of the included researches, the HWE should be firstly calculated and displayed. If the p-value of the HWE less than 0.05, there was considered significant imbalance of the studied objects and the study were excluded. Then the effect size of adjusted odds ratio (OR) with 95% CI of the additive model (C vs. A) and dominant model (CA + CC vs. AA) were pooled in order to assess the relationship between the A1166C polymorphism and PIH.

Heterogeneity among studies was evaluated by the Cochran Q test and the I² parameter [15]. In the tests, \( p < 0.05 \) or \( I^2 > 50\% \) was considered to be heterogeneous. When substantial heterogeneity was detected, the authors calculated summary OR and their 95% CI with the random effects model. If not, the pooled estimate was presented based on the fixed effects model.

The authors further conducted subgroup analysis according to ethnicity (Caucasians /Asian/ Chinese) to investigate the impacts on the present outcomes. Publication bias was also assessed by the funnel plot with Egger’s regression asymmetry test [16, 17]. In addition, HWE and Egger’s regression were performed using Stata 11.0, while the OR (95%CI) and funnel plot were displayed by software RevMan5.1.

Results

Literature retrieval

The procedures and the outcomes of the included literatures are clearly shown in Figure 1. According to the pre-established search strategies, the authors achieved 368 articles from PubMed, MEDLINE, Springer link, CNKI, and Wanfang database. A total of 345 repeated and obvious irrelevance articles were excluded out of the outcomes. Of the rest 23 studies, the authors reviewed the titles, abstracts, and the full texts only in ten articles (three reviews; two comments; two animal trials; five not about AT1R A1166C and PIH; one AC or CC genotype in both groups was zero) met the criteria and were included into the meta-analysis [1, 18-26]. Besides, all the ten included studies were consistent with the HWE, and there were no additional articles obtained from the manual search.
AT1R A1166C polymorphism and risk of pregnancy-induced hypertension: a meta-analysis of case control studies

Study characteristics and quality assessment

The characteristics and information of the included studies are shown in Table 1 [1, 18–26]. The ten included articles were all case control studies and were published between 2000 and 2008. A total of eight articles were studied in Asia, and the other two were conducted in Europe. Parts of the studies were lack of the age and the gestation of the pregnant woman. The total samples of the ten articles were 2,328 which included 920 PIH cases and 1,408 controls. Besides, the qualities evaluated of the included articles were arranged from six to eight, which meant all the included studies were high quality researches.

Meta-analysis

In overall analysis of the ten selected studies, the heterogeneity test of the additive model (C vs. A) showed that there were significant heterogeneities (\( p = 0.007, F = 61\% \)). Therefore, the random effect model could be applied to analyze the effect sizes. The pooled estimates of OR was 2.14 (95%CI: 1.54 - 2.98, \( p < 0.00001 \); Figure 2), indicating the

Table 1. — Characteristics of ten studies on AT1R A1166C and pregnancy-induced hypertension.

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<td>Kobashi 2004</td>
<td>8</td>
<td>Japan</td>
<td>Asian</td>
<td>29.7±0.5, 29.2±0.3</td>
<td>36.6±0.3, 39.1±0.1</td>
<td>102</td>
<td>108</td>
<td>71, 29, 2</td>
<td>96, 12, 0</td>
<td>0.370</td>
<td>0.5410</td>
</tr>
<tr>
<td>7</td>
<td>Liao 2007</td>
<td>7</td>
<td>China</td>
<td>Asian</td>
<td>27.8±2.9, 27.7±2.7</td>
<td>38.6±2.7, 39.0±1.9</td>
<td>102</td>
<td>108</td>
<td>71, 29, 2</td>
<td>96, 12, 0</td>
<td>0.370</td>
<td>0.5410</td>
</tr>
<tr>
<td>8</td>
<td>Jiang 2008</td>
<td>6</td>
<td>China</td>
<td>Asian</td>
<td>26.4±4.1, NP</td>
<td>NP</td>
<td>87</td>
<td>175</td>
<td>58, 28, 1</td>
<td>137, 38, 0</td>
<td>2.596</td>
<td>0.1071</td>
</tr>
<tr>
<td>9</td>
<td>Li 2008</td>
<td>6</td>
<td>China</td>
<td>Asian</td>
<td>27.0±6.5, NP</td>
<td>NP</td>
<td>88</td>
<td>113</td>
<td>43, 35, 10</td>
<td>64, 46, 3</td>
<td>2.806</td>
<td>0.0939</td>
</tr>
</tbody>
</table>

NP: Not provided; HWE: Hardy-Weinberg equilibrium.

Figure 2. — Forest plots of the frequency of allele C in AT1R A1166C in pregnancy-induced hypertension patients vs. the controls. Squares represent the effect size for the odds ratio of the frequency of allele C in AT1R A1166C in pregnancy-induced hypertension patients vs. the controls. Size of the squares is proportional to the size of the cohorts. Error bars represent 95% confidence intervals (CI). The diamond shape represents the pooled estimates within each analysis.
frequencies of allele C were significantly higher in PIH patients than those in control group.

The statistical heterogeneity of the studies of dominant model (CA+CC vs. AA) were also significant ($p = 0.003, I^2 = 64\%$), thus the random effects model was used for the analysis. Besides, the pooled OR (95% CI) of the dominant model was 2.22 (95% CI: 1.51 - 3.26, $p < 0.0001$, Figure 3) which demonstrated women with AC+CC genotypes were significantly higher in PIH patients.

### Subgroup analysis

The subgroup analysis by ethnicity (Caucasians/Asian/Chinese) of additive model and dominant model are presented in Table 2. According to the outcomes, the fre-
frequencies of allele C (Caucasians: \(OR = 1.49, 95\% CI, 1.11-1.98\); Asian: \(OR = 2.47, 95\% CI, 1.64 - 3.73\); Chinese: \(OR = 2.61, 95\% CI, 2.62 - 4.22\)) and women with AC+CC genotypes (Asian: \(OR = 2.62, 95\% CI, 1.67, 4.11\); Chinese: \(OR = 2.77, 95\% CI, 1.64, 4.68\)) were significantly higher in PIH patients, which were in accordance with the overall analysis above. In addition, the total estimate of the AC+CC genotypes of the Caucasians \(\left(OR = 1.37, 95\% CI, 0.95 - 1.98, p > 0.05\right)\) also indicated the PIH patients processed more AC+CC genotypes, but the result was not significant.

**Publication bias**

The funnel plots of the additive model (C vs. A) and dominant model (CA + CC vs. AA) in this meta-analysis are shown in Figures 4 and 5. The distribution of the points in the two figures seemed symmetrical which indicated there were no significant publication biases of the included studies. In addition, the Egger’s test of also additive model \((p = 0.451)\) and dominant model \((p = 0.623)\) also revealed no statistical significance for publication bias.

**Discussion**

Many studies on the association of the **AT1R A1166C** polymorphism and risk of PIH have been published in recent years [9, 27-29]. However these studies have shown mixed results due to small sample sizes or low statistical power. In the present meta-analysis, the authors combined and reanalyzed ten studies which contained 2,328 patients (920 PIH cases and 1408 controls cases) in order to achieve an integrative knowledge of **AT1R A1166C** polymorphism and risk of PIH.

The meta-analysis of the case control studies in present work indicated that PIH patients had higher frequencies of allele C and AC+CC genotypes compared to the controls. It was consistent with previous studies that C1166 of the **ATIR** gene was significantly associated with the risk of PIH in Polish [20] and Caucasian [8] subjects. In addition, a case-control study performed on some PIH patients in France also revealed a statistically significant increase in allelic frequency of C1166 in hypertensive subjects when compared to normotensive ones [9]. Therefore, this analysis confirmed that higher frequencies of allele C and AC+CC genotypes were related to the increasing risk of PIH.

It has been known that genes coding for components of the RA system involved in blood pressure regulation and vascular smooth muscle cell proliferation were considered to be candidate genes for risk factors for PIH as well as essential hypertension. **AT1R**, as one kind of the main components of the RA system were reported the mediators of vasoconstrictive function and salt distribution due to RA system [30]. Jiang et al. confirmed the associations between the C1166 allele of the **ATIR** gene and PIH [28]. Otherwise, the allele C1166 has been reported to be associated with aortic stiffness which might lead to high blood pressure [31], and the polymorphism was found to be associated with salt sensitivity in hypertensive patients [32]. Thus, the **AT1R A1166C** polymorphism might increase the risk of PIH. However, the molecular and biochemical mechanism by which the A1166C variant of the **ATIR** gene was involved in the manifestation of PIH was still obscure as the variable nucleotide was located in the 3’ untranslated region [33, 34].

In this meta-analysis, the included articles were all high quality researches which could decrease the selection bias and increase the reliability of our outcomes. Besides, the funnel plots and Egger’s tests proved there were no significant biases in the present authors’ studies. It signifies that the unpublished and missing retrieved articles would not significantly affect the present results. However some limitations of this study should be discussed. First of all, the articles included in the meta-analysis were few, especially studies of Europe subjects after they were stratified by ethnicity. Thus more high quality researches were needed to verify the stability of the results. Secondly, some information such as the age and gestation of the patient were not provided, and these factors might influence our outcomes. Last but not the least, there was no grey literature retrieved and included, so the study results may overstate the **AT1R A1166C** role on the risk of PIH.

**Conclusion**

The meta-analysis suggested that **AT1R A1166C** polymorphism may increase the risk of PIH development.

**References**


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Uterine artery pulsatility index and diastolic notch laterality according to the placental location

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² International Hospital Department of Gynecology and Obstetrics, Istanbul (Turkey)

Summary
Purpose of investigation: The authors aimed to determine the effect of placental site on uterine artery pulsatility index (PI) values and tendency for laterality of uterine artery diastolic notch in singleton pregnancies. Materials and Methods: All medical records of singleton pregnancies whose first trimester screening was done between years of 2004-2014, were evaluated retrospectively. Singleton pregnancies with detected/suspicious anatomical or genetic fetal anomalies, any systemic disease, familial genetic diseases, artificial reproduction techniques or missing data were excluded. Mean left and right uterine artery PI values and diastolic notch laterality ratios according to placental sites were determined and compared. Results: A total of 2,067 singleton pregnancies were included in data analyses. Mean gestational age was 12.57 ± 0.61 weeks. Left and right uterine artery PI was 1.42 ± 0.47 and 1.48 ± 0.56, respectively. Uterine artery notch was present in 18.86%. PI measurements did not show any statistical difference according to placental locations. Uterine artery notch laterality ratios according to common placental sites are as following; in anterior location (n=190) 67% bilateral, 21% left sided, 12% right sided; in posterior (n=136) 66% bilateral, 18% left sided, 16% right sided. Conclusion: The placental site has no effect on uterine artery PI values and the laterality of uterine artery notch in singletons.

Key words: Fetus; Singleton; Placenta; Uterine artery; Pulsatility index; Diastolic notch; Fetal ultrasonography.

Introduction

Imperfect invasion of endometrial tissue by trophoblasts results in an altered blood flow pattern and vascular dynamics of uterine artery as in case of preeclampsia [1]. Thus, evaluation of vascular dynamics of uterine artery using color Doppler ultrasonography (USG) takes a routine part in the prediction and follow of preeclampsia within gynecology and perinatology clinics [2-7]. In this retrospective study the authors aimed to determine the probable effect of fetal placental location on some parameters of vascular dynamics of uterine artery, namely magnitude of uterine artery pulsatility index (PI) and presence and laterality tendency of diastolic notch of uterine arteries on both sides.

Materials and Methods

All medical records of singleton pregnancies, whose first trimester screening was done between years of June 2004 and January 2014, were evaluated retrospectively. Singleton pregnancies with detected/suspicious anatomical or genetic fetal anomalies, any systemic disease, familial genetic diseases, artificial reproduction techniques or missing data were excluded. Sonographic examinations were performed transabdominally using one type of ultrasonography machine. As depicted previously in literature, by means of color Doppler USG, bilateral uterine arteries crossing the external iliac arteries were identified. Pulsed wave Doppler USG was performed to obtain three similar consecutive uterine artery waveforms and PI were measured with the angle of incidence less than 30 degrees. The uterine artery PI was measured and diastolic notch was noted if present, on both sides [2, 3]. Mean left and right uterine artery PI values and diastolic notch laterality ratios according to placental locations were determined and compared statistically. Statistical analyses were performed using SPSS statistics software. Ap value was set as < 0.05 for significance.

Results

In accordance with inclusion and exclusion criteria, a total of 2,067 singleton pregnancies were included in the data analyses. Mean maternal age was 29.44 ± 4.60 years. Mean crown-rump length was 63.05 ± 8.36 mm. Mean gestational age was 12.57 ± 0.61 weeks. Gender distribution was as female 44% and male 56%. Fetal placental sites and ratios were as following; anterior location 47%, posterior location 42%, lateral location 7%, and fundal location 4% (Figure 1). Mean uterine artery PI measurement was 1.46 ± 0.51. Left and right uterine artery PI values were accordingly 1.42 ± 0.47 and 1.48 ± 0.56; there was found no statistical difference between left and right PI measurements with paired t-test (p = 0.198). Both left and right PI measurements did not show any statistical difference according to the placental locations (Table 1).

Unilateral or bilateral uterine artery notch was present in 390 pregnant women (18.86%). Uterine artery notch later-
R.N. Ergin, M. Yayla

Table 1. — Uterine artery pulsatility indices according to the placental locations.

<table>
<thead>
<tr>
<th>Placental Location</th>
<th>Right uterine artery pulsatility index</th>
<th>Left uterine artery pulsatility index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>1.42 ± 0.54</td>
<td>1.51 ± 0.54</td>
</tr>
<tr>
<td>Posterior</td>
<td>1.42 ± 0.43</td>
<td>1.50 ± 0.47</td>
</tr>
<tr>
<td>Lateral</td>
<td>1.46 ± 0.40</td>
<td>1.53 ± 0.58</td>
</tr>
<tr>
<td>Fundus</td>
<td>1.37 ± 0.52</td>
<td>1.38 ± 0.65</td>
</tr>
</tbody>
</table>

Discussion

In the pathophysiology of preeclampsia, defective trophoblastic invasion of endometrial tissue alters blood flow pattern and vascular dynamics of the uterine artery [1]. This is the reason why during pregnancy both uterine arteries are routinely evaluated with color Doppler USG for any altered pattern in vascular dynamics, to predict the risk of preeclampsia within obstetrics and perinatology clinics [2-7].

Both transvaginal and transabdominal Doppler USG has proved to be effective in the evaluation of the vascular dynamics of the uterine arteries [4, 5]. In this present study the authors have performed uterine artery Doppler studies with transabdominal Doppler USG, in accordance with the methodological criteria described elsewhere [2, 3].

The evaluation of uterine artery PI at 11-14 gestational weeks has proved to be effective in identifying high ratio of pregnancies to develop severe preeclampsia or restricted fetal growth [5]. Although generally direct measurements of left or right uterine arteries’ PIs or mean of them are used to predict preeclampsia and some other pregnancy related adverse events, lowest PI measurement has been found to have higher detection rate for early preeclampsia compared to highest or mean PI measurements [6].

In a previous study, reference values for mean uterine artery PI were calculated in singleton pregnancies at 11-41 weeks of gestation [7]. Mean uterine artery PI measurements were slightly lower compared to this reference, which might be the result of the different populations studied. In a controlled study of 131 pregnancies with unilateral increased uterine artery PI and normal mean pulsatility at 20–22 gestational weeks, placental location, central versus lateral placenta, was not found to be associated with any effect on uterine artery PI measurements [8]. As well, unilaterally increased uterine artery PI with normal mean pulsatility was not found to be associated with any adverse pregnancy outcome compared to controls [8]. Similarly, with more detailed placental location, the present authors found no effect of placental location on uterine artery PI measurements.

One study, performed to assess the effect of placental location on uterine artery PI, concluded that ipsilateral artery PI measurements were consistently lower than contralateral ones in both singleton and twin pregnancies [9]. However, patterns of PI change throughout the pregnancy were found to be different between singleton and twin pregan-
cies; steady decrease in singleton pregnancies continued to term whereas it lasted until 27 weeks of gestation in twin pregnancies, and it remained same thereafter [9]. In addition, at any gestational weeks PI measurements were lower in twin pregnancies compared to singleton pregnancies on both sides [9].

In the literature, the presence of diastolic notch has been reported to be a predictor of poor maternal and perinatal outcome [10-13]. In a study in which 481 pregnancies with selected lateral placentas were evaluated at 22-24 weeks of gestation, the ones with mean resistance index above 90 percentile and diastolic notch were found to have higher risk of subsequent development of pregnancy induced hypertension and intrauterine growth retardation [10].

Presence of notch has been associated with increased resistance index of uterine arteries, higher risk of fetal growth retardation, and cesarean delivery due to fetal distress as well as need for neonatal intensive care unit more than 48 hours [11].

In a study, in which 31 selected preeclamptic women at a mean of 30.1 ± 3.0 gestational weeks were evaluated with their diastolic notch patterns as a prediction of any pregnancy related adverse effect [12]. Diastolic notches were classified as grade I notch (nadir in early diastole higher than half of peak diastolic notch velocity) and grade II (nadir in early diastole lower than half of peak diastolic notch velocity) [12]. The risk of delivery before 32 weeks and newborn spending more than 48 hours in neonatal intensive care unit were significantly higher in ones with diastolic notch [12]. In another study, presence of notch especially bilateral, alone or together with increased resistance index, was associated with increased risk for preeclampsia [13]. Likewise, some other parameters as notch depth index, representing the measured depth of the notch, has been shown to have higher positive predictive value for small for gestational age infant and preeclampsia [14]. In that study similarly, the pregnancies were evaluated for notch at a mean of 20.1 ± 2.0 gestational weeks and the presence of notch was noted in 20% of pregnancies [14]; 64% had midline and rest had lateral placental location and respective ratios of notch presence were 20% and 22% and an insignificant difference was found [14]. Ipsilateral uterine arteries however had significantly lower resistance index measurements compared to the contralateral ones [14]. Again, bilateral notch was associated with increased risk of preeclampsia [15]. Generally, the reported prevalence in the literature is between 4.5% and 38 % [15, 16].

In another study, 654 pregnancies were followed up for uterine artery PI plus waveforms and were grouped according to the placental location as midline, fully lateral, and in-between groups [17]. The ratios of diastolic notch were 14-16% at 20th gestational week and 5-6% at 32nd gestational weeks in placental groups with lower prevalence of preeclampsia (3-4%) [17]. Full lateral placental group notch was mentioned to be contralateral in more than half of the cases (no clear data given) [17]. The study concluded that preeclampsia can be predicted with diastolic notch taking gestational age and considering placental locations, highest sensitivity at 24th gestational week with bilateral notching, central, and in-between locations [17]. In this present study the diastolic notch was found to be 19% at a mean of 12.57 ± 0.61 gestational weeks and in the lateral group the laterality of the notches was ipsilateral in higher proportion without any statistical significance.

Conclusions

The placental site does not seem to have effect on uterine artery PI values and the laterality of uterine artery notch.

References


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Comprehensive effect assessment of medical nutrition guidance during pregnancy towards the health of mothers and children

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Department of Obstetrics, Tongzhou Maternal and Child Health Hospital, Beijing (China)

Summary

Objective: This study evaluates the effects of medical nutrition guidance provided to pregnant women to reduce pregnancy complications and occurrence of low birth-weight children and macrosomia. This guideline aims to provide the basis for improving birth delivery outcomes. Materials and Methods: A randomized controlled method was used. A total of 261 parturient women who enrolled in regular pregnancy testing were sampled and selected. The subjects were randomly divided into experimental group (EG, n = 124) and control group (CG, n = 132). The differences in maternal health, pregnancy outcomes, and newborn health between the two groups were compared. Results: Hypertensive disorders in pregnancy and gestational diabetes risks in EG were significantly lower than those in CG (p < 0.05). The cesarean section rate decreased (EG 36.29%, CG 51.50%), and the vaginal delivery rate increased (EG 63.71%, CG 51.50%). The incidence of macrosomia in EG was significantly lower than that in CG (p < 0.05). Conclusion: Medical nutrition guidance during pregnancy improves the perinatal outcomes of mothers and children.

Key words: Medical nutrition guidance during pregnancy; Pregnancy nutrition; Maternal health; Newborn health.

Introduction

Excessive intake of nutrients and nutritional imbalance during pregnancy can affect the perinatal outcomes of mothers and children. Given the increasing improvement of social living standards, nutrients during pregnancy have been excessively consumed in recent years. This excessive consumption has led to excessive weights of pregnant women. Maternal obesity significantly increases incidences of pre-eclampsia, gestational diabetes, and low birth weight. Macrosomia (high birth weight), shoulder dystocia, and abnormal labor progress increase incidences of cesarean delivery and postpartum hemorrhage [1-4]. A large number of studies have shown that nutrition guidance and weight management during pregnancy can effectively control weight increase, with high probability of preventing complications in mothers and children. Therefore, this guidance is conducive to the outcomes of mothers and children. Weight gain during pregnancy and nutritional status directly affect the process and outcome of pregnancy, fetal posterior development, and postnatal physical condition of women. Therefore, performing an individualized nutrition supply and weight management to pregnant women from early pregnancy stage is necessary. This individualized guidance reduces pregnancy complications and incidence of abnormal delivery and improves maternal and newborn health levels [5, 6], providing significance to perinatal work. This study provided medical nutritional guidance to pregnant women in the first trimester of pregnancy to achieve improved perinatal outcomes.

Materials and Methods

Data were obtained from 261 primiparous parturient women aged from 18 to 40 years. The subjects enrolled in regular pregnancy testing in the Department of Obstetrics, Tongzhou Maternal and Child Health Hospital from January 2011 to December 2011. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Tongzhou Maternal and Child Health Hospital. Written informed consent was also obtained from all participants.

Inclusion criteria included subjects who were healthy before pregnancy, had at least 12 weeks of live births pregnancy, and had live-birth newborns. Exclusion criterion was incomplete clinical data including one case of non-specific birth weight, two cases of abortion because of prenatal-diagnosed fetal malformations, one case of miscarriage during pregnancy (fetus was < 28 week gestation), and one case of non-gestational week. Exactly 256 cases had complete data.

The grouping design used the comparison research method. Based on the principles of voluntariness and informed consent, 124 pregnant women were randomly selected for the nutritional guidance. A total of 132 pregnant women whose conditions of age, economic status, and place of residence were roughly similar to those of the experimental group (EG) were selected and set as the control group (CG). The outcome indicators mainly included general demographic data, incidence of complications during pregnancy, birth weight, and newborn health.
A researcher was assigned to investigate and record the material age, pre-pregnancy weight, pre-pregnancy lifestyle, and eating habits of the subjects. The pre-pregnancy body mass index (BMI) was calculated based on height. The Abbott pregnancy nutrition software was used to develop a dietary regimen, which was modified according to different dietary habits, to perform individualized target nutrition guidance. The following data were obtained: basic situations of pregnant women, total weight gain during pregnancy, daily nutrient calorie intake per kilogram body weight during pregnancy, gestational weeks, pregnancy complications (including pre-eclampsia, anemia, and gestational diabetes), and delivery mode.

The main focuses of nutrition education were significance of reasonable diet during pregnancy, risk factors of nutrition imbalance, and daily nutrient intake during pregnancy. Stratified explanation was performed according to the pregnancy dietary pagoda recommended by the Chinese Nutrition Society. Pregnancy nutrition tips and precautions, such as health education matters, were completed by nurses. For parturient women detected with gestational diabetes during pregnancy, secondary maternal health education included the following: gestational diabetes effects on mother and child, using the food exchange chart to determine how to eat according to weight, cognition of food model, blood sugar control standards during pregnancy, and proper exercise. The education period was circa one hour per session and was completed by an obstetrician. Nutrition brochures were provided, and each parturient woman with gestational diabetes was required to record in detail her diet, daily weight, blood glucose, and movement.

The Abbott pregnancy nutrition software was used to develop a reasonable dietary regimen. The dietary calories and nutrient distribution were adjusted at any time in the middle and late stages of gestation.

Results

In this study, the minimum age of pregnant women in CG was 18 years and the mean age was 27.70 ± 3.73 years. The minimum age of pregnant women in EG was 20 years and the mean age was 27.84 ± 3.60 years. No statistically significant difference was observed between the age compositions of the two groups (p > 0.05); the age composition of each age segment was similar in both groups. BMI analysis showed that no statistically significant difference was observed between EG and CG groups (p < 0.05; Table 1).

The result showed that the pre-eclampsia complication rate in EG was 2.41%, which was significantly lower than that of CG (10.59%) with statistically significant difference (p < 0.05). Further stratified analysis indicated that the statistically significant difference mainly existed between subgroups of emaciated and normal (p < 0.05), but no statistical significance was achieved between the subgroups of overweight and obese (p > 0.05; Table 2).

The results showed that pregnancy nutrition guidance had a significant function in reducing the incidence of gestational diabetes, as shown in Table 3. The diabetes detection rate of EG was 17.88% lower than that of CG. The diabetes detection rates of EG, especially in the overweight and obese subgroups, were 30.77% and 25.00%, lower than those of CG (45.45% and 28.57%). The difference was statistically significant (p < 0.05; Table 3).

Whether in the emaciated, normal, or overweight subgroups, the cesarean section rate was significantly lower than that of CG (p < 0.05) throughout the study once nutritional guidance during pregnancy was established. However, the cesarean rate in EG (20.00%) was significantly lower than that in CG (71.43%) in the two obese subgroups (p = 0.215). This result may be attributed to the relatively small sample size of the pregnant women enrolled in these subgroups (Table 4).

In EG, only one case of macrosomia and no occurrence of low birth weight was observed, whereas 27 cases of macrosomia and seven cases of low birth weight were observed in CG. A statistically significant difference existed between the two groups (p < 0.05). The neonatal hyperbilirubinemia rates were 80.30% and 7.32% in CG and EG, respectively; the difference was statistically significant (p < 0.05). The hypoglycemic rate in CG was 83.21%, but not a single case occurred in EG. The comparison between the two groups showed a statistically significant difference (p < 0.05). Table 5 presents the detailed information of grouping, birth weight, and occurrences of hyperbilirubinemia and hypoglycemia.

According to results of univariate analysis, combined with relevant expertise analyses, newborn birth weight was set as a dependent variable. Whether or not to perform nutritional guidance, whether pregnancy complications would occur, and intrauterine growth were set as independent variables to establish logistic regression analysis for the main factors affecting newborn birth weight. The model was set at 0.05 and 0.10 as inclusion and exclusion criteria, respectively. The final analysis results showed that without the nutrition guidance, higher levels of gestational diabetes and pregnancy BMI were the risk factors of abnormal newborn birth weight. Among the risk factors, nutrition exhibited the greatest risk (OR = 42.327). Table 6 shows the main factors and the degrees of risks.

Discussion

This study showed that the incidence of pre-eclampsia (2.41%) in EG was significantly lower than that in CG (10.59%). No significant difference was observed between the emaciated subgroups of the two groups. The risk of pre-eclampsia in obese pregnant women (28.61%) was significantly higher than in peak, normal, and overweight women of the same group (9.09%, 8.89%, and 16.71%). This finding suggests that obesity is one of the adverse factors that cause gestational hypertension [7-9].

Diabetes obesity is an independent risk factor of gestational diabetes [10]. In this study, the risk of developing gestational diabetes by obesity in EG was significantly reduced when pregnancy nutrition guidance was performed (25%). The incidence rate in CG was 28.57%. This result indicates that reasonable dietary guidance is a protective factor in obese pregnant women. The diabetes incidence
Table 1. — Comparison of general information between EG and CG.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>CG</th>
<th>EG</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≤ 25</td>
<td>35</td>
<td>36</td>
<td>1.32</td>
<td>0.856</td>
</tr>
<tr>
<td>26-30</td>
<td>72</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>22</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-40</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI</th>
<th>Emaciation</th>
<th>Normal</th>
<th>Over-weight</th>
<th>Obesity</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emaciation</td>
<td>11</td>
<td>23</td>
<td>8.300</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>92</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over-weight</td>
<td>22</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>7</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Comparison of pre-eclampsia situation in EG and CG.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Normal</th>
<th>Pre-eclampsia (%)</th>
<th>Normal</th>
<th>Pre-eclampsia (%)</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emaciation</td>
<td>10 (90.91)</td>
<td>1 (9.09)</td>
<td>22 (95.65)</td>
<td>1 (4.35)</td>
<td>3.423</td>
<td>0.058</td>
</tr>
<tr>
<td>Normal</td>
<td>84 (91.11)</td>
<td>8 (8.89)</td>
<td>80 (96.41)</td>
<td>3 (3.59)</td>
<td>5.170</td>
<td>0.023</td>
</tr>
<tr>
<td>Over-weight</td>
<td>18 (83.33)</td>
<td>4 (16.71)</td>
<td>13 (100.00)</td>
<td>0 (0.00)</td>
<td>0.557</td>
<td>0.456</td>
</tr>
<tr>
<td>Obesity</td>
<td>5 (71.4)</td>
<td>2 (28.61)</td>
<td>5 (100.00)</td>
<td>0 (0.00)</td>
<td>0.569</td>
<td>0.428</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of GDM situations in EG and CG.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Normal</th>
<th>GDM</th>
<th>Normal</th>
<th>GDM</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emaciation</td>
<td>8 (72.73)</td>
<td>3 (27.27)</td>
<td>20 (86.96)</td>
<td>3 (13.04)</td>
<td>1.037</td>
<td>0.314</td>
</tr>
<tr>
<td>Normal</td>
<td>71 (76.67)</td>
<td>21 (23.33)</td>
<td>69 (83.13)</td>
<td>14 (16.87)</td>
<td>1.119</td>
<td>0.294</td>
</tr>
<tr>
<td>Over-weight</td>
<td>12 (54.55)</td>
<td>10 (45.45)</td>
<td>9 (69.23)</td>
<td>4 (30.77)</td>
<td>0.462</td>
<td>0.514</td>
</tr>
<tr>
<td>Obesity</td>
<td>5 (71.43)</td>
<td>2 (28.57)</td>
<td>4 (75.00)</td>
<td>1 (25.00)</td>
<td>0.016</td>
<td>0.789</td>
</tr>
</tbody>
</table>

Table 4. — Comparison of the delivery mode in EG and CG.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Natural delivery (%)</th>
<th>Cesarean (%)</th>
<th>Normal</th>
<th>Pre-eclampsia (%)</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emaciation</td>
<td>5 (45.43)</td>
<td>6 (54.55)</td>
<td>13 (56.62)</td>
<td>10 (43.48)</td>
<td>15.242</td>
<td>0.001</td>
</tr>
<tr>
<td>Normal</td>
<td>50 (55.56)</td>
<td>40 (44.44)</td>
<td>56 (67.47)</td>
<td>27 (32.53)</td>
<td>79.514</td>
<td>0.001</td>
</tr>
<tr>
<td>Over-weight</td>
<td>7 (29.17)</td>
<td>17 (70.83)</td>
<td>6 (46.15)</td>
<td>7 (53.85)</td>
<td>17.541</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td>2 (28.57)</td>
<td>5 (71.43)</td>
<td>4 (80.00)</td>
<td>1 (20.00)</td>
<td>3.077</td>
<td>0.215</td>
</tr>
</tbody>
</table>

Table 5. — Comparison of newborn health in EG and CG.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>CG</th>
<th>EG</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>Low birth-weight</td>
<td>7</td>
<td>5.30</td>
<td>0.00</td>
</tr>
<tr>
<td>Normal</td>
<td>98</td>
<td>74.24</td>
<td>123</td>
<td>99.19</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>27</td>
<td>20.45</td>
<td>1</td>
<td>0.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hyperbilirubinemia</th>
<th>Yes</th>
<th>No</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>26</td>
<td>106</td>
<td>8.241</td>
<td>0.004</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>109</td>
<td>22.615</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 6. — Multivariate logistic analysis results of newborn body weight.

<table>
<thead>
<tr>
<th>Factors</th>
<th>( \beta )</th>
<th>S.E.</th>
<th>( \chi^2 )</th>
<th>( p )</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition guidance</td>
<td>3.745</td>
<td>1.024</td>
<td>13.388</td>
<td>0.001</td>
<td>42.327</td>
<td>25.692</td>
</tr>
<tr>
<td>GDM</td>
<td>1.127</td>
<td>0.382</td>
<td>8.729</td>
<td>0.003</td>
<td>1.324</td>
<td>1.153</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>1.984</td>
<td>1.068</td>
<td>5.849</td>
<td>0.035</td>
<td>2.674</td>
<td>1.330</td>
</tr>
<tr>
<td>BMI</td>
<td>4.987</td>
<td>0.017</td>
<td>3.002</td>
<td>0.046</td>
<td>0.952</td>
<td>0.123</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.907</td>
<td>1.145</td>
<td>4.628</td>
<td>0.042</td>
<td>2.478</td>
<td>1.263</td>
</tr>
</tbody>
</table>
rate of pregnant women with normal BMI in CG was 23.33%, which was higher than that of EG (16.87%). This rate suggests that diabetes medical nutrition guidance [11-13] could also be applied to normal pregnant women.

Research data worldwide have shown that the weight gain of pregnant women influences the mode of delivery [14]. The dystocia rate of pregnant women who had rapid, excessive weight gain or significant weight gain during pregnancy and who needed assistance in the birth process was significantly higher than that of pregnant women whose body weight increased at a reasonable speed. The natural birth rate of EG was 63.71%, which was significantly higher than that of CG (48.5%). The cesarean section rate of EG was 36.29%, which was lower than that of CG (51.50%). The occurrence of labor abnormalities of EG in natural childbirth was 1.27%, which was significantly lower than that of CG (50%). A difference was observed between the two groups (p < 0.05). Thus, proper nutrition guidance and intervention during pregnancy could significantly improve pregnancy outcomes, improve natural birth rate, and reduce cesarean section rate [15]. This finding is consistent with the health policy advocated by the Ministry of Health, namely, “to promote natural childbirth and reduce cesarean section rate”, which could also improve the quality of perinatal medicine.

The medical nutritional guidance provided during pregnancy improved maternal intrauterine nutrition, thus preventing fetal-borne diseases. The theory states that balanced nutrition within the first 1,000 days of life can reduce the risk of offspring suffering from chronic non-infectious diseases, such as hypertension, diabetes, and heart disease [12]. Pregnancy nutrition guidance can significantly reduce the incidence of fetal-restricted growth and low birth weight [16]. In this study, the fetal-restricted growth occurred in CG, and the incidence rate in EG was 6.06%, indicating a significant difference between the two groups (p < 0.05). Good/adverse effects of pregnancy nutrition guidance mainly manifest in birth weight. In this study, the incidence of macrosomia in EG was 0.81%, which was significantly lower than that of CG (20.50%). Some studies have confirmed that incidence of childhood overweight is correlated with excessive increase of mother’s weight during pregnancy and macrosomia [17]. Reports have indicated that appropriate and reasonable pregnancy nutrition guidance is significant in reducing the rate of fetal macrosomia. Table 6 shows the logistic regression analysis which set newborn body weight as a dependent variable. Whether or not to perform nutrition guidance, whether pregnancy complications occurred, and intrauterine growth were set as independent variables. The result showed that gestational diabetes and high level of pregnancy BMI were risk factors of fetal abnormal birth weight. Among these factors, the effects of nutrition guidance showed the greatest risk (OR = 42.327). This regression model also showed that the incidence rate of abnormal neonatal weight in pregnant women with diabetes was 1.324 times that in pregnant women without diabetes [18]. Developing appropriate calorie intake, adjusting eating habits, and monitoring weight growth of pregnant women could improve the health conditions of newborns. This finding indicates that effective and reasonable nutrition guidance and intervention during pregnancy reduced the incidence of macrosomia and low birth weight. Based on these research results, a significant increase in maternal and perinatal outcomes were observed through nutritional guidance during pregnancy.

The problem of excessive nutrition intake during pregnancy is serious, as observed in recent years, and forms a contradiction with the lack of nutrition knowledge during pregnancy. Given these reasons, this study contrasted and evaluated the nutrition guidance during pregnancy toward the health of mothers and children. The study summarized the findings as follows.

Pregnancy nutrition guidance could significantly reduce the incidence of maternal complications. This study showed that medical nutrition therapy towards overweight and pregnancy obesity could significantly reduce the incidence of pre-eclampsia [19]. The comparison between EG and CG showed that the pregnancy nutrition guidance had a significant function in reducing the occurrence of gestational diabetes [20]. This guidance is especially important to overweight and obese pregnant women who had accompanying high-risk factors of diabetes.

After the pregnancy nutrition guidance, the mothers who exhibited reasonable weight gain and natural delivery had reduced risk of abnormal labor; the rate of cesarean section also significantly reduced [14, 21].

Nutritional guidance during pregnancy resulted in rare incidence of macrosomia and low-birth weight children, regardless of the previous body weight of the pregnant women. To maintain a stable intrauterine environment and reduce nutrient absorption and utilization, a stable blood sugar should be maintained, especially for pregnant women with gestational diabetes. A stable blood sugar would result in rare incidence of macrosomia.

Pregnancy nutrition guidance could significantly improve the health of mothers and children. Proper nutrient intake during pregnancy and fetal growth monitoring showed great significance in reducing maternal complications. Therefore, prenatal nutrition education should be carried out, especially in rural populations. Dietary survey and individualized nutrition therapy should be performed if necessary, and an obstetric nutrition department should be established. Pregnant women with gestational diabetes melitus diagnosed using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) standard should undergo positive nutrition intervention to control newborn weight, reduce incidence of macrosomia and low birth weight, as well as reduce the risk of fetal-borne diseases [22].
References


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The effectiveness of misoprostol or dinoprostone in neonatal outcome after labour induction in post-term nulliparas

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1 General Hospital Kavala, Kavala
2 Democritus University of Thrace, Department of Obstetrics and Gynecology, Alexandroupolis (Greece)

Summary
Objective: The object of this study was to investigate the efficacy of vaginal administration of misoprostol versus dinoprostone in neonatal outcome. Materials and Methods: The first Group A included 77 pregnant women, who requested pregnancy termination one week after labour term and received vaginally misoprostol 50 μg, while the other 69 pregnant women in Group B were vaginally administered three mg dinoprostone. According to the authors’ protocol this procedure was repeated after six hours for a maximum of two times. Results: The labour duration was longer in Group B (p = 0.000), while the APGAR score was better in Group A (p = 0.015). In Group A the labour modus was as follows: 86.9% normal vaginal labour, 3.8% vacuum extraction, and 9.3% cesarean section, while in Group B it was 82.83% normal vaginal labour, 3.07% vacuum extraction, and 14.1% cesarean section. Conclusion: Misoprostol has advantages according to neonatal outcome compared to administration of dinoprostone.

Key words: Misoprostol; Dinoprostone; Neonatal outcome; Post-term induction.

Introduction
Misoprostol is an analogue of prostaglandin E1 (PGE1) which was registered in many countries during the second half of the 1980s particularly those caused by non-steroidal anti-inflammatory drugs [1, 2]. Although misoprostol is not registered for reproductive health use, it is however widely used by gynecologists and obstetricians [3]. Misoprostol has been studied since 1993 for induction of labor in term pregnancies [4].

Labor induction may be indicated when the advantages outweigh the disadvantages of allowing the pregnancy to continue until spontaneous labor onset [5]. In cases of labor induction with an unfavorable cervix, either exogenous prostaglandins or mechanical methods are used to stimulate the production of endogenous prostaglandins through physical stretching of cervix for cervical ripening [6]. Recent studies reported the evaluation of the role of adjuvant interventions to shorten the duration of induced labor using misoprostol [7, 8]. The aim of this study was to certify and compare the efficacy and of vaginal administration of 50 μg misoprostol versus three mg dinoprostone for cervical ripening to neonatal outcome after labor induction in nulliparas or primiparas with singleton post-term pregnancies.

Materials and Methods
This study was a prospective, double–blinded observational trial of nulliparous or primiparous pregnant undergoing labor induction from March 2004 to June 2007 in Department of Obstetrics and Gynecology, Hospital Xanthi. All participants with a medical or obstetric indication for labor induction were eligible to participate in this study. Inclusions criteria were: gestational age measured from the first day of the least menstrual period according to menstrual history and vaginal ultrasonography longer than 40 weeks, age ≥ 17 years, singleton cephalic presentation of fetus, intact membrane, unfavorable cervical Bishop score < 6, and absence of spontaneous uterine contractions. Exclusions criteria were: ruptured membranes, parity more than two children, suspected chorioamnionitis or other serious infection, previous cesarean section, history of uterine myoma nucleation, risk factors for pregnancy, and fetal pathology. All study participants were admitted to labor induction and underwent cardiotocography (CTG) to rule out fetal distress and presence of uterine contractions. A cervical Bishop score was assigned and noticed on admission by attendance of the on–call physician. Prior to cervical ripening, a fetal ultrasound examination was performed to confirm the fetus presentation. The 77 pregnant participants from Group A, were administrated as a labor induction agent, misoprostol 50 μg, vaginally while other the 69 pregnant women from Group B received vaginally as agent three mg dinoprostone. Prostaglandin analogues were inserted in the left and right vaginal fornix and were previously moistened with two to three drops for injection. All participants remained on examination bed for one hour following insertion. CTG recordings were continued during the first hour of insertion and thereafter when the contractions occurred. CTG tracings were independently reviewed and noticed by the on–call physician and contraction abnormalities were noticed and coded as tachysystole and hyperstimulation. If obstructed labor was confirmed six hours after administration of the pharmacological agents, then the
same parameters were examined, dosing was repeated, and waited for another six to 12 hours. Time from induction to delivery, second dose administration in the two groups, neonatal APGAR score, and labor modus were determined and evaluated.

Statistical analysis

Statistical analysis was performed using the SPSS 11.5 statistical package for Windows. The quantity comparison from investigated parameters was described with statistical comparison of continuous variables, while the quality comparison from investigated parameters was described with the frequency variation from the continuous variables.

Test of normality from the quantity variables was performed by the one-way Kolmogorov–Smirnov test. The quantity variables had no normally distribution and due to this reason were described by median, maximum, and minimum values (Table 1). The comparison from the quantity variables between the two groups was performed using Mann Whitney statistical package, while the comparison from the quantity variables between the two groups was performed using Pearson Chi Square statistical package.

Figure 1. — Age distribution in Group A.

Figure 2. — Age distribution in Group B.

Figure 3. — APGAR score in Group A.

Figure 4. — APGAR score in Group B.
Results

The age difference was not statistically significant between the two groups; conversely the labor duration was longer in the dinoprostone group and the APGAR score was better in the misoprostol group. The differences were statistically significant (Table 1). The figures from age, APGAR score, second medication dose, and labor modus for the two groups are shown in Figures 1-6. The differences according to quality characteristics (second dose of medication, labor modus) were not statistically significant (Table 2). No serious side effects were noticed in both groups of the participants.

Discussion

Clinical trials have compared misoprostol with placebo, oxytocin, and other prostaglandins, primarily dinoprostone and prostaglandin gel E2 [9-14]. Misoprostol administered vaginally or orally is superior to placebo for inducing cervical ripening before induction of labor with oxytocin. In a Cochrane pregnancy and childbirth group which included 26 randomized trials, it was concluded that misoprostol is effective for labor induction, is associated with low incidence from side effects, however uterine hyperstimulation, changes of fetal heart rate, and the frequency of meconium stained amniotic fluid was higher in the misoprostol group compared to dinoprostone group [15]. It is unknown fetal distress is increased in the misoprostol group.

Main advantage of medical misoprostol is the combination of its uterotonic and cervical-ripening actions. Miso-
prostol is useful for elective medical abortion, cervical ripening before surgical abortion, evacuation of the uterus in cases of embryonic or fetal death, and induction of labor. The drug may also be used to treat and even prevent postpartum hemorrhage [15]. No differences were reported in the rates of caesarean section and neonatal maternal morbidity between women who received misoprostol and those who received prostaglandin E2. According to the present results no differences in the labor modus and the necessity of repeating a second dose were found. None of the participants in the present study had received oxytocin. The authors noticed the time of labor duration depended only on either misoprostol or dinoprostone and founded timely advantage in the misoprostol group (Table 1). According to current literature research, the indexes of neonatal effects (APGAR scores, admissions to neonatal intensive care, and meconium passage) are similar compared to those women with dinoprostone labor induction) [15, 16]. Based on the results of the current investigation, the authors revealed differences in the indexes of neonatal effects, which are statistically significant (Figures 3-4). However misoprostol is not recommended from American College of Obstetricians and Gynecologists for labor induction and cervical ripening [17]. Prostaglandin E2 or dinoprostone is the only prostaglandin approved by the USA Drug Administration for cervical ripening in pregnant women at or near term with a obstetric need for labor induction [18].

Although the sample of this study is moderate, it was proved that misoprostol intravaginally administration has no adverse effects in neonatal outcome by labor induction in post-term pregnant women. The effect of this medication should be investigated in third-trimester pregnancies beyond 38 weeks and in post-term singleton pregnancies with relatively high risk factors like hypertension. More detailed studies in the future are necessary to confirm any aspects not yet clarified and to compare this medical agent with other prostaglandins.

References


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The oocyte-to-baby rate of day 2, day 3 versus day 5 embryo transfer: a retrospective study

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2 Department of Reproductive Medicine, Jinjiang Hospital of Obstetrics & Gynecology, Chengdu, Sichuan (China)

Summary

Objectives: To investigate whether the time of embryo transfer (ET) affect the oocyte-to-baby rate. Materials and Methods: The database was retrospectively analyzed including total number of oocytes collected and corresponding oocyte-to-live baby born (LBB) rate. Then the relationship between different time of embryo transfer and oocyte-to-baby rate was compared. In a year period all patients undergoing infertility treatment were included in the study. The outcome parameters were total number of oocytes collected and corresponding oocyte-to-LBB. Results: For patients under the age of 35 years, there was no increase in oocyte-to-LBB regardless of the time of ET. For patients older than 35 years, the oocyte use rate increased significantly when embryo was transferred on day 2. Oocyte-to-baby rates were also analyzed after grouping patients on the number of oocytes retrieved per cycle. For patients < 35 years, the oocyte-to-LBB rate increased significantly on day 3 if < ten oocytes were obtained. whereas for patients > 35 years, the oocyte-to-baby rate was best on day 2 when about 15 oocytes were retrieved. Conclusions: This retrospective analysis demonstrated the relationship between the time of ET and oocyte-to-baby rate that is indicative of a more biologically efficient reproductive system.

Key words: Oocyte; In vitro fertilization; Time of embryo transfer; Live birth rates.

Introduction

For a long time, it was believed that the retrieval of multiple oocytes in in vitro fertilization (IVF) cycle was beneficial to the success because more embryos can be produced for transfer. However, despite collection of multiple oocytes and availability of many embryos, recent data demonstrate that the success rates of IVF treatment are still low, and that the majority of oocytes/embryos do not produce live births. It was reported recently that < 5% of the retrieved oocytes and about 15% of the transferred embryos gave rise to babies [1, 2].

A couple of papers have debated the most appropriate metrics to measure efficiency in assisted reproduction technology (ART) cycles. Live birth per retrieval cycle and live birth per embryo transfer (ET) are the standard metrics used. The denominator of these metrics can also be per cycle initiated [3] or per IVF treatment as a whole [4] that includes also the success of the derived frozen embryo transfer cycles [5]. However, these metrics do not assess the biological competence of the oocytes. A new metric, “oocyte-to-baby rate” was recently proposed to assess the efficiency of ART procedures. The metric takes into account all the oocytes collected and all the embryos obtained and used in each retrieval cycle [1, 6, 7]. Using this new metric, Patrizio and Sakkas found that only approximately 5% of the retrieved oocytes could produce a baby in young non-donor IVF patients, and that the oocyte-to-baby rate declined progressively to 1% for women 40 years or older. Surprisingly, the oocyte-to-baby rate in donor oocyte cycles was also low [1, 8].

Recently, some protocols have used better oocyte utilization rate, such as with minimal or mild ovarian stimulation protocols. However, there are no data in the literature evaluating the effect of time of ET on live-birth rates. This retrospective study was conducted to understand whether the time of ET would affect the biological efficiency of oocytes generated in IVF.

Materials and Methods

This study analyzed 1,360 cycles performed at the Shanghai First Maternity and Infant Hospital between 2008 and 2009. Oocyte donor cycles were excluded. Patients were divided into three groups according to the time of ET (group 1, ET on day 2; group 2, ET on day 3; group 3, ET on day 5). Two types of analyses were conducted. First, the authors analyzed the effect of timing of ETs on overall oocyte-to-baby rate in different age groups. Second, the relationships between oocyte-to-baby rate with the time of ET and the oocyte yield within each age group were studied.

The protocol of ovarian stimulation consisted predominantly of downregulation with leuprolide acetate at a dose of 1.25 mg starting in the midluteal phase of the preceding menstrual cycle followed by use of recombinant human follicle-stimulating hormone (hFSH). When two leading follicles had a mean diameter of 18 mm, recombinant human chorionic gonadotropin (hCG) was administered, and oocyte retrieval was carried out 36 hours later.
Table 1. — Oocyte-to-baby rate in patients without frozen embryos.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of oocytes</th>
<th>Day 2</th>
<th>LBB per Oocyte (%)</th>
<th>Day 3</th>
<th>LBB per Oocyte (%)</th>
<th>Day 5</th>
<th>LBB per Oocyte (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 35</td>
<td>585</td>
<td>123</td>
<td>19</td>
<td>16</td>
<td>2.6</td>
<td>261</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>35-37</td>
<td>189</td>
<td>69</td>
<td>7</td>
<td>6</td>
<td>3.2</td>
<td>68</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 38-40</td>
<td>282</td>
<td>119</td>
<td>6</td>
<td>5</td>
<td>1.8</td>
<td>80</td>
<td>36</td>
<td>1</td>
</tr>
</tbody>
</table>

ET: total number of embryos transferred; FHB: total number of fetal heartbeats; LBB: total number of live babies born

Table 2. — Oocyte-to-baby rate in patients with frozen embryos.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of oocytes</th>
<th>Day 2</th>
<th>LBB per Oocyte (%)</th>
<th>Day 3</th>
<th>LBB per Oocyte (%)</th>
<th>Day 5</th>
<th>LBB per Oocyte (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 35</td>
<td>5,099</td>
<td>793</td>
<td>242</td>
<td>207</td>
<td>4.05</td>
<td>2,969</td>
<td>320</td>
<td>120</td>
</tr>
<tr>
<td>35-37</td>
<td>344</td>
<td>109</td>
<td>22</td>
<td>21</td>
<td>6.1</td>
<td>349</td>
<td>79</td>
<td>19</td>
</tr>
<tr>
<td>&gt; 38</td>
<td>399</td>
<td>121</td>
<td>15</td>
<td>10</td>
<td>2.5</td>
<td>359</td>
<td>74</td>
<td>10</td>
</tr>
</tbody>
</table>

ET: total number of embryos transferred; FHB: total number of fetal heartbeats; LBB: total number of live babies born

*Final LBB per oocyte is not statistically significantly different between day 2 and day 3 group, p = 0.736.

Results

Oocyte-to-baby rate

There were a total of 19,075 oocytes in the 1,360 oocyte retrieval cycles analyzed (Group 1: 4,860 oocytes, 535 cycles; Group 2: 2,768 oocytes, 212 cycles; Group 3: 8,091 oocytes, 613 cycles). The total number of usable embryos, i.e. good quality embryos suitable for fresh ET and cryopreservation, were 9,339 (50% of the total oocytes collected), of which 3,131 (33.5%) were used for fresh ET and 6,208 (66.5%) were cryopreserved.

Oocyte-to-baby rate and age of patients

The outcomes of the oocytes used (oocyte-to-baby rate) relative to the day of ET are presented in Tables 1-3. In patients with no spare embryos suitable for cryopreservation, and the live babies born (LBB) rate per oocyte collected did not differ significantly among the three ET groups for patients under the age of 35 years (Table 1). For patients older than 35 years, the oocyte usage rate was higher in the day 2 group than the other groups, while there was no statistical difference (Table 1).

In patients with frozen embryos available, for patients younger than 35 years, oocyte-to-baby rate was similar in groups with ET performed on day 2 and day 3 (4.05% vs. 3.9%; p = 0.736) (Table 2). The rate was significantly lower in the day 5 ET group when compared to the other two group (p = 0.0018). For patients aged 35 to 37 years, the oocyte-to-LBB rate was significantly higher in group 1 than in the other two groups (6.1% vs. 3.4% vs. 3.0%, p = 0.033). In the patients older than 37 groups, the oocyte-to-baby rate was not significantly different among these three ET groups (p = 0.4).

Outcomes of live baby rate and number of oocytes retrieved

In patient younger than 35 years, the oocyte-to-LBB rate was significantly higher in the day 3 group and lower in the day 5 group when the number of oocyte retrieved was less than ten. In patients between age 35-37 years and with less than 15 oocytes retrieved, the oocyte-to-baby rate in the day 5 group was lower than in the other two groups, in patients between age 35-37 years, the oocyte-to-LBB rate was not different among the three ET groups.

Later. Progesterone supplementation (60 mg in oil) by intramuscular injection was started on the day of oocyte retrieval in all cases.

Fresh ETs were carried on day 2, day 3 or day 5 after oocytes retrieval using Wallace soft catheters under ultrasound guidance. Excess good quality embryos were cryopreserved on the day of ETs. Frozen ETs were performed in both hormone replacement cycles and natural cycles. The former involved 17β-estradiol with doses that increased progressively from two to six mg per day. The latter were performed on day 3 to day 4 after surge of serum LH. Luteal support consisted of daily take of 17β-estradiol (two to six mg) and intramuscular injection of progesterone (60 mg) for hormone replacement cycles or daily intramuscular injection of progesterone (60 mg) natural cycles.

A pregnancy test was performed 14 days after ET. If the test was positive, progesterone supplementation was continued for ten more weeks. The authors defined clinical pregnancies as those with visualization of fetal heartbeat on ultrasound at day 42 of gestation. The treatment outcome was presented as clinical pregnancies per oocyte retrieved and per ET. A spontaneous abortion was referred to pregnancy loss that occurred after visualization of the fetal heartbeat. The live birth event and the number of infants born were also assessed.

Statistical analysis comparing live births per oocyte in relation to the time of ET was performed using chi-square analysis using SPSS 15.0 software.
Discussion

In this study, the authors evaluated the effect of time of ET on the oocyte-to-baby rate. The result showed that the majority of oocytes retrieved and the majority of ETs fail to produce live births, which is in agreement with previous study [1]. However, the oocyte-to-baby rate in young patient (< 35 years) was significantly higher in the day 3 group than the other groups, while in those between 35-37 years, the day 2 group was significantly higher than the other groups. These data indicates that despite the retrieval of an equivalent number of oocytes, it may better the oocyte-to-baby rate by changing the time of ET according to patients' age and number of oocytes retrieved. The following hypotheses may explain the relationship between time of ET and oocyte-to-baby rate.

First, in a natural cycle, only one antral follicle becomes dominant while the remainder of developing follicles undergo atresia. In contrast, during ovarian stimulation for ART these follicles are rescued, whose cytoplasm and nucleus is intrinsically abnormal [9, 10]. In vitro culture systems can affect the oocyte's development competence, especially for those oocytes from older patients. The present study found that reducing culture time in vitro in patients older than 35 years have higher oocyte-to-baby rate, which may due to the impact of suboptimal laboratory conditions on both oocytes and embryos. These may have substantial effect on older patients. The present data show that a significantly low LBB rate on day 5 and majority of oocytes retrieved are wasted.

Secondly, the present study showed that the oocyte-to-baby rate is low in all groups. During blastocyst culture, few embryos develop into blastocyst while most embryos undergo the fate of developmental retardation, which may be due to the impact of suboptimal laboratory conditions on both oocytes and embryos. These may have substantial effect on older patients. The present data show that a significantly low LBB rate on day 5 and majority of oocytes retrieved are wasted.

Identification of the best ET time can have many important clinical applications. Current oocyte quality parameters do not necessarily correlate with competence, in spite of more and more refined ways to assess oocyte's biological potential, however, none of these is perfect to select the best oocytes to inseminate. The present data show that ET time affect oocyte-to-baby rate. If these data are confirmed by others, it may be a better and simple choice in clinic to change ET time according to age and acquired oocytes, which may better oocyte wastage.

The long-held assumption that more oocytes are better is definitely contradicted by the studies of Patrizio et al. [1, 8]. The present data showed that the retrieval of 15 or fewer oocytes was associated with the highest oocyte-to-baby rate, which indicates that referring to oocyte-to-baby live rate, more oocytes may have a disadvantage. It is the time to better control ovarian stimulation protocol to acquire appropriate oocytes. Changing control ovarian stimulation protocols and ET time may have better oocyte-to-baby rate.

In summary, the authors retrospectively identified that whether transfer is performed on day 2, day 3 or day 5 does make a difference on the oocyte-to-baby rate. Moreover, these findings indicate that embryos can be safely scheduled according to patient’s age and oocytes acquired.
Acknowledgements

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References


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Ultrasound-guided intrauterine insemination versus blind intrauterine insemination: a randomized controlled trial

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Summary

Purpose: This study was performed to determine the effects of ultrasound (US) guidance during intrauterine insemination (IUI) on pregnancy rate. Materials and Methods. This study is a prospective randomized controlled trial which was performed in Women’s Health Research and Education Hospital, Infertility Unit. The study enrolled 130 couples who were scheduled to undergo IUI. The couples were randomized according to a computer-generated list into two groups; 1) the ultrasound-guided IUI group included 64 couples (n = 64) treated for 99 cycles 2) blind IUI group included 66 couples (n = 66) treated for 104 cycles. All women underwent controlled ovarian stimulation before IUI. The study’s main measurements were pregnancy rate per cycle; pregnancy rate per woman. Results: The pregnancy rates were similar in both the ultrasound-guided (USG) (16.2%, 16/99) and non-ultrasound-guided (NUSG) (12.5%, 13/104) groups (p = 0.386). Conclusions: The present results suggest a routine ultrasound guidance during IUI is not essential as it does not increase pregnancy rates but it can be used in such cases to overwhelm some sort of difficulties.

Key words: Intrauterine insemination; Ultrasound guidance; Pregnancy rate.

Introduction

Intrauterine insemination (IUI) is a simple first-base treatment for infertile couples [1]. In comparison with other assisted reproductive techniques (ART), this treatment has been widely used to treat infertile couples with a variety of indications. Common indications include cervical factors, mild endometriosis, mild to moderate male factors, ovulatory dysfunction, and unexplained infertility [2]. The reported pregnancy rate per cycle ranges from 8% to 22% [3-5]. The reported pregnancy rates per IUI cycle are variable due to differences in cause and duration of infertility, ovarian stimulation and methodology, sperm preparation techniques, treatment cycles, and number of times that IUI is performed during a cycle (once or twice) [3-5]. Engels et al. reported that pregnancy development is not associated with endometrial volume, but women who developed pregnancy have a significantly higher subendometrial flow index (FI) [6].

In cycles of in vitro fertilization (IVF), the embryo transfer process is generally done under abdominal ultrasound (US) guidance. Ultrasound visualization of the endometrial cavity prevents the catheter from touching the uterine fundus. US also allows us to visualize the cervico-uterine angle, reducing the number of difficult cervical catheterizations, as well as cervical manipulation [7]. Many studies have indicated that US guidance during embryo transfer increases the rates of implantation and pregnancy [7,8].

However, only two studies evaluated the use of US during IUI. The catheter striking the uterine wall or cervical manipulation with tenaculum placement increases uterine contraction [9] due to the release of oxytocin or prostaglandin and expulsion of > 40% of the volume introduced into the uterine cavity has been reported [10]. Balci et al. reported that uterine contractions during IUI were significantly more frequent with tenaculum usage [11]. However, while uterine contractions increase with tenaculum, pregnancy rates do not decrease; even they may increase [11,12]. Considering the role of seminal prostaglandins in natural fertilization, vaginal application of misoprostol before the IUI, in order to increase the ratio of conception, did not result with an increase in the ratio of pregnancy [13].

This study was performed to investigate whether the US guidance during IUI improves pregnancy rates. Extra attention was performed to not to touch the fundus during the IUI procedure [14]. The authors utilized US in order to perform IUI by observing that catheter does not get in touch with the uterine fundus and compare the pregnancy results with IUI without US.

Materials and Methods

The study protocol was approved by the Dr. Sadi Konuk Education and Research Hospital Ethical Committee on February 7, 2011 (approval number 2011/2-05). This was a randomized, single-blind, controlled trial comparing the efficacy of US guided (USG) IUI with non-ultrasound guided (NUSG) IUI on pregnancy rate.

The participants were recruited from 173 consecutive couples undergoing infertility counseling at the present ART Unit from February 2011 to July 2011. Figure 1 shows a flowchart of the...
A total of 130 eligible infertile couples – with mild male factor, idiopathic or ovulatory infertility – were enrolled for the study. All patients were counseled about the nature of the study and gave their informed consent. They were then randomized according to a computer-generated list in sealed, opaque envelopes into two groups; the USG IUI and NUSG IUI groups.

All the women underwent a standard gynecological examination and cervical screening by the Papanicolaou test. The following infertility work-up was performed on all women; hysterosalpingography, transvaginal ultrasonography, basal hormonal assays (on the third day of the spontaneous cycle) and determination of the number of antral follicles. Semen analysis was performed on the samples from all male partners.

Ultrasonic scans were performed via a 200 ultrasound unit equipped with a five to seven MHz endovaginal probe. The numbers of follicles in both ovaries were added up to the total antral follicle count. The follicles visualized and counted by transvaginal sonography in the early follicular phase (on day 3 of spontaneous cycle) were two to ten mm in size. All women had the following basal hormonal assays: estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and prolactin. Serum levels of progesterone were measured on day 21-24 by the chemiluminescence method.
Hysterosalpingography was performed to investigate the shape of the uterine cavity and the shape and patency of the fallopian tubes. Semen analysis was performed by employing visual estimation by microscope.

The exclusion criteria were: older than 40 years, not having a normal uterine cavity and/or bilaterally obstructed fallopian tubes, to be diagnosed as hypogonadotrophic hypogonadism or hypergonadotrophic hypogonadism or any dysfunction of thyroid gland or any other coexisting chronic disease, basal FSH level higher than 12 IU/L; male partner’s sperm count of less than five million on a washed sample, and being unable to provide fully informed written consent.

Pre-intervention procedures
A low-dose step-up protocol was used in all women. Daily subcutaneous injection of 75 IU of recombinant follicle-stimulating hormone (rFSH) was started on day 3 on the menstrual cycle. On the seventh day, US was performed. If the US image showed ≥10 mm follicular development, patients were followed-up without changing dosage until one or two follicles reached a diameter of 17-20 mm. If the US image showed <10 mm follicular development, dose of rFSH was increased to 112.5 IU/day on the eighth day and the patients were followed-up with every one to three days intervals until one or two follicle reached ≥17-20 mm in diameter. Subsequent dose augmentation was performed with 37.5 IU per week (up to 225 IU/day). The duration of treatment was 28-35 days. IUI was carried out 36 hours after hCG injection. A maximum of three cycles were carried out in all women.

The aim of stimulation was to achieve a mono-follicular response. In the study, IUI was also applied to women who developed double follicles. After one or two follicles with 17-20 mm in a diameter were detected sonographically, 250 μg of recombinant human chorionic gonadotropin (rhCG) was administered subcutaneously. If three or more mature follicles (≥17 mm) developed or there was no follicular development, IUI was cancelled due to legal restriction for IUI. IUI of 12, 11, and five, and three women in the NUSG group were cancelled at first, second, and third cycle, respectively. No OHSS was observed, because IUI was cancelled when women had three or more mature follicles (≥17 mm). The authors assessed endometrial thickness (ET) which reflects estrogen level at the β-hCG day. All ETs were ≥8 mm. Three couples underwent an unplanned course of four cycles of IUI, although a maximum of three cycles were planned. One patient in the USG group and two patients in NUSG group underwent four cycles of IUI. The USG group included 64 couples treated for 99 cycles, and the NUSG group included 66 couples treated for 104 cycles.

Intervention procedures
All the patients had full bladders during IUI and the procedure was performed using the identical catheters in both groups. The catheters had a rigid outer channel and a flexible inner channel. The authors carried out one single insemination per treatment cycle 36 hours after hCG injection. All IUI procedures were performed by the same two gynecologists.

During IUI procedure in USG group, the internal flexible catheter was put into the cervical canal and passed through the internal cervical orifice. The inner catheter channel was pushed forward into the uterine cavity under abdominal US guidance until it reached within one to 1.5 cm of uterine fundus. Hence, the catheter was prevented from getting in touch with the fundus. When the flexible inner sheath did not pass through the cervical canal, the rigid outer channel was firstly passed through the cervical canal and then the IUI procedure was preceded as described above. Outer sheaths were used in three patients from each group.

When it was indubitable that the catheter was in place, sperm collected in the Andrology Laboratory, by the swim-up method, were injected into the uterine cavity. It was difficult to pass the cervical canal in some patients. Abdominal ultrasound was performed using an ultrasound unit with a 3.5-MHz abdominal probe.

In NUSG group, the catheter’s inner channel was pushed into the uterine cavity until the resistance of the internal cervical orifice was passed. The catheter was pushed forward two to 2.5 cm into the uterine cavity after passing the internal cervical os. If the flexible inner sheath could not pass the cervical canal, the cervical canal was passed using the rigid outer channel and the process was continued as described above. When it was thought that the catheter was in place, sperm prepared using the swim-up method in the Andrology Laboratory was introduced into the uterine cavity.

Post-intervention procedures
After the procedure, the women in both groups were advised to rest in bed for 20 minutes. No cervical tenaculum or hysteroscope was used in any cases during the procedure. The β-hCG levels were measured 12 days after IUI. Pregnancy was confirmed by transvaginal US ten days after the β-hCG was positive.

Statistical Analysis
All the statistical calculations were performed with the Statistical Package for Social Sciences (SPSS) statistical software package. The number of women to be recruited was determined using a sample size calculation, and was based upon the primary outcome measure of PR per cycle. Assuming a per cycle PR of 16.8% (on the basis of the study conducted by Ramón et al. [15], 98 cycles in each group would be needed to detect a 17% difference between the groups (α=0.05 and power [1-β]=0.8). risk in a bilateral contrast; employing the arcsin calculation method.

The present authors used the Shapiro Wilk test to test variables for normality. Results of descriptive analysis were presented as median (minimum-maximum) and percentage. Mann Whitney U test for continuous data and Fisher’s Exact test, Yates Chi-Square test and Pearson Chi-Square test for categorical data were used for comparisons between the USG and the NUSG groups. Odds ratios (OR) with 95% confidence interval (CI) were calculated for pregnancy rates (PR).

In all statistical comparisons, a p value ≤0.05 was used to indicate a significant difference.

Results
A total of 203 stimulated cycles in 130 patients were included. For each peer, the partner’s sperms were used. USG group included 64 women and NUSG group 66. Ninety-nine cycles in USG group and 104 cycles in NUSG group were carried out.

Baseline and pre-intervention clinical characteristics of the study groups are shown in Table 1. The study groups were similar in terms of age (p = 0.710) and BMI (p = 0.631). There were no differences between the groups in any of baseline outcomes including duration of infertility (p = 0.636), total motile sperm counts (p = 0.531), basal FSH levels (p = 0.227), and rates of infertility types (p > 0.050 for all infertility factors). There were no differences between the groups in number of cycles (p = 0.448), cumulative dose of rFSH (p = 0.088) and number of follicle ≥17 mm (p = 0.299) as pre-intervention outcomes (Table 1).
Pregnancy rates are shown in Table 2. The PR per cycle was 16.2% (16/99) and 12.5% (13/104) in the USG and NUSG groups, respectively. There was no difference between the groups in terms of PR per cycle (odds ratio [OR]=1.35; 95% confidence interval [95% CI]: 0.61-2.97; p=0.548). The first cycles PR were 18.8% (12/64) in the USG group and 13.6% (9/66) in the NUSG group. There was no difference between the groups in first cycle PR (OR=1.06; 95% CI: 0.23-4.92; p = 1.000). There was no difference between the groups in second (OR=1.36; 95% CI: 0.78-25.14; p = 1.000) and third cycle PR (OR=1.40; 95% CI: 0.52-3.12; p = 0.238) (Figure 2). The PR per woman was 25% (16/64) in the USG group and 19.7% (13/66) in the NUSG groups. There was no difference between the groups in PR per woman (OR=1.26; 95% CI: 0.52-3.12; p = 0.606). The ongoing PR was 62.5% (10/16) in the USG groups and 84.6% (11/13) in the NUSG groups (Figure 2).
Abortion rates (OR=0.82; 95% CI: 0.01-68.63; \( p = 1.000 \)), chemical PR (OR=0.82; 95% CI: 0.01-68.63; \( p = 1.000 \)) and multiple PR (OR=1.91; 95% CI: 0.17-137.64; \( p = 0.606 \)) of the groups were similar.

Discussion

The combination of controlled ovarian hyperstimulation (COH) with IUI is an important option in infertility treatment. IUI is similar to the embryo transfer procedure in IVF. Several authors have studied the factors affecting implantation rate in the embryo transfer procedure. For example, the presence of blood on the catheter is associated with a lower implantation rate [16]. US guidance in embryo transfer significantly increases the pregnancy and implantation rates in IVF [8]. The use of abdominal US during transfer avoids catheter trauma to the endometrium and decreases the damage via direct visualization of the catheter, thereby decreasing uterine contraction.

Although in recent years plenty of studies regarding to embryo transfer technique and US guided embryo transfer had been made, there is few studies dealing with the insemination technique in IUI procedure. These studies are about tenaculum, bougie, and hard catheter usage [17], but there are only two studies corresponding to USG IUI [15,19]. The embryo transfer and IUI techniques are similar in that both require cervical catheterization and release material into the endometrial cavity. Using USG during the embryo transfer procedure in IVF has been studied in detail. It is generally agreed that USG increases the pregnancy rate, although one study indicated that the pregnancy rate did not increase with USG during the embryo transfer procedure [16]. Therefore, USG during IUI should theoretically improve the pregnancy rate in controlled ovarian hyper stimulation with IUI cycles. The present authors tested this hypothesis using a methodology similar to that used by Ramón et al. [15], although they differed in the COH protocol and follicle limits used.

In the present study, the authors aimed for monofollicular development. A low-dose step-up protocol was used in COH, starting with 75 IU rFSH daily on day 3. Ramón et al. started on day 2 and administered 150 IU rFSH and one subcutaneous dose of 0.25 mg cetrorelix daily after one follicle had a diameter of 16 – 20 mm. They added 75 IU/day of recombinant luteinizing hormone (rLH) to the FSH stimulation protocol from the start of antagonist administration.

The present authors believe that Ramón et al. [15] had used cetrorelix for synchronizing high number of follicles formed by high dose FSH. However in a low dose step up protocol, there is no need for such an effort they did not use cetrorelix or rLH. IUI was cancelled if three or more follicles developed and they were excluded from the study since ratio of multiple pregnancy increase in these patients [18], while Ramón et al. [15] used five follicles as the cycle limit.

The need for tenaculum is extremely rare. Although tenaculum use does not affect pregnancy ratios, the present authors did not use it as Ramón et al. did [15]. They had no difficult IUI case (tenaculum, hysterometry, need for bougie). They needed to use rigid outer channel of the catheter in three patients in each group. Since there is no difference between soft and firm catheters in terms of pregnancy results [18], these cases were not evaluated as difficult IUI.

The pregnancy rates per cycle in the USG IUI 16.2% (16/99) and classical IUI 12.5% (13/104) groups were similar to those in the study reported by Ramón et al. (16.0%, 17/106 vs. 16.8%, 21/125, respectively) [15]. The pregnancy rates per woman in the present study were low compared to those reported by Ramón et al. for both USG IUI and classical IUI (25% and 19.7% vs. 51.5% and 52.5%, respectively). These differences were likely due to the differences in the cycle cancellation criterion and the number of ovulation induction cycles per woman. The rates of multiple pregnancies in the present study (6.3% (1/16) in the USG IUI and 7.7% (1/13) in classical IUI) were low compared to that reported by Ramón et al. [15] but the abortion rates were similar in both studies.

In contrast with the study done by Ramón et al. [15], recently published trial done by Oztekin et al. indicates there might be an increase in the pregnancy rates with the IUI procedure under US guidance [18]. Through this retrospective study, randomization was provided with applying IUI under US guidance to those patients who had a full bladder, and blindly IUI application to those who had an empty bladder. Tenaculum usage was defined as hard IUI. The pregnancy rates of the groups US used and blind IUI are reported as 23.4 (34/145), 13.9 (17/122), respectively. Difficult IUI rates were 9.7 (14/145) in the US used group and 26.2 (32/122) in the blind IUI group. A statically significant difference (\( p < 0.001 \)) was determined between the hard IUIs. Although using a tenaculum infers a hard IUI, there have been studies that show there is no significant change with the pregnancy rates [11, 12]. All the patients were with a full bladder at the very beginning of the present study. Tenaculum usage was not needed at all. The present authors defined the cases as difficult IUI where we used the outer rigid outer channel of the catheter. In both groups there were only three cases where they used that rigid outer channel, so that there was no statistical difference. The present authors can relate the lack of difference between the groups to the following: uterine fundus is not touched also in the NUSG group; the uterine contractions observed in both groups are probably similar and their effects on pregnancy is equal; uterine contractions do not decrease pregnancy ratios as in embryo transfer.

Conclusion

In conclusion, the present results suggested that routine US guidance during the IUI procedure is not essential, as
routine US does not increase pregnancy rate. The present results suggest a routine US guidance during IUI is not essential as it does not increase pregnancy rates but it can be used in such cases to overwhelm some sort of difficulties.

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The association between anti-Müllerian hormone and IVF-ICSI outcome in poor responder patients performing long protocol

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Summary

Purpose of investigation: Ovarian reserve reflects the capacity of the ovaries for a successful pregnancy. Anti-Müllerian hormone (AMH) could be a useful marker to predict ovarian reserve and to adjust controlled ovarian stimulation. The aim of this study was to assess the relationship between AMH and intracytoplasmic sperm injection-in vitro fertilization (IVF-ICSI) outcome in poor responder women. Materials and Methods: This study was conducted prospectively for a period of 12 months. Inclusion criteria were FSH value > 15 iu/l or antral follicle number < 4, on the 2nd day of cycle. All patients underwent GnRH agonist stimulation with long protocol. Serum AMH levels were measured in the treatment cycle just before the stimulation. After the treatment, patients who were pregnant formed the study group and patients who were not pregnant formed the control group. Serum AMH level was the main outcome measure. Results: The study and control group consisted of 34 and 70 patients, respectively. No significant difference was found in duration of infertility, antral follicular count, basal E₂ and FSH levels. The mean serum AMH level was significantly higher in study group (p = 0.005). The retrieved oocyte number, metaphase 2 oocyte number, and fertilization rate were also significantly higher in the study group. Discussion: Evaluation of serum AMH seems to be a useful marker to predict IVF-ICSI outcome in poor responder patients.

Key words: IVF-ICSI; Anti-Müllerian hormone; Poor responder; Ovarian reserve.

Introduction

Intracytoplasmic sperm injection-in vitro fertilization (IVF-ICSI) is associated with yielded oocytes’ number and quality. Poor responder patients are one of the most important problems in IVF-ICSI management. Success of the treatment decreases in such patients [1]. Evaluation of ovarian reserve could help to predict the patients who will not become pregnant. Thus, the duration of treatment and the management of controlled ovarian hyperstimulation (COH) might be easily performed. Numerous tests and methods have been available to assess ovarian reserve. However, there is no ideal test demonstrating ovarian reserve and pregnancy estimation [2]. Among these tests only measurement of antral follicle count (AFC) and serum anti-Müllerian hormone (AMH) levels have found to be beneficial. Broer et al. reported that AFC and AMH were useful to predict poor response [3]. But both of them were not successful to estimate a pregnancy.

AMH is a dimeric glycoprotein produced by granulosa cells in pre-antral and antral follicles [4, 5]. Serum AMH levels remain unchanged in conditions in which endogenous gonadotropin levels are low such as with pregnancy, ovulation induction or treatment with oral contraceptive pills [6]. An ideal ovarian reserve test should rapidly assess the chance of pregnancy. Therefore, the cost of the treatment would decrease due to inclusion of convenient patients into assisted reproductive treatment (ART). Although AMH is the best marker demonstrating poor response, there is no cut-off value for the patients whether to start a treatment in relation with IVF or not [7]. Thus, this study was designed to detect the association between serum AMH levels and IVF-ICSI outcome and to determine a cut-off level for AMH to predict IVF-ICSI success.

Materials and Methods

This study was conducted prospectively for a period of 12 months. The study protocol of this clinical trial was reviewed and approved by the Ethical Committee of Bozok University Medical Faculty. Informed consent was taken from all participants. Inclusion criteria for poor responders were FSH value > 15 iu/l or antral follicle number < 4, on the 2nd day of cycle. Serum AMH levels were measured in the treatment cycle just before the stimulation. After the treatment, patients who were pregnant formed the study group and patients who were not pregnant formed the control group. Serum AMH level was the main outcome measure. Results: The study and control group consisted of 34 and 70 patients, respectively. No significant difference was found in duration of infertility, antral follicular count, basal E₂ and FSH levels. The mean serum AMH level was significantly higher in study group (p = 0.005). The retrieved oocyte number, metaphase 2 oocyte number, and fertilization rate were also significantly higher in the study group. Discussion: Evaluation of serum AMH seems to be a useful marker to predict IVF-ICSI outcome in poor responder patients.

Key words: IVF-ICSI; Anti-Müllerian hormone; Poor responder; Ovarian reserve.
was below 50 pg/ml, COH was started with highly purified-urinary FSH on the 2nd day of the cycle. Then GnRH agonist dose was decreased to 0.5 mg/day. The beginning FSH dose was 300 iu and the dose was individually adjusted according to the previous treatment cycles, body mass index (BMI), and age. COH was monitored by using E2 measurement and TVUSG. Human chorionic gonadotropin (hCG) (5,000 IU × 2) was administered when the dominant follicle reached 17 mm. Oocytes were retrieved by TVUSG-guided needle aspiration. IVF-ICSI was performed in all cases. Luteal phase support was given by vaginal progesterone (8% vaginal gel) beginning on the oocyte pick-up (OPU) day and lasted for 12 days (until the serum β-hCG measurement day). If pregnancy occurred, progesterone was given until the 12th gestational week. Clinical pregnancy was accepted as a pregnancy when the gestational sac or fetal heartbeat was confirmed by TVUSG.

Statistical analysis
Statistical analysis was performed using SPSS 17.00. The Chi-square test was used for categorical variables, independent sample t-test was used for continuous variables, and Mann Whitney U test was used to compare median values. A p-value < 0.05 was considered significant. The area under the receiver operating characteristic curve was used to determine discriminative power of serum AMH level in prediction of IVF/ICSI outcome.

Results
One hundred and four women were included the study. All patients were on their first cycle of IVF-ICSI treatment. The long protocol was well tolerated by the patients. No systemic adverse effects were observed and no severe ovarian hyperstimulation syndrome (OHSS) occurred. After the treatment 34 patients (32.6%) became pregnant and formed the study group. Control group consisted of seventy patients (67.4%), pregnancy could not be achieved.

In control group, the mean age was 32.12 ± 6.01 years and it was significantly higher than study group (p = 0.01). No significant difference was found in duration of infertility, antral follicle count, basal E2, and FSH levels (Table 1). The mean serum AMH level was significantly higher in study group (p = 0.005). The retrieved oocyte number, metaphase 2 oocyte number and fertilization rate were also significantly higher in study group. Comparison of the groups in relation with IVF-ICSI outcome is shown in Table 2.

Table 1. — Characteristics of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Not pregnant (n=70)</th>
<th>Pregnant (n=34)</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.12 ± 6.01</td>
<td>29.14 ± 3.96</td>
<td>0.01</td>
<td>-0.72 to 5.23</td>
</tr>
<tr>
<td>DI (years)</td>
<td>6.29 ± 0.63</td>
<td>7.20 ± 1.02</td>
<td>0.38</td>
<td>-0.42 to 1.27</td>
</tr>
<tr>
<td>Basal E2 level (pg/ml)</td>
<td>63.04 ± 13.31</td>
<td>58.93 ± 11.27</td>
<td>0.95</td>
<td>9.25 to 21.66</td>
</tr>
<tr>
<td>Basal FSH level (iu/l)</td>
<td>19.31 ± 0.71</td>
<td>18.73 ± 0.62</td>
<td>0.44</td>
<td>-1.33 to 2.85</td>
</tr>
<tr>
<td>AFC</td>
<td>3.04 ± 0.46</td>
<td>2.76 ± 0.29</td>
<td>0.56</td>
<td>-0.35 to 0.77</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD. CI: confidence interval; DI: duration of infertility; FSH: follicular stimulating hormone; AFC: antral follicle count.

Table 2. — Comparison of IVF-ICSI outcome according to the pregnancy status.

<table>
<thead>
<tr>
<th></th>
<th>Control group (not pregnant, n=70)</th>
<th>Study group (pregnant, n=34)</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>1(&lt;1-3&gt;)</td>
<td>1(&lt;1-4&gt;)</td>
<td>0.402</td>
<td></td>
</tr>
<tr>
<td>RON</td>
<td>4.71±3.55</td>
<td>6.67±2.85</td>
<td>0.006</td>
<td>-3.34 to -5.76</td>
</tr>
<tr>
<td>MON</td>
<td>3.34±2.46</td>
<td>5.26±2.53</td>
<td>0.001</td>
<td>-2.95 to -0.89</td>
</tr>
<tr>
<td>FR, (%)</td>
<td>(48.9)</td>
<td>(57.86)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>AMH</td>
<td>0.45±0.28</td>
<td>0.61±0.23</td>
<td>0.005</td>
<td>-0.264 to -0.047</td>
</tr>
<tr>
<td>E2 on hCG day</td>
<td>1949.55±2264.21</td>
<td>2264.62±2680.64</td>
<td>0.53</td>
<td>-1312.70 to 683.40</td>
</tr>
<tr>
<td>P on hCG day</td>
<td>0.65±0.22</td>
<td>0.69±0.21</td>
<td>0.48</td>
<td>-0.123 to 0.059</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SD, (percentages) and median <range>. IN: intervention number; CI: confidence interval; RON: retrieved oocyte number; MON: metaphase 2 oocyte number; FR: fertilization rate; P: progesterone.

Figure 1. — The ROC analysis of AMH. Area under the curve for AMH: 0.686. Cut off value: 0.4 (sensitivity: 0.85, 1-specificity: 0.44).

Area under the curve for AMH: 0.686 (Figure 1). The cut-off value for AMH was 0.4 ng/ml (sensitivity 85%, specificity 44%). The cumulative ongoing pregnancy rate (OPR) was 24% (25/104) with this cut-off level. The cumulative ongoing pregnancy rate (OPR) was 24% (25/104) with this cut-off level.

Discussion
In this clinical study, the association between serum AMH levels and IVF-ICSI outcome was evaluated. Also, a cut-off level for AMH was attempted to predict the success of IVF-
AMH level will take its rightful place for predicting the success in poor responder patients. Therefore, measurement of serum AMH level seems to be a useful marker to predict IVF-ICSI outcome in poor responder patients. The cut-off value for AMH was found to be 0.4 (sensitivity 85%, specificity 44%).

Poor ovarian reserve and advanced maternal age are the most important factors influencing the success of IVF-ICSI. Management of COH and administration of convenient protocol are critical. Therefore, prediction of a poor response status helps to individualize the treatment regime and to yield the best results [8]. Numerous static tests such as FSH, inhibin β, ovarian volume, AFC, or dynamic test as clomiphene citrate challenge test (CCCT), GnRH agonist stimulation test (GAST), and exogenous FSH ovarian reserve test (EFTOR) have been utilized in the past [6, 7, 9]. However, the values of these tests are limited.

AMH has been introduced as a novel marker predicting ovarian reserve [9-11]. Hazout et al. reported that 1.1 ng/ml was a cut-off level for AMH. They founded that IVF-ICSI outcome was poor with an AMH level less than 1.1 ng/ml. Also, AMH measurement was found to be more prognostic than age, serum FSH, inhibin B or estradiol [9]. La Marca et al. demonstrated that AMH level remains unchanged throughout menstrual cycle. However, other steroids secreted from oocytes exhibit important variability [11]. Streuli et al. showed that AMH levels exhibit alterations during the cycle [12], but these fluctuations were found to be slight and clinically negligible. Therefore, serum AMH levels were accepted as stable during the cycle when they compared with other ovarian reserve markers.

Various cut-off levels were described for AMH. Buyuk et al. reported that IVF-ICSI outcome was better with random serum AMH levels ≥ 0.6 ng/ml [13]. Gleicher et al. identified an AMH cut-off of 1.06 ng/ml [14]. They affirmed that poor responder patients could have increased pregnancy rates with an AMH level > 1.06 ng/ml. Celik et al. detected that an AMH cut-off level ≥ 1 ng/ml had a sensitivity of 58.7% and specificity of 95.1% in poor responder women [15]. In the present study, the authors found an AMH cut-off level ≥ 0.4 ng/ml (sensitivity 85%, specificity 44%). The patients had basal FSH level > 15 IU/ml and AFC < 4.

In conclusion, the present authors analyzed the association between serum AMH level and IVF-ICSI outcome. The pregnancy rates were significantly lower with AMH levels < 0.4 ng/ml. AMH was found to be a useful parameter to predict poor ovarian reserve. Therefore, measurement of serum AMH level will take its rightful place for predicting the success of IVF-ICSI in near future. However, large prospective randomized controlled studies are required to show the importance of AMH in predicting ovarian reserve and IVF-ICSI outcome.

References


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Introduction

Mother and child health is one of the important subjects of public health. A healthy child is born to a healthy mother as a result of a well-managed pregnancy. Adequate and qualified antenatal care (ANC) services are required for having a healthy and safe gestation period [1]. The objective of ANC services is to prevent, mitigate or treat/manage the health problems or diseases (including those directly related to pregnancy) that are known to have negative effects on pregnancy and to provide the necessary information and give advice to women and their families/husbands about a healthy pregnancy, birthing, and postnatal period including baby care and breast feeding practices. According to the World Health Organization (WHO), a healthy pregnant woman should be monitored at least four times during her pregnancy [1]. There are many factors influencing the birthweight (BW) of an infant. One of these is the level and quality of ANC.

The number of ANC visits varies according to income levels of countries. Pregnant women who are believed to be receiving inadequate number of ANC visits are visited 8.2 to 12 times on the average in countries with high level of income, but usually less than five times in countries with low and moderate income. It was found in the researches conducted that prenatal mortality was higher in those who received inadequate ANC, but there was not a significant relationship between preterm birth and infants with a low birthweight (LBW), and the level of ANC. There is no strong evidence that the level and quality of ANC has an impact on BW [2]. It was found in a study that those pregnant women who had a preterm birth and those who delivered babies with a LBW had more ANC [3].

It is recommended in the United Kingdom (UK) that ANC start in the early weeks of pregnancy (ideally within the first ten weeks) and those who are parous with an uncomplicated pregnancy be visited seven times, and those who are nulliparous with an uncomplicated pregnancy be visited ten times for ANC [4]. The percentage of those receiving ANC in Turkey has increased in time, reaching 92% in 2008. While it is recommended that pregnant women receive ANC ideally ten times in Turkey, 73.7% of them have received ANC four times and more. It is also recommended that weight measurement, blood pressure measurement, urine and blood tests, and ultrasonic examination of the abdomen or listening of fetal heart rate be carried out at least once during ANC [5]. The ANC management guideline prepared by the Ministry of Health also mentions that pregnant women should be visited at least four times and explains in detail what should be done during each visit [6].

The purpose of this research was to measure whether the level and quality of ANC has any effect on the BW of infants of a singleton birth in a hospital.

Materials and Methods

This research was a retrospective causal study. The research was carried out in the provincial center of Yozgat, a city located in the middle of Turkey. The population of the research consisted of women who had singleton live births at the state hospital and a pri-
vate hospital in the center of the province. The sample of the research included those who had deliveries at the mentioned hospitals in the period of conducting the research. Before administering the questionnaire, those included in the research were explained the purpose of the research and that they were free to participate in the research or not and that their verbal consent were obtained. N=788 women were included in the research who agreed to participate by giving a verbal consent. Institutional permission was obtained for the research from the Governorship of Yozgat and an institutional permission was obtained for the research from the Ethics Committee of Yozgat State Hospital.

The data were collected by filling out the questionnaire prepared by the investigator with the help of the interviewers. The interviewers were third and fourth year students of the nursing department, and were trained by the investigator. Care was taken to administer the questionnaire to have the mothers in a resting state able to respond to the questionnaire. The variables found to be statistically significant as per the independent samples T test and ANOVA were made subject to multiple ANCOVA and linear regression analyses.

The independent samples T test, ANOVA, ANCOVA, and linear regression analysis were used in statistical evaluation of the data. The variables found to be statistically significant as per the independent samples T test and ANOVA were made subject to multiple ANCOVA and linear regression analyses. BW (g) was taken as the dependent variable in the multiple analyses. From the independent variables, mother’s age (years), height (cm), duration of pregnancy (weeks), weight gained during pregnancy (kg), and the number of problems experienced during pregnancy were taken as covariates and having received adequate-qualified ANC was taken as the categorical variable.

**Results**

Of those who participated in the research, 33.6% lived in the provincial center, 35.5% of them in borough centers, and 30.8% in villages; 54.5% of them had nuclear family structure, household size was 5.1±2.2 and the mean age was 25.7±5.8, the youngest being 15 and the oldest 47 years of age. Of the women participated in the research, 8.9% stated that they had no social security and 91.7% of them that they were not working in any job; 74% of the mothers stated that they became pregnant willingly. The mean BW was not found different according to willingness for pregnancy (t = 0.81, p = 0.42). In the women studied, 93.4% received adequate ANC, 66.4% qualified ANC, and 65.7% adequate and qualified ANC (Table 1); 84.6% of those who received ANC stated that they began receiving ANC in the first three months of their pregnancy (95.7% in the first 20 weeks). The mean

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
<th>x ¯ ± SD</th>
<th>Characteristics</th>
<th>n (%)</th>
<th>x ¯ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age</td>
<td></td>
<td></td>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>117 (14.8)</td>
<td>3212.0±531.6</td>
<td>Vaginal</td>
<td>260 (33.0)</td>
<td>3290.5±445.8</td>
</tr>
<tr>
<td>20-24</td>
<td>259 (32.9)</td>
<td>3233.5±557.2</td>
<td>Caesarean section</td>
<td>528 (67.0)</td>
<td>3278.5±574.8</td>
</tr>
<tr>
<td>25-29</td>
<td>217 (27.5)</td>
<td>3329.1±488.7</td>
<td>Gestational age</td>
<td>788 F=86.1, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>120 (15.2)</td>
<td>3301.9±548.4</td>
<td>&lt; 37 weeks</td>
<td>81 (10.3)</td>
<td>2667.9±629.5</td>
</tr>
<tr>
<td>≥ 35</td>
<td>75 (9.5)</td>
<td>3395.5±553.9</td>
<td>37–39 weeks</td>
<td>320 (40.6)</td>
<td>3247.4±475.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 40 weeks</td>
<td>387 (49.1)</td>
<td>3440.1±460.2</td>
</tr>
<tr>
<td>Mother’s height</td>
<td></td>
<td></td>
<td>Maternal weight gain</td>
<td>731 F=5.62, p=0.004</td>
<td></td>
</tr>
<tr>
<td>≤ 150 cm</td>
<td>58 (7.8)</td>
<td>3176.7±392.2</td>
<td>5–9 kg.</td>
<td>194 (26.5)</td>
<td>3204.6±612.3</td>
</tr>
<tr>
<td>151–155 cm</td>
<td>114 (15.4)</td>
<td>3244.3±552.2</td>
<td>10–14 kg</td>
<td>320 (43.8)</td>
<td>3275.2±499.3</td>
</tr>
<tr>
<td>156–165 cm</td>
<td>422 (57.1)</td>
<td>3286.7±558.5</td>
<td>≥ 15 kg</td>
<td>217 (29.7)</td>
<td>3379.8±516.9</td>
</tr>
<tr>
<td>≥ 166 cm</td>
<td>145 (19.6)</td>
<td>3359.8±492.2</td>
<td>Fe, vitamins use</td>
<td>788 t=1.90, p=0.058</td>
<td></td>
</tr>
<tr>
<td>Mother’s education</td>
<td></td>
<td></td>
<td>Yes</td>
<td>684 (86.8)</td>
<td>3189.7±530.1</td>
</tr>
<tr>
<td>≤ Elementary</td>
<td>411 (52.2)</td>
<td>3247.0±565.0</td>
<td>No</td>
<td>104 (13.2)</td>
<td>3352.9±475.8</td>
</tr>
<tr>
<td>Primary (8 years)</td>
<td>173 (22.0)</td>
<td>3322.1±524.6</td>
<td>Medical problems</td>
<td>778 t=3.60, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>≥ High school</td>
<td>204 (25.9)</td>
<td>3320.3±477.7</td>
<td>Adequate</td>
<td>736 (93.4)</td>
<td>3295.0±533.5</td>
</tr>
<tr>
<td>Mother’s economic level</td>
<td></td>
<td></td>
<td>Adequate-qualified</td>
<td>518 (65.7)</td>
<td>3313.6±541.0</td>
</tr>
<tr>
<td>Good</td>
<td>101 (12.8)</td>
<td>3283.4±444.6</td>
<td>Inadequate</td>
<td>52 (6.6)</td>
<td>3105.2±536.1</td>
</tr>
<tr>
<td>Middle</td>
<td>457 (58.0)</td>
<td>3288.9±542.3</td>
<td>ANC level</td>
<td>788 t=2.48, p=0.013</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>230 (29.2)</td>
<td>3269.2±559.5</td>
<td>Adequate</td>
<td>736 (93.4)</td>
<td>3295.0±533.5</td>
</tr>
<tr>
<td>.interval between pregnancies</td>
<td></td>
<td></td>
<td>Adequate-qualified</td>
<td>518 (65.7)</td>
<td>3313.6±541.0</td>
</tr>
<tr>
<td>Primigravida</td>
<td>300 (38.9)</td>
<td>3252.9±504.0</td>
<td>Inadequate-unqualified</td>
<td>270 (34.3)</td>
<td>3222.7±520.4</td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>110 (14.2)</td>
<td>3260.6±542.1</td>
<td>Total</td>
<td>788 (100.0)</td>
<td>3282.5±535.4</td>
</tr>
<tr>
<td>2 ≤ 3 years</td>
<td>120 (15.2)</td>
<td>3277.5±580.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 3 years</td>
<td>284 (36.8)</td>
<td>3325.2±558.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Percentages are based on the sum of the column of those who responded. x ¯ ± SD: mean ± standards deviation.
Does level of antenatal care affect birthweight? Study of a Central Anatolian Region

The mean BW was 3282 ± 535.4 g, the minimum being 900 g and maximum 5,000 g. Of the babies in the study, 5.7% had LBW (< 2,500 g) and 10.3% of them were born prematurely (< 37 weeks); 30.9% of those who were born preterm and 2.8% of those who were born term were babies with LBW. The mean BW was not found to differ according to the age group of mothers, their height, their living place, mode of delivery, time between pregnancies, use of vitamin F during pregnancy or the mother’s education and economic status. The mean BW differed according to the weight gained by the mother during pregnancy, gestational age, having problems during pregnancy, and the level and quality of ANC (Table 1). When an ANCOVA analysis was made after standardizing the status of having had problems during pregnancy, the number of ANC visits was not statistically significant (Table 2). While the mean BW was not significant according to the mother’s age and height in the singular analysis, it was found significant in the multiple analyses (Table 2). When the level of receiving ANC was standardized, BW increased as the mother’s age, height, weight gained by her during pregnancy and gestational age increased, and the number of problems experienced during pregnancy decreased. In 25.9% with change in BW can be explained by these six variables (20.9% by gestational weeks). Among these six factors, the effect of the level of receiving ANC is minimal (0.6%) (Table 3). When an ANCOVA analysis was carried out after standardizing the status of receiving adequate-qualified ANC, the effect of this variable on BW was not significant, whereas the woman’s age, height, weight gained by her during pregnancy, gestational age, and experiencing problems during pregnancy were significant (Table 3). The variables that seemed to have impact on BW depending on the level and quality of ANC were analyzed separately using linear regression. BW increased in those who received ade-

Table 2. — Factors affecting BW after standardizing experiencing of medical problems during pregnancy.

<table>
<thead>
<tr>
<th>Source *</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>27.214</td>
<td>0.000</td>
<td>0.039</td>
</tr>
<tr>
<td>Mother’s age (year)</td>
<td>23.697</td>
<td>0.000</td>
<td>0.034</td>
</tr>
<tr>
<td>Mother’s height (cm)</td>
<td>6.462</td>
<td>0.011</td>
<td>0.009</td>
</tr>
<tr>
<td>Numbers of ANC</td>
<td>2.713</td>
<td>0.100</td>
<td>0.004</td>
</tr>
<tr>
<td>Maternal weight gain (kg)</td>
<td>10.762</td>
<td>0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>178.979</td>
<td>0.000</td>
<td>0.210</td>
</tr>
<tr>
<td>Medical problems during pregnancy</td>
<td>8.848</td>
<td>0.003</td>
<td>0.013</td>
</tr>
</tbody>
</table>

* General Linear Model Univariate analysis (ANCOVA).

Table 3. — Factors affecting BW after standardizing the level and quality of receiving ANC.

<table>
<thead>
<tr>
<th>Source *</th>
<th>Adequate ANC / Inadequate ANC</th>
<th>Adequate-qualified ANC / Inadequate-unqualified ANC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>40.099</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>25.102</td>
<td>0.000</td>
</tr>
<tr>
<td>Mother’s age (years)</td>
<td>24.376</td>
<td>0.000</td>
</tr>
<tr>
<td>Mother’s height (cm)</td>
<td>5.238</td>
<td>0.022</td>
</tr>
<tr>
<td>Maternal weight gain (kg)</td>
<td>10.845</td>
<td>0.001</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>178.705</td>
<td>0.000</td>
</tr>
<tr>
<td>Number of med. prob. during pregnancy</td>
<td>6.309</td>
<td>0.012</td>
</tr>
<tr>
<td>ANC’s level</td>
<td>4.304</td>
<td>0.038</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td></td>
<td>0.259</td>
</tr>
</tbody>
</table>

* General Linear Model Univariate analysis (ANCOVA).

Table 4. — Factors affecting BW according to the level of receiving ANC.

<table>
<thead>
<tr>
<th>Model *</th>
<th>Inadequate ANC (n=52)</th>
<th>Adequate ANC (n=736)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standardized Coefficients β</td>
<td>t</td>
</tr>
<tr>
<td>(Constant)</td>
<td>-1.331</td>
<td>0.197</td>
</tr>
<tr>
<td>Mother’s age (years)</td>
<td>0.438</td>
<td>3.214</td>
</tr>
<tr>
<td>Mother’s height (cm)</td>
<td>0.063</td>
<td>0.480</td>
</tr>
<tr>
<td>Maternal weight gain (kg)</td>
<td>0.002</td>
<td>0.017</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>0.543</td>
<td>3.991</td>
</tr>
<tr>
<td>Number of med. prob. during pregnancy</td>
<td>-0.146</td>
<td>-1.083</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td></td>
<td>0.560</td>
</tr>
</tbody>
</table>

* Linear regression.
quate or adequate-qualified ANC as primarily gestational age and then the mother’s age and height, and weight gained by her during pregnancy increased and the number of problems experienced during her pregnancy decreased. The mother’s age and gestational age were significant in those who did not receive adequate ANC and the mother’s age, weight gained by her during pregnancy, and gestational age were significant in those who did not receive adequate-qualified ANC. While adequate receipt of ANC was significant, although slight, for BW, the number of ANC visits and whether or not the ANC was adequate-qualified were not significant. However, two of the factors effecting BW, namely, weight gained during pregnancy and experiencing problems during pregnancy were both more significant in those who received adequate ANC and adequate-qualified ANC (Tables 4 and 5). This shows that the level and quality of ANC also affects BW indirectly.

**Discussion**

In this study, the effect of ANC on BW was examined independent of other factors. The mean BW in the research group (3,282.5 g) was slightly higher than the mean BW found in locally performed researches in Turkey (2,955 g, 3,222 g, and 3,152 g, respectively) [7-9]. The mean BW was 3,492 g in Finland (2007), 3,414 g in Germany (2007), and a research conducted in the USA revealed the mean BW to be 3,264 g [10-12]. While the mean BW in those born term and singleton in the research group (3,352.9 g) was close to those born term and singleton in the USA (3,389 g in 2005), it was higher than the mean BW of Latin American countries (3,156 g) [13, 14]. A meta-analysis of the researches made between 1970 and 1980 showed that intrauterine growth retardation was an important problem in developing countries and prematurity in developed countries. This analysis also revealed that the mean BW was 3,299 g in the USA, 3,162 g (Hungary), 3,500 g (Norway) in Europe, 3,208 g in Japan, 3,285 g in China, 3,250 in Iran, and 3,540 in Iraq [15].

Of the women in the research group, 93.4% received adequate ANC and 65.7% of them adequate-qualified ANC (Table 1). The mean weeks of beginning to receive ANC was 8.74 ± 5.65. Those taking part in the research have received more ANC (73.7% of them for four times and more) as compared to pregnant women in Turkey in 2008 [5]. In a research carried out in France, it was found that 93.3% of women received ANC at least four times and 72.6% of them received adequate ANC (whose first visit was before 15th week or who received ANC once in a month) and the mean weeks of beginning to receive ANC was 12.3 ± 5.37 and pregnant women in Finland (2007) received ANC 16.5 times on the average and the mean weeks of beginning the first visit was 9.6 weeks [10, 16]. It was found in the USA that 61.1% of pregnant women received adequate ANC in 1980 and 38.9% of them received inadequate (16.7% totally inadequate) ANC [17]. The percentage of those who received adequate ANC is similar to the results of the researches performed in France and USA.

The mean BW of the women in the research group did not differ according to their living place, mode of delivery, time between pregnancies or the mother’s educational and economic status (Table 1). When receipt of adequate and adequate-qualified ANC was standardized, it was found that as the age, height, weight gained during pregnancy, and gestational age of those participated in the research increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age. It was found in a study conducted in Brazil that as the weight gained during pregnancy increased, BW also increased; 26.6% of the change in BW can be explained by five variables (22.5% by the weeks of pregnancy) (Table 3). The fact that 30.9% of those who were born premature and 2.8% of those who were born term were babies with LBW better shows the importance of gestational age.
and LBW, qualified ANC intervention to adolescent pregnant women increased BW and the mean weeks of pregnancy [21, 22]. In another Cochrane review study, prenatal mortality was found higher in those who received inadequate ANC and no relationship was found between preterm delivery and LBW, and the level of ANC [2]. The present results are similar to those of other researches. In a review study, of the 28 studies, 24 reported an effect on birth outcomes, five (21%) found a significant positive effect on gestational age, and seven of 17 (41%) found a significant positive effect on birth weight [23].

No significant relationship was found between the socioeconomic condition of the mothers in the research group and BW. The reason for this could be that the majority of the mothers (93.4%) had received adequate ANC. As in other researches, the present authors also did not find any significant relationship between the level and quality of ANC and BW. However, the fact that the level and quality of ANC can affect the factors influencing BW shows that ANC may have an impact on BW indirectly. Both adequate and qualified ANC must be received for a healthy pregnancy and healthy delivery of a baby. Gestational age is essentially the factor that affects BW. It is recommended that the necessary interventions be made to minimize the factors causing premature births.

References


Resolution of pelvic pain related to adenomyosis following treatment with dextroamphetamine sulfate

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Summary

Purpose: To determine if treatment with dextroamphetamine sulfate can reduce pelvic pain that was attributed to adenomyosis. Materials and Methods: Dextroamphetamine sulfate was given to a 32-year-old woman who suffered on a daily basis from severe chronic pelvic pain that was not relieved by laparoscopic removal of endometriosis by oral contraceptive and ibuprofen. The adenomyosis was diagnosed by magnetic resonance imaging. Results: Within three months the pain was completely gone and has remained absent for six months. Conclusions: Dextroamphetamine sulfate relieved pain from adenomyosis similar to its effect on endometriosis.

Key words: Adenomyosis; Chronic pelvic pain; Dextroamphetamine sulfate; Endometriosis; Sympathetic hypofunction.

Introduction

There are various types of pelvic pain including dysmenorrhea, dyspareunia, middle schmertz, chronic pelvic pain, introital pain (vulvodynia or vulvovaginosis), and pelvic pain of bladder origin. Dextroamphetamine sulfate has been found to be a very safe and very effective treatment for all of these disorders [1-5].

For years surgical therapy especially for endometriosis, either through laser vaporization or surgical removal, was the primary treatment [6]. However, even when there is absolute laparoscopic confirmation of absence of return of endometrial implants by repeat laparoscopy, a high percentage of females will experience a return of their pain [7]. In contrast, treatment with dextroamphetamine sulfate provides long lasting relief of pain which rarely ever returns as long as treatment is continued [8]. It is believed that a lot of other chronic treatment refractory disorders, including but not limited to pain, also responds to sympathomimetic amine therapy [9, 10].

The sympathetic nervous system controls cellular permeability. Hypofunction of the sympathetic nervous system may then allow the absorption of chemicals and toxic material into tissues that would normally be impervious which then evokes an inflammatory response, that in turn, leads to pain [10]. The sympathomimetic amine dextroamphetamine sulfate may replace the diminished neurotransmitter or stimulate dopamine and thus correct the permeability defect [10].

Many of the treatments for endometriosis do not work as well for adenomyosis. One possible reason for failure of surgical removal of endometriosis to work or even some medical therapies is because of concomitant existence of adenomyosis. The authors present a case of endometriosis and adenomyosis that showed only temporary relief of pain with surgical removal of endometriosis, but where the woman had extremely good relief from treatment with dextroamphetamine amine sulfate.

Case Report

A 32-year-old woman presented to discuss any options short of the proposed hysterectomy for her severe chronic pelvic pain of five years duration. She had a laparoscopy and was found to have endometriosis. Laser vaporization reduced the pain intensity from a 10 to a 5, but it returned to a full 10 within six months.

Subsequently an MRI study found a thickened junctional zone along the inferior uterine body measuring 14 mm and was thus diagnosed as segmental adenomyosis. She was advised that only a hysterectomy would relieve the pain. Since she had not had any children as yet, she wanted to preserve her uterus. Despite taking oral contraceptives continually for three months at a time before allowing menses and despite 800 mg ibuprofen every four to six hours, she had pain every day with periods of waxing and waning with most of it left-sided. On 15-mg dextroamphetamine sulfate extended release capsules, the pain markedly improved. An increase in dosage to 30-mg extended release capsules once daily was initiated. Six months following the increased dosage, the woman remained pain-free and does not even require ibuprofen.

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Discussion

Adenomyosis can be added to the list of pain disorders that respond very well to sympathomimetic amines. One could argue that MRI diagnosis may not be the ultimate way to diagnose adenomyosis, but it is the best tool available short of pathological evaluation after hysterectomy.

One could argue that the persistent pain was not from the adenomyosis but merely related to the continued absorption of chemicals and toxins into the pelvic tissues. Nevertheless the presumptive diagnosis and considered etiology for the pain was adenomyosis based on the MRI. Treatment with dextroamphetamine sulfate saved her from having a hysterectomy and no hope for children, except for adoption or surrogacy or embryo transfer into a gestational carrier. Dextroamphetamine sulfate is safe to use during pregnancy in the dosages employed and may even be used to obviate infertility and/or miscarriage in treatment refractory cases [11].

References


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Hashimoto thyroiditis onset after laparoscopic removal of struma ovarii: an overview to unravel a rare and intriguing finding

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Summary
Struma ovarii is an uncommon type of ovarian tumor derived by germinal cells, characterized by the predominance of thyroid tissue (>50%); 90-95% of these formations are benign and mainly affect the left ovary, while in 6% of the cases struma ovarii is bilateral. The malignant transformation is a rare condition that often occurs after 50 years. In most instances, diagnosis of malignant struma ovarii is made postoperatively during histological analysis. This tumor appears to derive by one germinal cell through loss of heterozygosity of the androgen receptor gene and of the X chromosome. Clinical symptoms comprise abdominal pelvic mass, lower abdominal pain, abnormal vaginal bleeding, and ascites (the occurrence of this condition has been observed in one-third of the cases). The patients with struma ovarii generally do not manifest symptoms related to thyroid hyperfunction, reported only in 8% of the cases, and due to hyperstimulation of the thyroid by auto-antibodies. Thyroid tissue of the struma ovarii, often embedded in a teratoma, may be papillary, follicular or with mixed pattern and it can include elements of mucinous cystoadenomas, Brenner’s tumor or carcinoid or melanomas cells. Here the authors report their experience with an unusual case of Hashimoto thyroiditis onset after laparoscopic removal of struma ovarii.

Key words: Struma ovarii; Ovarian tumor; Laparoscopy; Hashimoto thyroiditis.

Introduction
Definition and epidemiology
Struma ovarii is an uncommon type of ovarian tumor derived by germinal cells (mature teratoma), characterized by the presence of thyroid tissue for more than 50% of the overall mass [1-5]. It uncommonly occurs in pre-pubertal girls and the peak of incidence is the fifth decade [6]. The first Authors who described struma ovarii were Von Kalden in 1985 and Gottschalk in 1899 [7]. It comprises 1% of all ovarian tumors and 2.7 % of all dermoid tumors [8]. Ninety to 95% of these formations are benign [9] and mainly affect the left ovary, while in 6% of cases it is bilateral[10]. Sometimes, thyroid tissue is organized in small foci on the peritoneal surface: this particular and rare condition is defined as “strumosis” [8, 11-13]. The malignant transformation is a rare condition that often occurs after 50 years [10], and causes metastasis in 5% of cases [14]. In most instances, diagnosis of malignant struma ovarii is made postoperatively during histological analysis [15]. The etiology of this tumor is still not clear. Ciccarelli et al. [16] have shown loss of heterozygosis (LOH) for the androgen receptor and on X chromosome (LOH on Xp22.1-Xq21.32), suggesting the monoclonality of this tumor. Furthermore, they have identified multiple loss of region in chromosomes 4, 7, 10, 13, 14, 16, and X, but they could detect no chromosomal anomalies by using comparative genomic hybridization (CGH). There are useful genetic tests to address the diagnosis of malignant struma ovarii:
1) immunohistochemical staining of Hector Battifora mesothelial-1 (HBME-1) cells and Galactin 3, often positive in papillary thyroid carcinoma [17, 18];
2) BRAF mutation (v-raf murine sarcoma viral oncogene homolog B1) including V600E, K601E, and TV599-600M variants, found in two-thirds of malignant struma ovarii with papillary histology [19, 20];
3) REarranged during transfection (RET) rearrangements, found in 70% of follicular variant papillary thyroid cancer [20, 21];
4) RAt sarcoma (RAS) mutation;
5) Neurotrophic tyrosine kinase receptor 1 (NTRK1) mutation [22, 23];
6) Cytokeratin 19 expression [21].

Clinical characteristics and diagnosis
Benign struma ovarii’s symptoms and signs comprise palpable pelvic/abdominal mass, pelvic/abdominal pain, abnormal vaginal bleeding, and ascites (observed in one-third of the cases), although most of the cases are totally asymptomatic [24-26]. As the benign counterpart, malignant struma ovarii are often asymptomatic: when symptomatic, the patient usually presents palpable abdominal mass (45%), pelvic pain (40%), menstrual irregularities (9%),
hypeothyroid symptoms (8%), ascites (17%), and deep vein thrombosis (4%) [27, 28]. In laboratory tests it is not rare to find, both in benign and malignant form, serum increased carbohydrate antigen (CA) 125 levels [29]. Clinical manifestations of hyperthyroidism occurs in 5% - 8% of patients [26, 30, 31], rarely with overt thyrotoxicosis [15]. Generally, this tumor is functionally inactive, and when hyperthyroidism occurs, it is due to the presence of thyroid-stimulating hormone receptor stimulating antibodies (TSHR-ab), which stimulate hormone production in the thyroid [6, 25]. Struma ovarii may be suspected also by radiologic imaging:

- Pelvic ultrasonography (especially performed by transvaginal approach) could identify adnexal mass characterizing the size, the location, the presence of solid areas, and relationships of contiguity. Savelli et al. [32] have shown that the most characteristic ultrasonographic feature is the presence of one or more “struma pearls”, i.e. well circumscribed roundish areas of solid tissue with a smooth surface. However, as literature reports, the ultrasonographic pattern of struma ovarii are usually non-specific; it is possible to observe a multicellular cystic or solid adnexal mass [33, 34] and the presence of low resistance blood flow in the central portion of tumor and free fluid in the pelvis are frequent findings [35-37].

- Computerized tomography (CT) of pelvis could evidence a complex mass containing various size of cystic and solid components [34, 36]. After intravenous (i.v.) injection of contrast medium, the solid portion intensely enhance [34, 36], while most cysts contain non-enhancing fluid with Hounsfield values (HU) > 20 and calcifications [38].

- Magnetic resonance imaging (MRI) clearly shows a strong enhancement of the solid component in the tumor after i.v. injection of gadolinium- diethylenetriaminepentaacetic acid (Gd-DTPA) [39], and also cyst with a low signal on T1-weighted images and a very low signal on T2-weighted are a typical findings in struma ovarii [33].

- a I 131 scan is useful to evaluate active thyroid tissue when struma ovarii is suspected [16, 40]. Occasionally, struma ovarii is associated with Meigs’ syndrome for the presence of ascites, pleural effusion, and serum increased CA 125 levels. In these cases, it is important to make a differential diagnosis with other malignant ovarian neoplasms [41-49]. Overt hypothyroidism rarely follows the resection of struma ovarii tumor [50], as well as it is uncommon to find evidence of concurrent autoimmune thyroiditis. The following has been reported: struma ovarii with patient’s thyroid tests normal [51]; struma ovarii with the presence of antithyroid antibodies [52]; struma ovarii with severe postoperative hypothyroidism [53, 54]; struma ovarii with Hurte cells, positive antithyroid antibodies, and normal thyroid function [55], and struma ovarii without Hurte cells [56].

Literature describes rare cases of association of ovarian struma with toxic thyroid adenoma [16, 25] or Graves’s disease [57]. Particularly, an interesting uncommon clinical case was reported that showed the coexistence of Graves’s disease, papillary thyroid carcinoma incidentally found after thyroidectomy, and unilateral benign struma ovarii accompanied by ascites, pleural effusion, and elevated CA 125 levels, with the immunohistochemical identification of TSHR-ab that supported the functional struma ovarii tissue [58].

Histologic findings and prognosis

On gross examination, the struma is brown or green-brown, partly or entirely cystic, filled with gelatinous fluid [59]. Ninety-four percent of malignant struma ovarii is monolateral, commonly affect left ovary, and is macroscopically similar to the corresponding benign counterpart [60]. Thyroid tissue is the major component of the mass; it may be papillary, follicular or mixed pattern and it can include elements of mucinous cystadenoma, Brenner tumor, carcinoid or melanoma. Birefringent crystals of calcium monohydrate are present in most cases. Immunohistochemical staining for thyroglobulin, triiodothyronine, thyroxine can confirm the diagnosis [61]. Malignancy is defined by histological features of tumor, including cellular atypia and hyperplasia, nuclear pleomorphism, high mitotic activity, microinvasion into surrounding vessels and/or ovarian capsule, and discovery of metastasis by imaging [62, 63]. Nevertheless, there is still controversy about the defying characteristics of a malignant struma ovarii by using the pathological criteria applied in diagnosing thyroid carcinoma [14, 21, 62]. As Bhansali et al. [40] revealed, in malignant struma ovarii histopathology, the presence of solid areas mixed with cysts without teratomaticous elements. Final pathology review is diagnostic for this condition in 5-37% of cases [11]. Malignant form is classified into different categories by histology [28, 30, 64]: papillary type is the most common and identified by ground glass or overlapping nuclei; follicular variant of papillary carcinoma, which shares the same nuclear characteristics as the papillary type but has a follicular architecture; follicular carcinoma; “highly differentiated follicular carcinoma of ovarian origin” (HDFG) characterized by metastasis with innocuous histological appearance, resembling that of non-neoplastic thyroid [65]; undifferentiated anaplastic carcinoma, and medullary carcinoma.

In general, malignant struma ovarii appears to have good prognosis with low metastatic potential (5-6% of the cases). Upon review of literature [56, 64], patients with follicular carcinoma have a survival rate of five years after diagnosis, those with papillary carcinoma eight years, and those with undifferentiated carcinoma 13 months, whereas, patients with HDFG seem to have a survival rate similar to the general population. Papillary and follicular carcinoma are the most frequent of malignancy to occur in ovarian struma, while other
forms occur only rarely. Typical follicular carcinoma is more aggressive than the somewhat more common papillary carcinoma, the HDFC is the least aggressive of these tumor types, while the undifferentiated carcinoma is the most aggressive. The most common metastatic sites reported include other pelvic structures as the contralateral ovary, or other sites as omentum, liver, lungs, bone, and brain [11]. While follicular cancer preferentially metastasizes to the lung, liver and brain, papillary carcinoma metastasizes to the organs of the abdominal cavity, lymph nodes, and rarely to the liver. Roth et al. [65] reported that in malignant struma ovarii bone metastases occur in 26% of patients with typical follicular carcinoma, in 17% of patients with HDFC, and in 4% of patients with papillary carcinoma. These tumors may metastasize up to ten years after initial surgery. In view of the long period between the initial diagnosis of malignant struma ovarii and the possibility of recurrence (typically within four years), as reported in the literature, it should be used for long term follow up to ten years [28].

Treatment
Therapy for benign struma ovarii is surgical resection. Due to rarity of malignant struma ovarii, there is a paucity of data in past literature regarding the optimal treatment modality for such patients. For women desiring further childbearing, unilateral salpingo-oophorectomy may be a feasible option in the absence of capsular invasion or distant metastasis [27]. Total abdominal hysterectomy and bilateral salpingo-oophorectomy should be performed in postmenopausal women or premenopausal women in which fertility is not desired. In any cases in which disease has spread outside the ovary, complete staging for ovarian cancer with pelvic washings, omentectomy, and pelvic and para-aortic lymph node dissection should be performed [40]. Adjuvant therapy consisting of total thyroidectomy and 131 ablation should only be considered in cases of residual and metastatic disease. In these cases, sequential serum thyroglobulin level measurement as follow-up every six to 12 months to detect recurrence has been recommended. Postoperative work-up should include serum thyroglobulin level and total body 131 scan to evaluate residual intra-abdominal disease in patients who have undergone total thyroidectomy. In malignant ovarian struma, iodine-131 ablation has been applied both as a therapeutic and preventive measure against local and distant recurrence [28]. This is supported by the evidence of 50% recurrence rate in those women who were treated with pelvic surgery only [27]. In these cases, medical therapy with synthetic levothyroxine appears to be appropriate in order to suppress the secretion of TSH and maintain inactive any residual cells [10, 50]. However, for patients with multiple metastatic lesion or those who do absorb radioiodine poorly, external beam radiation has been proposed. Chemotherapy and radiotherapy have been used for recurrent metastatic struma ovarii which does not concentrate radioiodine [10, 50].

Case Report
Patient’s data and anamnesis
The patient was a 67–year-old Caucasian female. Anthropometric parameters: weight 72 kg, height 154 cm (BMI: 30, first degree obesity). Familiarity for metabolic disease (her father had type 2 diabetes). First menstrual cycle when she was ten-years-old, followed by regular menstrual cycles for all characters. Parity 2012. Physiologic menopause at age 50. Personal history positive for tobacco use (ex-smoker of 20 cigarettes/day, suspended seven years ago). Remote pathological history: tonsilllectomy, cholecystectomy, uterine curettage for abortion, rectal prolapse surgically treated, knee prothesis, surgical correction of carpal tunnel syndrome, essential hypertension, neuropathic pain, gastro-esophageal reflux, anxious depressive syndrome. Drug history: acetylsalicylic acid, Omeprazole, Pregabalin, Sotalol, Rosuvastatin, Venlafaxine, perindopril/indapamide, alprazolam, and felodipine. She presented herself to the current authors’ observation for the incidental finding on pelvic ultrasound of right ovarian cyst. She, therefore, underwent preparation for laparoscopic surgery. Prior to surgery, she underwent routine blood tests and assay of tumor markers, chest x-ray, electrocardiogram (ECG), and pelvic ultrasound re-evaluation.

At the admission, the patient was informed in a comprehensive and complete way regarding her clinical condition and procedures that the authors were going to perform, and signed an informed consent to allow the data collection for research purposes (a subsequent formal approval by the Institutional Review Board was obtained before initiating the report). The following report is in accordance with the Helsinki Declaration, conforms with the Committee on Publication Ethics (COPE) guidelines (http://publicationethics.org/) and the CARE (Consensus-based Clinical Case Reporting) Statement [66], available through the EQUATOR (Enhancing the Quality and Transparency Of Health Research) network (http://www.equator-network.org/).

Preoperative tests
The laboratory tests were all within normal range except for a slight increase of the enzyme lactate dehydrogenase (LDH), which was 682 U/L [normal value (n.v.) 152-460]. Chest X-ray and ECG were negative. Tumor markers were are all within normal range: alpha feto protein (AFP) was 1.6 U/L (n.v. 0-10), carcinoembryonic antigen (CEA) 1 ng/ml (n.v. <5), CA 15.3 13.8 U/ml (n.v. < 39,5), CA 19-9 6.7 U/ml (n.v. 0-37), CA 125 4.1 U/ml (n.v. 0-35). The transvaginal pelvic ultrasound (Figure 1 a-e) showed retroverted uterus in moderate flexion, nonhomogeneous echo pattern, widespread fibrosclerosis, and atrophic involution (longitudinal diameter: 58 mm; anteroposterior diameter: 62 mm; transverse diameter: 50 mm.). Endometrium presented finely uneven margins and greater echogenicity at the isthmus (Figure 1a). Normal vascularity at color Doppler sampling. Left ovary was 19.7 x 12.9 mm (Figure 1b) and right one 56.2 x 56.9 mm (Figure 1c). In particular, right ovary was occupied by multiple irregularly roundish formations: the greatest was 67.4 x 49.8 mm and presented transonic ecopattem and finely corpuscular aspect (Figure 1d). Other lesions in the right ovary showed complex structure, characterized by alternating hyperechoic and vacuolar anechoic areas (Figure 1e). No free fluid in Douglas.

Gynecological examination, surgery, and histologic findings
The preoperative gynecological examination evidenced external genitalia and vagina of primipara, regular uterine body, right ovary increased in volume, no appreciable left ovary, small cervix, and no atypical bleeding. Subsequently, the patient underwent laparoscopic surgery. During surgical procedure, the authors performed lysis of adhesion between the right ovary, the peritoneal wall and the bowel, and then bilateral salpingo-oophorectomy.
Figure 1. — Transvaginal ultrasonographic imaging. a) Left ovary: 19.7 x 12.9 mm. b) Endometrium. c) Right ovary: 86.2 x 56.9 mm. d) Major roundish irregular formation in the right ovary: 67.4 x 49.8 mm. e) Minor formations in the right ovary.

Figure 2. — Histologic examination. a) Thyroid tissue with dilated follicles (asterisk). Haematoxylin-Eosin staining. Original magnification x 40. b) Thyroid tissue with evident papillae (arrow) protruding into dilated follicles (asterisk). Haematoxylin-Eosin staining. Original magnification x 150.
Histologic examination of the left ovary showed diffuse fibrofollicular hyperplasia and albicans bodies. Both fallopian tubes were sclero-atrophic. Right ovarian cyst showed the presence of a typical teratoma with prevalence of thyroid tissue with dilated follicles (Figure 2 a-b).

**Follow-up**

The patient had regular post-surgical course and was discharged home on the second day, in full health. Two and a half months after surgery, patient underwent morpho-functional thyroid evaluation. During this follow-up, she reported since the surgery, the onset of hyperthyroidism-like symptoms as flushing, profuse sweating, and tachycardia. The indices of thyroid function were normal: thyroid-stimulating hormone (TSH) was 2.010 mIU/ml (n.v. 0.27 - 4.2), free thyroxine (FT4) 14.04 pm/L (n.v. 12 - 22), but free triiodothyronine (FT3) was modestly increased (5.12 pg/ml; n.v. 2-4). The research of organ-specific antibodies was positive for anti-thyroid peroxidase (AbTPO < 5 IU/ml; n.v. 0 - 34) and anti-TSH receptor (Trab 0.10 IU/L; n.v. <1). The thyroid gland sonogram, postoperatively performed, was also normal. Transvaginal pelvic ultrasonography performed after three and a half months after surgery did not reveal any significant findings. The patient was therefore sent to the attention of the endocrinologist. Considering the clinical and laboratory findings, the authors diagnosed Hashimoto thyroiditis in euthyroidism, and patient is currently not taking any drug and undergoes periodic biochemical and instrumental checks.

**Discussion**

Struma ovarii in an uncommon ovarian tumor is very rarely identified. Since the paucity of symptoms and signs, diagnosis occurs in most cases postoperatively after histologic analysis. As was evidenced from the present literature review, struma ovarii is difficult to diagnose on the basis of clinical manifestations or imaging studies; moreover, the presenting clinical features are widely nonspecific, and even if secondary conditions such as thyroid hyperfunction, ascites or hydrothorax are present, these usually regress spontaneously upon surgical removal of the primary tumor. Infrequently, it is possible to evidence clinical manifestations of hyperthyroidism or hypothyroidism associated or not with thyroid specific antibodies’ positivity. In the present case, the authors report a case of coexistence of struma ovarii associated with post-surgical onset of thyroid autoimmune phenomenon (AbTG) and hyperthyroidism-like symptoms. Considering the clinical and laboratory data, they diagnosed Hashimoto thyroiditis. This scenario is similar to few others found in literature [54, 67-69].

In the present case, the correlation between excision of struma ovarii and post-surgical onset of Hashimoto thyroiditis remain to be elucidated: the authors could hypothesize that the laparoscopic removal of struma ovarii caused the activation of the antigen presenting cells (APCs), which processed and displayed thyroid-like antigens to T helper lymphocytes type 2 (Th2) and provoked the massive production of autoantibodies by B lymphocytes. Since the authors did not evidence any significant increase of T3 and T4, the cause of hyperthyroidism-like symptoms is still to be determined.

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avarii with marked ascites and elevated CA-125 levels: case report
Complete resolution of frozen shoulder syndrome in a woman treated with dextroamphetamine sulfate for chronic urinary urgency

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Summary

Purpose: To evaluate the efficacy of dextroamphetamine sulfate for idiopathic frozen shoulder in a woman being treated for bladder urgency and inability to lose weight despite dieting. Materials and Methods: Dextroamphetamine sulfate was initiated at 15 mg extended release capsules increasing to 25 mg extended release capsules to a 47-year-old woman. Results: She lost 19 pounds in four months, her bladder urgency disappeared, and she had complete resolution of the idiopathic frozen shoulder problem. Conclusions: Idiopathic frozen shoulder syndrome can be added to the long list of conditions that are related to hypofunction of the sympathetic nervous system and all respond to dextroamphetamine sulfate therapy. They gynecologist is more familiar with this syndrome because of it being the main cause of pelvic pain. Thus the gynecologist may become the physician who subsequently treats orthopedic or rheumatological problems or other health issues.

Key words: Idiopathic frozen shoulder; Urinary urgency; Obesity; Sympathomimetic amines.

Introduction

Pelvic pain of bladder origin, or as it is sometimes called interstitial cystitis, is part of a pelvic pain syndrome which includes dysmenorrhea, dyspareunia, mittelschmerz, chronic pelvic pain, and vulvodynia or vulvovaginitis. These conditions are linked by having at the core of the problem hypofunction of the sympathetic nervous system [1]. The sympathetic nervous system controls cellular permeability and hypofunction allows absorption of chemicals and toxic materials into the tissues which evoke an inflammatory response and pain [2]. All these entities respond quickly and effectively to treatment with sympathomimetic amines especially dextroamphetamine sulfate [3, 4]. Interstitial cystitis resistant to standard therapy has been shown to respond very well to treatment with dextroamphetamine sulfate [5, 6].

Despite many articles written about different chronic treatment refractory disorders that respond very well to sympathomimetic amine therapy, most physicians in other fields of medicine seem to be unaware of the condition of sympathetic nervous system hypofunction. Some of these disorders include a variety of chronic gastrointestinal disorders, skin disorders, rheumatologic disorders, and disorders of the nervous system to name a few [1, 2]. Sometimes a woman will seek help from the gynecologist or gynecologic subspecialist for problems of the pelvis and will not only notice improvement of the pelvic disorder but also some other chronic disorders. Since the gynecologist is the most likely type of physician to treat this disorder referred to as the sympathetic neural hyperalgesia edema syndrome they may inadvertently find that other conditions may respond. The physician should then report these findings so other physicians can benefit from this knowledge.

In this case report, a woman who sought help for refractory urinary urgency and inability to lose weight despite dieting found complete relief of the idiopathic frozen shoulder syndrome.

Case Report

A 47-year-old woman came in for her annual check-up. She complained of increasing hirsutism, inability to lose weight despite dieting, and chronic urinary urgency. A cystoscopy was negative. She also mentioned that she has had a frozen left shoulder unable to elevate her right arm. Extensive testing revealed no etiology related to ligament, tendon, or muscle damage or inflammation. She had stopped physical therapy one year before because it had not proven to improve her condition.

She showed improvement in her weight symptoms when returning in one month on 15 mg dextroamphetamine sulfate extended release capsules. Since she had no side effects, she was...
increased to 25 mg extended release capsules. Three months later she lost 19 pounds (she was 5’8” and 185 pounds), no longer had urinary urgency, and her idiopathic frozen shoulder syndrome completely disappeared. She had elected not to treat the hirsutism.

Discussion

Frozen shoulder is a condition in which movement of the shoulder becomes restricted. Primary frozen shoulder infers an unknown etiology [7]. It is commonly a self-limiting condition of approximately 1.3 years of duration though incomplete resolution can occur [8]. Thus it is possible that the frozen shoulder resolved spontaneously and coincidentally with dextroamphetamine sulfate therapy. However, in view of the many other disorders that respond so quickly to this drug after years of not responding to other therapies, it seems likely that the dextroamphetamine sulfate was responsible for her improvement [8]. It is not surprising she lost weight since this drug has proven very effective for women who cannot lose weight despite appropriate dieting [9].

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Isolated fallopian tube torsion during pregnancy: a case report

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Summary
Isolated fallopian tube torsion is a rare pregnancy-related complication. It is frequently misdiagnosed as acute appendicitis or ovarian torsion owing to the lack of specific symptoms or signs. Here, the authors report a case of a 35-year-old primigravida at 30 weeks and six days of gestation who had presented with right isolated fallopian tube torsion and a history of right oophorectomy. The authors propose that isolated fallopian tube torsion should be included in the list of differential diagnosis when encountered with patients complaining of lower abdominal pain.

Key words: Fallopian tube; Torsion; Pregnancy; Isolated; Ipsilateral oophorectomy.

Introduction
Among the possible causes of acute abdominal pain during pregnancy, the most common is acute appendicitis [1]. Many non-obstetric conditions mimic acute appendicitis, such as adnexal torsion, pyleonephritis, urinary calculi, cholecystitis, and bowel obstruction. If the patient presenting with abdominal pain is pregnant, obstetrical causes such as preterm labor, abruptio placenta, chorioamnionitis, and torsion or degeneration of a leiomyoma should be considered [1].

Because isolated fallopian tube torsion usually presents with right lower quadrant abdominal pain, it is difficult to differentiate it from acute appendicitis. Thus, it may be initially misdiagnosed as acute appendicitis. An accurate diagnosis may be obtained only in the operating room.

Case Report
A 35-year-old woman, gravida 0, para 0, was referred to the present hospital with mild consistent right lower abdominal pain, which had commenced five days prior. She was at 30 weeks and six days of gestation, and antenatal care had been well performed at a local clinic. At onset, she visited the local clinic due to vague lower abdominal pain, which was considered suggestive of preterm labor and she was thus admitted for tocolysis. During the first few days, tocolytic therapy was effective in relieving pain. However, on the fifth day of therapy, she complained of right lower abdominal pain with increasing intensity and was referred to the present hospital for a second opinion. On reviewing her history and physical examination, no noticeable symptoms or signs were found, except for mild right lower quadrant abdominal pain and tenderness. Her vital signs were stable, and initial laboratory findings revealed mild leukocytosis (white blood cell, 11.34 K/µl [range, 4–10 K/µl] and elevated C-reactive protein, 3.497 mg/dl [range, 0–0.5 mg/dl]). Routine urinalysis results were normal.

Cardiotocography revealed no uterine contractions. Preoperative ultrasonography revealed no adnexal cyst or signs of acute appendicitis. Because the patient had undergone right oophorectomy due to a mature cystic teratoma six years prior, right adnexal torsion was not included in the differential diagnosis at first. For further assessment, magnetic resonance imaging (MRI) was performed. MRI showed a normal appendix, and no abnormal focal lesion was identified. Because the correct diagnosis could not be established, an exploratory operation was performed in light of possible acute appendicitis. Laparotomy with small right paratubal incision was performed by the general surgical team. The findings revealed a normal appendix; however, a hyperemic edematous right fallopian tube was observed. In addition, the right fallopian tube had twisted twice counterclockwise (Figure 1). Right salpingectomy was performed by the on-call gynecology team. No adhesion band was found around the right fallopian tube despite having undergone surgery previously. The right fallopian tube showed no abnormalities such as cysts, fluid-filled hydrosalpinx, or pyosalpinx. Although the appendix seemed to be grossly normal, appendectomy was performed to rule out microscopic inflammation. Histopathology confirmed a slightly congested fallopian tube, measuring 7.5 × 1.2 cm. Pathological reports showed fallopian tube congestion and hemorrhage resulting from ischemic damage and normal appendix. After the operation, the radiologist revisited the MRI and attempted to locate the twisted right fallopian tube, but was unable to do so.

After surgery, the patient was kept under observation by using cardiotocography. Preterm labor occurred on the first postoperative day and intravenous infusion of ritodrine was maintained until the fourth postoperative day. She was discharged on the seventh postoperative day. At the two-week follow-up at the outpatient clinic, both the patient and fetus were doing well.

Discussion
Isolated fallopian tube torsion during pregnancy is extremely rare; Origoni et al. retrieved only 19 cases treated surgically from 1936 to 2009 [2]. In 2011, isolated torsion of the left normal fallopian tube during pregnancy and laparoscopic management of an isolated fallopian tube torsion with a large right paratubal cyst at 35 weeks of gestation were reported [3, 4].
Torsion generally occurs in abnormal fallopian tubes; however, it may develop in normal ones as well [3]. The main predisposing factors for isolated fallopian tube torsion during pregnancy are paraovarian cysts such as Morgagni hydatids and hydrosalpinges and ovarian cysts [2,5].

Torsion without an apparent adnexal mass is uncommon. It is, however, seen in children in whom the adnexa are especially mobile, allowing torsion at the mesosalpinx [6].

In the present case, the left fallopian tube was normal and the left ovary was absent due to an oophorectomy performed six years prior. This fact is noteworthy. Generally, torsion of the adnexal structures involves the tube or the ovary, but more often it involves both and a common predisposing factor is an ipsilateral adnexal mass [7]. Hence, the present authors did not consider adnexal torsion as a diagnosis at first. However, it is possible that the fallopian tube had moved about freely without the ovary and thus had a greater chance for twisting.

Fallopian tube torsion has been reported more often on the right side, and this may be related to the presence of the sigmoid colon, which prevents excessive adnexal movements or slow venous flow on the right side resulting in congestion of the tube. Surgeons correlate right lower abdominal pain with appendicitis, and this often leads to related operations [8, 9].

The most common symptom of tubal torsion is lower abdominal pain with or without nausea, vomiting, and sometimes uterine bleeding. Abdominal tenderness is observed in almost all patients [5]. However, these features are not pathognomonic, and imaging studies can help in diagnosing acute abdomen. In previous reports, transvaginal ultrasonography, computed tomography (CT), and MRI have been used [7,10]. In the present case, the authors avoided CT.

Ultrasonography is usually the first examination performed in an emergency setting [7]. Color Doppler ultrasonography can be used to reveal arterial and venous flow to the adnexal structures. However, it is limited in its capacity for displaying adnexal torsion [11]. The present authors were unsure of the existence of any abnormality in the pelvic cavity as detected by ultrasonography; hence, MRI was performed. CT or MRI is recommended for the detection of appendicitis, twisted vascular pedicle, thickened fallopian tube, and pelvic mass [7].

Even though imaging studies play an important role in diagnosis, the correct diagnosis is not usually established prior to operation, and it is almost always necessary to remove the tube [12]. Early diagnosis and treatment make it possible to conserve the twisted adnexa by untwisting the pedicle and resecting the cysts or tumors. However, when the patient presents with nonspecific clinical and laboratory findings, surgery is often delayed and irreversible necrotic change has occurred in majority of the cases, thereby requiring salpingectomy [13].

In conclusion, although isolated tubal torsion is extremely rare, it should be included in the list of differential diagnoses of pregnant women with lower abdominal pain, even if the ipsilateral ovary had been removed previously.

References


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Introduction

Polycystic ovarian syndrome (PCOS) is a common and ill-understood endocrine disorder with multisystem sequelae. Surgical treatment of PCOS associated with infertility actually consists of laparoscopic ovarian drilling (ovarian electrocautery) which displaces ovarian wedge resection performed by laparoscopy and laparotomy. Ovarian wedge resection, first reported by Stein and Leventhal [1], has been largely abandoned because of its possible consequences, like adhesions formation and ovarian tissue loss which can finally lead to premature ovarian failure. In this paper the authors present a case of a pregnancy after ovarian wedge resection by laparotomy after unsuccessful ovarian drilling.

Case Report

A 20-year-old patient was admitted to the present outpatient clinic because of infertility, secondary amenorrhea, and hirsutism. Telarche was at the age of ten. Menarche was at the age of ten, however menses were irregular (occurring every two to three months) with the last menstruation present at about two months before the admission. The patient was never pregnant before; she tried to become pregnant for about eight years prior to the admission. Three years prior she had undergone laparoscopic ovarian drilling with no effect on menstrual pattern and fertility. After clinical and ultrasonographic examinations, polycystic ovarian syndrome (PCOS) was diagnosed and consequently laparotomy with ovarian wedge resection was decided upon. Six weeks later patient was diagnosed with an early pregnancy.

Discussion

Ovarian wedge resection was first reported by Stein and Leventhal in 1939 [1]. Authors reported regular menses in 80% and spontaneous pregnancies in 50% of patients treated with this method [2]. Later the procedure was associated with high percentage of ovarian and periadnexal adhesions [3, 4] and with substantial loss of ovarian tissue and ovarian blood supply, which can finally lead to prema-
ture ovarian failure [5, 6]. This is why the procedure has been largely abandoned and replaced by other procedure - laparoscopic ovarian drilling.

Laparoscopic ovarian drilling has a well documented efficacy in relation to ovulation induction and pregnancy rate. It is established that majority of patients who are clomiphene resistant, ovulated after drilling (56-94%) and that at least half of them obtained a pregnancy (43-84%) [7, 8]. Still there is a group of patient who fail to respond to ovarian drilling (20-30% of patients) [9]. Amer et al. established that patients with BMI higher than 35 kg/m², serum testosterone concentration higher than 4.5 nmol/l, free androgen index (testosterone x100/sex hormone binding globulin) more than 15, and duration of infertility longer than three years are poor responders to ovarian drilling [10]. van Wely et al. established that early menarche, low LH/FSH ratio, and low serum glucose levels predict failure of laparoscopic ovarian drilling [11].

Laparoscopic ovarian drilling is the leading method in surgical treatment of PCOS. Introduction of effective new treatment options for PCOS (insulin sensitizers [12, 13] and aromatase inhibitors [14, 15] may further diminish the need for surgery in patients with PCOS in the future. The present case showed that there are still patients in which ovarian wedge resection is the most effective method in the treatment of PCOS with infertility.

References


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Severe ascites as the primary symptom of fulminant postpartum HELLP syndrome: a case report

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Summary
Previous studies show that HELLP syndrome occurring in postpartum period was more dangerous to both fetus and the mother than if presented before delivery. So far, there is a lack of screening or predictive tests for postpartum HELLP syndrome in clinical practice. Case: Here the authors report a rare case of postpartum HELLP syndrome with severe ascites as the primary symptom. The patient was diagnosed with severe preeclampsia and fetal growth retardation at 34+2 weeks gestation and received anti-hypertensive therapy. Severe ascites were found intraoperatively during emergency caesarean section. On the second postoperative day, complete HELLP syndrome was diagnosed. This case gradually complicated by hypoproteinemia, acute renal failure, severe anaemia, and infection and required renal haemodialysis, blood transfusion, and other supportive treatments for about one month. Conclusion: Although this case has a fulminant and long course, it has a well clinical prognosis and also shows that severe ascites may be a clue for postpartum HELLP syndrome in patient with severe preeclampsia.

Key words: HELLP syndrome; Sever ascites; Renal failure; Severe preeclampsia; Postpartum.

Introduction
The acronym of HELLP syndrome was devised by Weinstein in 1982 and was a serious, life-threatening severe preeclampsia complication [1]. H stands for hemolysis indicating microangiopathic hemolytic anemia, EL for elevated liver enzymes indicating a pathological increase of liver enzymes, and LP for low platelet count indicating thrombocytopenia. HELLP syndrome afflicts many pregnant women and results in a large percentage of maternal and perinatal complications [2]. Maternal mortality of the syndrome is 3.9% and perinatal mortality varies between 7.7% and 37% in worldwide [3]. Seleuk et al. reported that HELLP syndrome is the most frequent cause of acute renal failure in pregnancy [4].

The most common used classifications of HELLP syndrome were developed at the Universities of Tennessee and Mississippi. The Tennessee Classification [5] defined the “complete HELLP syndrome” which met all of the following criteria: (1) platelets 100,000/ml or less, (2) AST 70 IU/L or greater, and (3) abnormal peripheral smear in addition to either total serum LDH 600 IU/L or greater or bilirubin 20.4 umol/L or greater. The patient who displays some but not all of these was defined as “partial HELLP syndrome”. The syndrome also can be divided into three classes or groups primarily according to the platelets’ count [6]: class I requires severe thrombocytopenia (platelets’ count is less than 50,000/ml), class II requires moderate thrombocytopenia (platelets’ count is 50,000 to less than 100,000/ml), and class III requires mild thrombocytopenia when (platelets’ count is 100,000 to 150,000/ml).

This syndrome accounts for 0.17% to 0.85% of all live births [7]. Preeclampsia or eclampsia can increase the incidence of the syndrome [8]. The classical presentation of HELLP syndrome is right upper quadrant pain or epigastric pain accompanied with malaise, nausea or vomiting, and these symptoms accounts for 82-96% of the cases [9]. When the condition arises in the postpartum period, it will pose more serious risk to both fetus and the mother than before delivery [10]. Therefore, it is important for clinicians to make an accurate diagnosis of the syndrome to prevent any adverse outcome. Its occurrence is rare in the postpartum period, even rarer without any malaise except for severe ascites.

Woods et al. reported that severe ascites portend a patient at high risk for cardiopulmonary complications [11]. However, severe ascites as the primary symptom of HELLP syndrome is rare. Here the authors report a class I fulminant postpartum HELLP syndrome with severe ascites as a primary symptom complicated by renal failure in a patient who was under intensive care for severe preeclampsia in obstetrics department.

Case Report
The patient was a 35-year-old women, Yellow race, gravida 3, para 1. Her antenatal care was conducted in the maternity and children’s healthcare center in Xindu City. She was seen regularly by the doctors at this center. The care was uneventful until 30 weeks
plus six days gestation when the doctor found her blood pressure was 189/120 mmHg and prescribed nifedipine and magnesium sulfate for her in the center for seven days. Then, she went home and did not continue her therapy. In this period, her blood pressure was about 150/90 mmHg (measured by her family members). After 10+ days, she went to the center again and the doctor referred the patient to the present hospital with blood pressure fluctuated at a high level (178-203/113-120 mmHg) and 3+ of proteins in the urine. The patient was admitted to the present hospital at 34 weeks plus two days gestation with a chief complaint of elevated blood pressure and albuminuria. She had no past medical or surgical history. On admission, her blood pressure was 180/120 mmHg and her the reflexes were brisk. The blood cell count, creatinine, serum urea nitrogen, urates, albumin, and creatinine clearance rate was normal with only mild raised liver enzymes. Both lower extremities showed mild pitting edema and the urterine fundus height was less than that of a date. Obstetric ultrasound showed that biparietal diameter and femur length of fetus was 7.7 cm and 5.9 cm, respectively, which was below the 5th percentile for the gestational age. The ultrasound test did not find any ascites. Overall, the diagnoses were severe preeclampsia and intrauterine growth retardation. The authors administered intensive care in obstetrics department and ten mg of nifedipine three times per day, magnesium sulfate, and ten mg of dexamethasone once per day for three days. However, the blood pressure also ranged between 140/100 and 170/120 mmHg.

Continuous fetal monitoring showed that the non-stress test (NST) indicated fetal distress. Therefore, the authors give the patient an emergency lower segment caesarean section immediately after ten days from admission. The process of the operation was smooth except for about 2,000 ml clear yellowish ascites which was inconsistent with mild pitting edema in her both lower extremities. The patient gave birth to an unhealthy baby with Apgar’s scores (3-5/7/1-5-10 minutes) and a birth weight of 1,730 g and the baby required neonatal care. The baby was then transferred to Children's Centre of Chengdu. The patient had a postpartum haemorrhage of 300 ml. In this operation the authors give the patient 1,500 ml liquid (normal saline + colloid).

After operation the patient was asymptomatic and had normal blood cell count (Hb:126 g/L: PLT:100×10⁹/L), creatinine, serum urea nitrogen, urates, creatinine clearance rate, and liver enzymes were also normal with ALT: 54 IU/L, AST: 44 IU/L, GGT: 336 IU/L, ALP: 165 IU/L, TBA: 2.9 IU/L and the urine output was normal. However, the serum albumin dropped to 26.4 g/L. The blood pressure of the patient was still at a high level and the highest was 180/120 mmHg. The authors called cardiovascular specialist for a consult who suggest to give the patient furosemide and double increment of nifedipine dosage to control hypertension. At the end of the first post-operative day, the urine of the patient's disease. Usually, it manifests in the right upper quadrant or epigastric pain, nausea and/or vomiting or malaise [6]. Only about 13-30% manifests itself on postpartum up to six days after delivery [9, 12]. The present case was diagnosed with severe preeclampsia before delivery, on the first day after cesarean section, and the patient had acute renal failure. Both severe ascites and acute renal failure complicating HELLP syndrome is not infrequent, in the Rath et al. review, the incidence was 8% respectively. However, in all cases of the syndrome generalized edema account for 50%-69% [9, 13]. Some doctors argue that severe ascites are caused by hypoproteinemia which may be a reason for the ascites according to the clinical experience. However, it was not inconsistent with general edema. Currently we are still not sure of the accurate etiology of the ascites and the difference between generalized edema and severe ascites. Perhaps severe ascites are predictors of postpartum HELLP syndrome.

HELLP syndrome cases with low platelet counts have been found to be at increased risk for adverse maternal outcomes. Among all the deaths in patients with HELLP syndrome, 60% of cases were class I [2, 14]. The present case was a complete and class I HELLP syndrome, however at the beginning, it was not typical of complete HELLP syndrome which is sometimes referred to as partial HELLP syndrome.

According to the present authors’ previous knowledge, the only cure for HELLP syndrome is delivery and all the other treatments are palliative, even at early gestational ages. They also discuss the early delivery for this case in the present hospital. However, pediatricians in the hospital did not suggest
to conduct the delivery for the safety of the baby according to the poor conditions of neonatal care in the present district. Basama et al. also reported a postpartum HELLP syndrome case and they reported that this type had casted some doubt about the traditional idea and highlighted that there are no current means of prediction and early detection the syndrome [15]. The present case is consistent with their statement, but in the present case severe ascites may have been the early prediction of the syndrome. Then the present authors recognised the increased of the liver enzymes (AST, ALT), anuria, and acute renal failure. They also observed haematuria which indicated haemolysis in this case.

The pathophysiology of HELLP syndrome is still unknown; among forming hypotheses, role of imbalance between vasoconstrictive and vasodilation hormones was very important in this condition. Previous study showed that abnormal placentaion resulting in placental ischemia and then produced some circulating toxic factors which react aggressively with endothelium causing endothelial injury, especially the increased production of the vasoconstrictor thromboxane A2 [16]. These toxic factors can promote platelet aggregation and results in endothelial lesions, local vascular constriction, and thrombocytopenia. In the present case, these vasoconstrictive substances may contribute to the difficulties in controlling blood pressure. The acute renal failure and severe ascites in the present case can be explained by the fact that endothelial lesions mainly occur in organs with high blood flow and lead to severe maternal complications.

Even though a recently systemic review shows that there is no sufficient evidence to support using the corticosteroids for HELLP syndrome [17], the present authors still used aggressive corticosteroids treatment besides symptomatic treatment in this case according to some studies that concluded that aggressive corticosteroids clearly benefit the mother with HELLP syndrome according to stage of disease, especially for the cases of class I or II [14].

Conclusion

In summary, the authors reported a rare case of postpartum HELLP syndrome with severe ascites as the primary symptom. Although the case had a fulminant and long course, it had a well clinical prognosis with multidisciplinary ICU approaches. So far, there is a lack of screening or predictive tests for postpartum HELLP syndrome in clinical. This case shows that severe ascites may be a clue for postpartum HELLP syndrome in patient with severe preeclampsia.

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Successful term delivery following second-trimester excision of a massive hydrosalpinx presenting as an adnexal mass in pregnancy: management and considerations

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Summary
To the best of the authors’ knowledge, a massive hydrosalpinx presenting as an adnexal mass complicating pregnancy has never been reported. They report the case of a 26-year-old female diagnosed with a persistent right adnexal cyst in a pregnancy resulting from spontaneous conception, confirmed to be a 30-cm hydrosalpinx at the time of surgery. Though laparoscopy was envisioned, due to the size of the mass, a right salpingectomy was performed during the second trimester by laparotomy, and the patient had an uncomplicated course of her pregnancy following the intervention, delivering a healthy infant at term. Herein, the authors explore the potential etiologies and different considerations when faced with an adnexal mass in pregnancy. They emphasize that, though rare and uncommon, a hydrosalpinx should be included in the differential diagnosis of persistent adnexal cysts in pregnancy.

Key words: Adnexal mass in pregnancy; Persistent ovarian cyst; Hydrosalpinx; Laparoscopy; Surgery in pregnancy.

Introduction
A hydrosalpinx is characterized by a dilated fallopian tube filled with serous or clear fluid, leading to tubal occlusion. This finding is a direct consequence of infectious salpingitis, and is often associated with tubal disease and tubal factor infertility. A hydrosalpinx is a well-known cause of a persistent adnexal mass.

Case Report
A 26-year-old woman, gravida 1, para 0 following spontaneous conception, was referred to the present tertiary care institution at 13 weeks’ gestation because of sonographic evidence of a 25-cm right ovarian cyst. The adnexal mass was diagnosed at 11 weeks of gestation during an initial screening ultrasonography for measurement of nuchal translucency. The patient was completely asymptomatic, and physical exam was unremarkable except for an increased abdominal girth, which was inconsistent with her gestational age. On physical examination, the mass was non-tender, non-mobile, and easily palpable, reaching the abdominal upper right quadrant near the epigastrium.

Repeat transabdominal ultrasound in the present antenatal imaging center at 13 and 15 weeks revealed a right ovarian cyst measuring 24-cm in its longest axis. A viable intrauterine singleton pregnancy was confirmed at that time. The patient was counseled that the size and the persistence of the ovarian cyst through the second trimester warranted surgical exploration and intervention, to prevent gestational complications, as well as to obtain a pathologic diagnosis which may alter the management of her pregnancy.

Though given the trimester in question, the option of laparoscopic excision was envisioned, the size of the mass in question precluded this approach. Laparotomy was performed at 17 weeks' gestation. With the patient under general anesthesia, a midline vertical incision was undertaken. Pelvic washings were collected and the entire abdomen and pelvis were explored. No ascites or suspicious lesions were identified. Given the localization of the cyst, a right ovarian-sparing salpingectomy via laparotomy was performed with no complications. The right adnexal cyst was found to be a large hydrosalpinx, measuring 25-cm in diameter. The right ovary and the left adnexa were inspected and determined to be disease-free. The final pathologic report confirmed the diagnosis of right hydrosalpinx, the mass weighing 2,437 g and measuring 30 x 26 x 10 cm. The patient received perioperative prophylactic indomethacin. She developed no fever, no preterm premature rupture of membranes, and no preterm labor along the course of her pregnancy.

At 41 weeks of gestation, she was admitted to the present labor and delivery unit in spontaneous, active labor. She had an uncomplicated vaginal delivery of a 3,990-g female infant with APGAR scores of 9 - 9 - 9 at one, five, and ten minutes of life, respectively.

Discussion
Adnexal masses during pregnancy are a rare occurrence. Observational studies evaluating adnexal pathology during pregnancy estimate a 1% - 4% incidence of sonographically detectable adnexal masses, with the majority of masses resolving spontaneously with increasing gestational age [1].
Given that the overwhelming majority of adnexal masses in pregnancy are benign and a good percentage will spontaneously resolve, an appropriate option for management of adnexal pathology in pregnancy is serial observation with ultrasound performed each trimester [1-3]. However, the active management of large, persistent, or complex adnexal masses is often warranted and may even require surgical intervention in the second trimester, where the risks of both first-trimester loss and preterm labor can be avoided. This approach is indicated for two major reasons. First, dangers of obstetrical and surgical complications from the adnexal pathology such as torsion, rupture, hemorrhage, ascites, among others, may complicate the course of the pregnancy, and jeopardize both the maternal and fetal stability. Secondly, given the size and often-complex appearance of these cysts, the suspicion of malignancy is raised, in which case further evaluation would be indicated. Indeed, the incidence of malignant tumors in pregnant patients with adnexal masses is reported to be between 1% to 6% [4]. With the increasing use of routine obstetrical ultrasonography, a more conservative approach has been proposed as a potential option [5]. A study comparing diagnostic imaging to surgico-pathological analysis showed that diagnostic ultrasonography was able to correctly identify 95% of dermoid tumors, 80% of endometriomas, and 71% of simple cysts based solely on their sonographic appearance [6]. Moreover, MRI can be helpful in differentiating leiomyomas and complex cysts [7]. According to several reviews, the best predictors of persistence of the masses are ultrasound appearance (complex cyst, septations) and size [5, 8]. The Risk Malignancy Index (RMI) [9], an algorithm utilized to determine the suspicion of adnexal malignancy, takes into account the maternal menopausal status, the serum level of CA-125, as well as the sonographic appearance of the mass in question. Though limited by the screening inaccuracy of CA-125 markers during the gestational period, and more generally due to its poor specificity in the premenopausal patient, the RMI can guide further management based on ultrasound findings. Characteristic sonographic findings that warrant further investigation include multilocular cysts, the presence of solid areas, metastases, ascites, and bilateral lesions [9].

The most common ovarian neoplasms associated with pregnancy are mature cystic teratomas and benign cystadenomas. Less frequently encountered tumors are functional cysts, endometriomas, paraovarian cysts, leiomyomas, malignant neoplasms, and others. Women diagnosed with ovarian malignancy during pregnancy are typically diagnosed with early stage disease making them ideal candidates for fertility sparing surgery. The present patient had sonographic appearance of a simple cyst with two thin septations, compatible with a benign ovarian cystadenoma. Because of its 25-cm size, surgical exploration by laparotomy was indicated. A large hydrosalpinx was found and a right salpingectomy was performed. This is, to the best of the authors’ knowledge, the first reported massive hydrosalpinx presenting as a persistent adnexal mass in pregnancy.

Hydrosalpinx is known to reduce fertility and impair IVF outcome. Though the reason is unclear, salpingectomy is effective in improving birth rates after IVF. It is theorized that embryotoxic properties of the hydrosalpinx fluid may be detrimental to the growth of the embryo and fetus, so that in its absence, normal embryonic development and placentation can occur [10]. This is mainly true when hydrosalpinx is bilateral and visible by ultrasonography.

It is essential to keep in mind that a hydrosalpinx may be unilateral, with sonographic appearance of a simple ovarian cysts or cystadenomas. Despite its association with infertility, a hydrosalpinx may be co-existing with an intrauterine viable pregnancy. Therefore, it should be considered in the differential diagnosis of persistent adnexal cysts complicating pregnancy. The present authors emphasize that because complications of abdominal surgery are increased in pregnancy, surgical management of adnexal masses in pregnancy, including hydrosalpinx, may need to be evaluated on a case-by-case basis.

References

Giant cervical myoma associated with urinary incontinence and hydroureteronephrosis

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Summary
Cervical leiomyomas compromise fewer than 5% of all uterine leiomyomas. Cervical myomas exacerbates surgical difficulties, such as poor operative field, difficult suture repairs, and blood loss. When performing myomectomy for cervical myomas, care must be taken to avoid injuries to neighboring structures in the pelvic cavity. These structures include the bladder in front of the cervix, the rectum behind the cervix, and the uterine arteries and ureters on both sides. Myomectomy for cervical myoma is empirically difficult and frequently problematic. The authors report a case of giant cervical myoma presenting with urinary incontinence.

Key words: Cervical myoma; Hydroureteronephrosis; Urinary incontinence.

Introduction
Uterine myoma is a common gynecological disorder occurring in 20-50% of women, of late reproductive age. A majority of myomas are associated with uterine corpus. Cervical myomas account for less than 5% of uterine myomas [1]. The uterine cervix is adjacent to the uterine arteries, ureters, rectum, and bladder. Therefore, cervical myomectomy has the surgical limitations of poor operative field, and vulnerability of the neighboring organs to injury [2]. The authors report a case of giant cervical myoma presenting with urinary incontinence.

Case Report
A 51-year-old woman was admitted to the present clinic with ongoing urinary incontinence for two months. A solid mass was revealed extending to the umbilical level in bimanual examination. Cervix could not be recognized due to the compression and distortion of the mass. Ultrasonography showed vesical globe and bilateral hydroureteronephrosis and 1,500 cc of urine was drained via a Foley catheter. MRI showed a cervical mass measuring 19x11x10 cm that compressed the posterior portion of the bladder (Figure 1). Uterus and cervical myoma was removed by laparotomy through a midline incision (Figure 2). Myomectomy cavity was sutured without leaving empty space after bleeding control. Bilateral hydroureteronephrosis and incontinence regressed in the post-operative period. Patient was discharged on the fifth postoperative day without any complications.

Discussion
Cervical leiomyomas compromise fewer than 5% of all uterine leiomyomas. They may be categorized as those that occur at a subserosal location (i.e., extracervical type) and those that occur within the cervix (i.e., intracervical type) [3]. Most patients with uterine myomas are symptom-free. When symptoms occur, they usually correlate with the location of the myomas, their size, or concomitant degenerative changes. As myomas grow, pressure is exerted on adjacent viscera with manifestations from the urinary tract, such as frequency, outflow obstruction, and compression of the ureters. Gastrointestinal symptoms such as constipation or tenesmus may be the result of a posterior wall myoma that is exerting pressure on the recto-sigmoid [4]. Cervical myomas exacerbates surgical difficulties, such as poor operative field, difficult suture repairs, and blood loss. When performing myomectomy for cervical myomas, care must be taken to avoid injuries to neighboring structures in the pelvic cavity. These structures include the bladder in front of the cervix, the rectum behind the cervix, and the uterine arteries and ureters on both sides. Myomectomy for cervical myoma is empirically difficult and frequently problematic [5]. The most difficult part of cervical myomectomy is suturing the base of the wound following enucleation. Complete dissection of the surrounding organs such as the bladder and ureter near the base of the wound, is difficult. Bleeding makes the visual field difficult to maintain; thereby increasing the possibility of damage during suturing [6].

Surgical excision of cervical myomas can be performed through laparotomy or laparoscopy. The present authors preferred laparotomy due to the extreme size of the myoma. Paying attention to the relationship between fibroids and adjacent organs, to avoid damage to those organs through careful dissection and suturing from the
base of the myomectomy cavity, without leaving empty space after bleeding control, are important factors in reducing morbidity.

References


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Treatment of pregnant patient with disseminated intravascular coagulation (DIC) due to placental abruption – a case report

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Summary
A primigravid woman at 29th gestational week with placental abruption causing fetal death, that underwent instant cesarean section, developed a disseminated intravascular coagulation (DIC), revealed by hemoperitoneum and hematoma of the abdominal wall. After re-laparotomy and transfusion of blood, fresh plasma, and platelets, the patient was discharged from hospital on the 14th postoperative day completely recovered. To conclude, conservative surgical approach for DIC treatment is possible and safe. Novel antifibrinolitic drugs are recommended for obstetrical patients with DIC to enable a healthy subsequent pregnancy.

Key words: Placental abruption; DIC; Congenital thrombophilia; Antifibrinolitic drugs; Tranexamic acid; Desmopressin.

Introduction
Placental abruption is one of the most common causes of antepartum hemorrhage as well as the coagulation failure like disseminated intravascular coagulopathy (DIC). It is as the leading cause of maternal morbidity and mortality, despite modern improvement in obstetric practice [1].

Case Report
A primigravid woman aged 36 years was administered to Clinic for Gynecology and Obstetrics Clinical Center of Serbia at 29th gestational week due to uterine hyper-tonus and moderate to profuse hemorrhage that had begun two hours prior, suddenly, in the middle of the night. She complained of intermittent uterine contractions and headache lasting 24 hours.

After clinical and ultrasound examinations, fetal death and placental abruption were diagnosed. Blood pressure on admission was 120/70 mmHg and pulse was 96 bpm. Laboratory analysis showed altered glucose, creatinine, bilirubin, AST, LDH, creatinine kinase, uric acid, serum protein, and sodium levels. Other biochemical parameters were within the referral range.

Due to maternal vital indications, urgent cesarean section was performed. A dead (Apgar score 0) male fetus weighing 1,200 grams was delivered. A partial placental abruption was confirmed. During operation patient received ten IU oxytocin bolus, two blood pools (545ml), two doses of fresh frozen plasma (FFP), and ten doses of cryoprecipitate. Manual revision of uterine cavity was performed and uterus was sutured in two layers. Hemostasis was achieved and uterus was appropriately contracted. Before closing the abdomen sub-fascial drainage was inserted. After clinical examination and palpation, uterus was adequately contracted. Ultrasound scan revealed anticipated size, clear contours, and empty cavity of the uterus. In the right adnexal region a hypo-echogenic area approximately 35mm in diameter was seen. This finding could correspond to free fluid (possibly blood) in the abdomen. Another a hypo-echogenic area approximately 20 mm in diameter was registered in the anterior abdominal wall between fascia and muscles. According to its ultrasound characteristics, it resembled a hematoma.

Laboratory analyses after caesarean section were altered and anemia was confirmed. Blood pressure was 116/68 mmHg and pulse 115 bpm. Therefore, despite therapy, the symptomatology worsened one hour later. Consequently, due to patient’s vital indications, a re-laparotomy performed was straightaway. The operative findings confirmed diffuse intra- and retro-peritoneal hemorrhage with hematoma. Uterus was showing signs of Couvelair’s syndrome, but the stitches on the uterine incision were intact with adequate hemostasis. A conservative approach was decided due to age and parity of the patient. Described hematomas were evacuated and revision of hemostasis was done. There was no visible active bleeding, either in the abdomen, or in the anterior abdominal wall. Nonetheless, because of the existing right infundibulopelvic ligament hematoma, a ligature was performed on the right ovarian artery. During surgery the patient received seven blood pools (1,700 ml), three doses of SSP (955 ml) and 25 doses of cryoprecipitate, 20 doses of platelets, and cell saver 1,775 ml.

Patient spent the following seven days in the intensive care unit. For the first three days she was on mechanical respiration due to impaired auscultator sound in the basal lung regions. Chest radiography showed pleuroperticardial effusions. The patient was medically treated with oxygen, aminophylline, urbasone, lasix, enalapril, presolol, nifelat, bensedine, uterotonic treatment, and desmopressin.

Postoperatively, patient received uterotonic treatment (oxytocin and prostaglandin M15) and antibiotics daily. She also received treatment with anti-fibrinolitic tranexamic acid (one mg/kg/hour infusion) and emosint (desmopressin, DDAVP). She was given additional three blood pools (720 ml), two doses of SSP (455 ml)


and five doses of crio-precipitate. On the tenth postoperative day clinical findings were significantly improved. Biochemical analyses were all in referral range. Coagulation factors stabilized: PT 12.9s, INR 1.10, aPTT 29.1s, D-dimer 13.1, AT III 108.8, and fibrinogen 3.3.

Patient was discharged from hospital on the 14th postoperative day, completely recovered with appropriately contracted uterus and laboratory data in referral range. On the control examination 21 days after surgery, all clinical and laboratory findings were normal, therefore the stitches were removed.

Six months later, as a part of detailed diagnostics made in order to discover the true etiology of described complications, a hematological examination was performed. Patient was diagnosed with congenital heterozygote thrombophilia type FIH 20210A. She also had positive anti beta 2 GP I antibodies in low titer. Patient was advised that in case of pregnancy, antithrombotic therapy must be immediately begun. One year later patient became pregnant again. Throughout the whole pregnancy she was gynecologically and hematologically regularly controlled and received anticoagulant therapy. On the July 19th, 2013, the patient underwent a planned cesarean section and gave birth to a healthy child weighing 3050 grams. Postoperative course was uneventful for both mother and child.

Discussion

The most important causes of antepartum hemorrhage (vaginal bleeding from the 20th week of gestation until delivery) are placenta praevia and placental abruption, which together account for more than 50% of cases [1, 2]. Placental abruption is defined as premature separation of a normally sited placenta and occurs in one in 100 pregnancies. Risk factors include pre-eclampsia, blunt abdominal trauma, smoking, cocaine use, multiple pregnancies, increasing maternal age and parity, polyhydramnios, and previous history of abruption [2, 3].

Placental abruption can be easily diagnosed if it occurs in the lower part of the placenta and blood escapes through vagina. However, bleeding can occur at times between the placenta and the uterine wall and in that manner can remain concealed for significant time. The only symptoms apparent in these cases are sudden onset of abdominal pain, persistent abdominal pain, and marked uterine hyper-tonus. Maternal tachycardia with decreased blood pressure and oliguria from end-organ insult and hypovolemia/hypotension, PT < 60%, aPTT of more than 30 seconds, fibrinogen < 200 mg/ml, platelet count < 100,000/ml3, and antithrombin III activity of < 80% are all indicative of DIC [8, 9].

In the current treatment of severe obstetrical hemorrhage, first-line therapy includes transfusion of packed cells and fresh frozen plasma in addition to uterotonic medical management and surgical interventions [6, 11]. The present patient received significant amounts of transfused blood and blood derivates.

The most important treatment is elimination of underlying triggering mechanism. Even in the case of a pronounced clotting defect, significant bleeding occurs only when the anatomical integrity of the vascular system is disrupted [10]. Consequently, adequate hemostasis is essential for DIC treatment. Bilateral hypogastric artery ligation is proven to be effective if there is no active bleeding or infection [12]. The authors also proved that conservative treatment is safe and hysterectomy can be avoided.

Treatment of DIC consists of replacement of volume, blood products, coagulation components, cardiovascular, and respiratory support. The correction of clotting factors deficiencies (evident by prolonged PT/PTT) is done with FFP. Early fibrinogen replacement should be made in women who have low fibrinogen where worse outcomes can be expected. On the other hand, if the initial fibrinogen level is < 50 mg/ml, infusion of cryoprecipitate is strongly indicated. Hematocrit levels should be maintained with transfusion of packed erythrocytes, while platelet transfu-
agulation components, cardiovascular, and respiratory symp-

tomatology. Treatment of pregnant patient with disseminated intravascular coagulation (DIC) due to placental abruption – a case report

References


[16] Tranexamic acid is a synthetic derivative of the amino acid lysine that exerts its antifibrinolytic effect [16]. It is a competitive inhibitor of plasminogen and plasmin which reversibly blocks lysine binding sites on plasminogen molecules. In that manner, it inhibits the interaction of plasminogen and plasmin with lysine on the surface of fibrin. Although plasmin can still be formed under these circumstances, it is unable to bind to and degrade fibrin [17, 18].

It can be administered both orally and intravenously and it is eliminated through urine. Tranexamic acid is well toler-

ated [19]. Increased risk of thrombosis with the drug has not been demonstrated in clinical trials. It is still con-

traindicated in patients with a history of thromboembolic disease, and dosage reductions are recommended in pa-

tients with renal insufficiency. The drug crosses the blood-

brain barrier and the placenta, but excretion into breast milk is minimal. Therefore it is a safe treatment postpartum [17, 18].

Tranexamic acid significantly reduced mean blood losses with statistically significant reductions in transfusion re-

requirements [19]. Efficacy of tranexamic acid in the control of bleeding has also been reported in numerous studies re-

garding cesarean section, placental abruption or postpar-

 tum hemorrhage [16]. It the presented case, it was proven that tranexamic acid can be a beneficial treatment for ob-

stetrical patients with DIC.

Literature data show that there is a strong connection be-

 tween hematological disorders, especially congenital thrombophilia and dismal pregnancy outcomes as miscar-

riage, placental abruption, or peri-neonatal ischemic stroke [20].

Placental abruption should be considered if hard and painful uterus is found and especially if the patient is bleed-

ing. Ultrasound scan should always be performed in order to determine both retro-placental hematomas and the fetal condition. If bleeding is heavy, delivery should be carried out as soon as possible. Conservative approach is possible and safe. Disseminated intravascular coagulopathy is a common complication of placental abruption. Treatment of DIC consists of replacement of volume, blood products, co-

agulation components, cardiovascular, and respiratory sup-

port. Novel drugs, as tranexamic acid and desmopressin, are good treatment for obstetrical patients with DIC and therefore the present authors recommend their use. Opti-

mal treatment of pathological conditions that lead to DIC can enable a healthy subsequent pregnancy.
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Selective termination in discordant twin pregnancy with early onset preeclampsia: case report

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Summary
Objective: To study the effectiveness of selective termination for discordant twins in treating early onset preeclampsia. Materials and Methods: After literature review, ethical review, and discussion with the couple, one patient with early onset preeclampsia complicated with a lethal condition in one twin, was performed selective termination by intracardiac injection of potassium chloride at 27 weeks' and four days' gestation in an effort to reverse preeclampsia and prolong the pregnancy. Results: The clinical manifestation of preeclampsia was alleviated in this patient. At 29 weeks, the stillborn fetus was delivered because of spontaneous preterm labor. A live birth was achieved five days later. All procedures allowed continuation of the pregnancy for an additional two weeks and one day of the remaining fetus. Conclusion: Selective termination may be an option for treating early onset preeclampsia in discordant twins, instead of termination of whole pregnancy.

Key words: Dichorionic pregnancy; Early onset preeclampsia; Discordant twins; Selective termination; Delayed-interval delivery.

Introduction
Preeclampsia is associated with fetal growth restriction. The management of severe pre-eclampsia focuses on prevention of seizures and control of blood pressure, but the ultimate method remains to terminate the pregnancy. Twin pregnancy is one of the high risk factors for preeclampsia. Some twin gestations with preeclampsia are in the extremely immature period and complicated restricted fetal growth of only one fetus. In these cases, pregnancy termination is accepted to resolve the maternal illness and results in poor neonatal outcome for both twins. The authors explored an alternative treatment in the management of these pregnancies that might improve survival of the normal developing twin.

Case Report
A 24-year-old woman conceived dichorionic twins naturally, gravida 2, para 0. Gestational age was determined by ultrasound. Normal nuchal translucency and growth in twins were shown by ultrasound at 12 weeks' gestation. Serum screening at 16 weeks' gestation was normal. Ultrasound showed twin A appropriate for gestational age (AGA) and twin B lagging by three weeks with oligohydramnios at 20 weeks' gestation and no anatomic abnormalities on either twin. Gestational diabetes was revealed by oral glucose tolerance test (OGTT) at 24 weeks.

At 26 weeks' gestation, the woman developed pre-eclampsia, and was hospitalized with a blood pressure of 180/100 mmHg, proteinuria (5.48 g/24 hours) and elevated serum transaminases (aspartate aminotransferase [AST] 54 U/L, alanine aminotransferase [ALT] 72 U/L). Ultrasound showed twin A AGA, twin B anhydramnios and absent end diastolic velocity in the umbilical artery with growth less than 3 percentile. Magnesium sulfate and calcium channel blocker (nifedipine gastrointestinal therapeutic system (GITS) 60 mg oral per day) were used to control pre-eclampsia. Dexamethasone (12 mg/d in divided doses) was given for fetal lung maturatation. The poor prognosis of the restricted twin was discussed with the parents and they decided the expectant management.

During expectant management, the woman complicated with developing preeclampsia as serum transaminases elevated (AST 122 U/L, ALT 180 U/L), proteinuria increased (from 5.48 g to 6.99 g/24 hours). Ultrasound evaluation revealed no growth of twin B, still absent end diastolic velocity in the umbilical artery and severe oligohydramnios, while evaluation of twin A was reassuring.

The couple was counseled of the poor prognosis for the restricted twin and selective termination of twin B as an option to prolong the pregnancy on behalf of twin A. After detailed informed consent, the procedures was performed by the transabdominal intracardiac injection of potassium chloride into twin B at 27 weeks' and 4 days' gestation.

After selective termination, the symptoms of pre-eclampsia alleviated. Oral nifedipine GITS was decreased to 30 mg per day and diastolic blood pressure was stable, the proteinuria decreased to 3.56 g/24 hours at 29 weeks. She had spontaneous preterm labor occurring at 29 weeks and a stillborn girl weighing 550 g was delivered. Thirty minutes after delivery of this twin, the umbilical cord was ligated as high as possible in the cervix under aseptic conditions. Ritodrine was performed as prophylactic tocolysis. Ceftezole (one g iv three times per day for five days) was administered. Vital signs, complete blood counts, C-reactive protein, and coagulation studies were normal afterwards. Fetus was monitored by non-stress test (NST), ultrasound, and umbilical artery Doppler. No clinical signs of chorioamnionitis were noted.

At 29 weeks and five days, a 1,300 g female neonate was delivered vaginally with Apgar scores of 9 at one minute and 9 at five minutes because of spontaneous labor. The neonate received full resuscitation and immediate life-support intervention and was transferred to NICU. Macroscopic and histologic examination of...
the placenta confirmed a dichorionic pregnancy, with velamentous insertion of umbilical cord in twin B. The postpartum recovery of the mother was uneventful and was discharged four days later after delivery. The neonate was given pulmonary surfactant because of respiratory distress syndrome. Two days after birth, the neonate presented progressive dyspnea and was diagnosed with patent ductus arteriosus (PDA) by echocardiography. Then ibuprofen was given to close the PDA. The neonate died five days after birth because of massive pulmonary hemorrhage.

Written informed consent was obtained from the patient for publication of this case report.

Discussion

Preeclampsia remains a major cause of maternal mortality worldwide with adverse perinatal outcomes. Twin gestations occur in 3.26% of pregnancies and the risk of preeclampsia in twin pregnancies has been shown to be more than twice that in singleton gestations [1, 2]. Treatment in preeclampsia is aimed to prolong pregnancy and prevent severe maternal complications, until now still focuses on blood pressure regulation, seizure prophylaxis, and monitoring fetal condition. However the ultimate method remains delivery of the fetus and placenta. Because of the high maternal morbidity and the extremely low perinatal survival rates, expectant management in severe preeclampsia is not recommended in cases of less than 24 weeks and/or in those with severe fetal growth restriction at any gestational age < 26 weeks [3].

In this case, the patient complicated with developing preeclampsia and with worsening condition of the restricted twin during the expectant management. Termination of the pregnancy would resolve the maternal condition and affect the co-twin’s survival. To the authors’ knowledge, multifetal pregnancy reduction (MPR) was associated with higher birth-weight and lower rates of preterm deliveries [4]. They hypothesized that preeclampsia might be reversed by selective termination of restricted twin in this case. They investigated the database of Cochrane Library, PubMed, EMBASE, et al. and found several reports published about resolving preeclampsia by selective fetocide of the abnormal twin. Audibert et al. reported the first case of resolution of preeclampsia after selective termination of the worsened twin at 32 weeks’ gestation and the pregnancy was uneventful until delivered at 38 weeks [5]. Heyborne et al. reported three patients with second trimester preeclampsia treated with selective fetocide of the twin in lethal condition. Preeclampsia resolved in all three patients, allowing an additional 9 to 23 weeks before delivery of the remaining fetus [6].

In the present case, after the authors performed the selective termination of the restricted twin, severe preeclampsia was alleviated and allowed the pregnancy to continue for an additional two weeks and one day before delivery of the AGA fetus. Although there are few cases in the literature about the selective termination to the restricted twin, the present case further confirmed that selective termination may be an option for treating early onset preeclampsia in discordant twins, instead of termination of whole pregnancy.

In the present case a delayed interval of five days was achieved between delivery of the dead fetus and retained fetus after selective termination. Delayed delivery in multiple gestation is of very rare occurrence. While it is decided, the cord of the first born twin should be ligated under aseptic conditions with an absorbable suture, as close to the cervix as possible. Prophylactic tocolysis may be used after first twin’s birth, or later during uterus contractions with no evidence of chorioamnionitis [7]. Antibiotics should be provided immediately after the birth of the first child. There is no clear indications for the use of prophylactic cervical cerclage [8]. Therefore, the authors did not perform cerclage after the stillborn fetus was delivered.

Although the neonate in the present case died by massive pulmonary hemorrhage complicated with PDA, based on the literature and the authors’ experience, selective termination may be an option for treating preeclampsia in discordant dichorionic twins in an effort to prolong the pregnancy, instead of termination of whole pregnancy. Further studies are required for a better understanding of the pathophysiological mechanisms of this procedure.

References


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Right angular pregnancy at seven weeks’ gestation: a case report treated by laparoscopic approach

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Summary
Angular pregnancy (AP) or implantation of the embryo in the lateral angle of the uterine cavity close to the internal ostium of the fallopian tube is a very rare event. In fact, angular pregnancy refers to implantation of the embryo just medial to the uterotubal junction, in the lateral angle of the uterine cavity. AP must be distinguished, anatomically, from interstitial pregnancy by its position in relation to the round ligament, which crosses the Müllerian duct at the side of the uterotubal junction. AP is associated with a high rate of complications such as bleeding and ruptured uterus due to delayed diagnosis. The authors present a clinical report of AP at seven weeks’ gestation without uterine rupture. They performed directly operative laparoscopy because of acute intra-abdominal hemorrhage. Laparoscopy was useful in the treatment of early angular pregnancy and could avoid the need for invasive surgery or hysterectomy.

Key words: Angular pregnancy; Laparoscopy.

Introduction
The majority of ectopic pregnancies are tubal (95%), most commonly in the ampulla of the fallopian tube [1, 2]. Two to five percent of ectopic pregnancies are interstitial; less than one percent are angular [2].

The term interstitial pregnancy is used if the implantation occurs in the interstitial part of the fallopian tube that is embodied within the muscular wall of the uterus. Angular (AP) pregnancy, on the contrary, occurs by embryo implantation in the medial to the uterotubal junction, in the lateral angle of uterine cavity. Anatomically, it is distinguished from interstitial pregnancy by its position in relation to the round ligament, which crosses the Müllerian duct at the side of the uterotubal junction [3]. AP must be differentiating from others forms of ectopic pregnancy such as interstitial, and intramural pregnancy in order to make a correct clinical and histopathological diagnosis, to identify the signs when the uterine rupture appears imminent, and to take the best therapeutic decision according to gestational age at presentation. Undetected, AP may develop until the second trimester with the risk of catastrophic hemorrhage and greater maternal mortality risk [2, 3].

The authors present a case of AP at seven weeks gestation without uterine rupture, treated directly by laparoscopic approach. The rarity and clinical management of this condition are discussed, as well as the importance of ultrasound investigation for early diagnosis.

Case Report
A 31-year-old Nigerian woman (para 1), with a history of two prior voluntary terminations of pregnancy, one spontaneous vaginal delivery, and a prior ectopic pregnancy of the right fallopian tube followed by right unilateral salpingectomy, came to the present authors’ attention for routine ultrasound control. She denied fever, nausea, vomiting, diarrhea, vaginal bleeding, abdominal pain or voluntary guarding.

She had a screening ultrasound made by transvaginal ultrasoundography (TVUS), performed at seven weeks’ gestation, which revealed a gestational sac, reported as extrauterine and containing a live embryo of 5-mm CRL with heart beats, corpus luteum of the left ovary, and intraperitoneal free fluid. The pregnancy was located in the right angular region and surrounded by thin (five-mm) asymmetric myometrium (Figure 1). The value of beta-hCG was 10,057 mIU/ml at recovery.

The case was treated directly by laparoscopy because of acute intra-abdominal hemorrhage. At laparoscopy, the AP with an in-
tact amniotic sac and embryo was on the right side and the uterine wall was about to break (Figure 2). The endometrial cavity, round ligaments, extrauterine parts of the left fallopian tube, and ovaries were normal, and the right fallopian tube was absent.

The pregnancy could not be saved, and thus the authors removed the pregnancy and coagulated by bipolar forceps the myometrial tissue bleeding without suture (Figure 3). A uterine curettage was performed afterwards. The patient was discharged on the third postoperative day.

**Discussion**

The majority of ectopic pregnancies are tubal (95%), most commonly in the ampulla of the fallopian tube [1, 2]. Two to four percent of ectopic pregnancies are interstitial and less than one percent are angular [2].

Risk factors associated with the higher incidence of angular ectopic pregnancy include congenital uterine anomalies, previous ectopic pregnancy, in vitro fertilization and ovulation induction, pelvic inflammatory disease, previous intrauterine procedure, and use of intrauterine contraceptive devices [4, 5].

APs are usually represented by rupture, which is associated with severe abdominal pain, the syndrome of internal haemorrhage, and often a state of shock, mostly occurring usually between the 6th and the 12th week of pregnancy [6].

In the present case, the pregnancy was implanted in the right angle part of the uterine wall, with a prior ectopic pregnancy of the right fallopian tube followed by right unilateral salpingectomy.

The patient had an unremarkable postoperative course and was discharged after 72 hours when the serum hCG level dropped to 10 IU/L and the hemoglobin value was 11.5 g/dL. Over the next four weeks, the serum hCG level appropriately declined to a negative value and TVUS revealed a normal uterine wall. The histology of surgical specimen confirmed the presence of pregnancy.

Treatment of patients with ectopic pregnancy is not standardized regarding level of β-hCG, gestational age, and size of gestational mass. There are several treatment strategies for ectopic pregnancies: expectant management, systemic or local medical treatment with methotrexate (MTX), surgical treatments including laparotomy, laparoscopy, hysteroscopy or dilatation and curettage. In many cases MTX alone or a combination therapy with MTX before surgical intervention has been recommended. The surgical approach will depend on the surgeon’s experience in laparoscopy and the gestational week. In early gestational weeks, angular, interstitial or abdominal pregnancies can be treated by laparoscopy.

Management of AP is faced by many unresolved questions such as whether the intervention is always necessary, or how long is too long for a wait-and-see approach. Data from randomized trials regarding the future fertility and obstetric outcome are also lacking. There have been no prospective studies comparing outcomes of medical and surgical treatment options for APs. In recent years, the tendency towards conservative medical treatment has been increasing.

According to the present authors’ experience, laparoscopy should be the first-line treatment of early APs in case of acute intra-abdominal bleeding.

**References**


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Delayed diagnosis of ureteral injuries following gynecological laparoscopic surgery: three case reports and review

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Summary

Purpose of investigation: The clinical manifestations, causes, and methods for the treatment and prevention of ureteral injuries presenting late after gynecological laparoscopic surgeries were investigated. Materials and Methods: The medical records of patients who experienced delayed recognition of ureteral injuries after gynecological laparoscopic surgeries in the present hospital between 2008 and 2012 were reviewed retrospectively. Results: There are three cases of ureteral injuries which are diagnose-delayed. The incidence of the malignant tumor groups was 0.99%, and for malignant patients undergoing laparoscopic lymphadenectomy it was 1.3%. Conclusion: The main way to prevent delayed ureteral injuries is to avoid adhesion between the ureter and surrounding tissues as well as eliminate electrothermal trauma during laparoscopic operations. Surgical repair is effective for such patients.

Key words: Laparoscopic surgeries; Ureter; Iatrogenic injuries.

Introduction

Ureteral injury is a severe complication that can occur during or after gynecological surgery. Since the female ureters are closely associated with the female genital organs, the ureters are likely to be injured during pelvic surgeries, especially during complex procedures [1]. Ureteral injury may cause urinary fistula, infection, renal failure, and even death [2]. In recent years, the incidence of perioperative iatrogenic ureteral injuries has increased as more gynecological laparoscopic procedures are being performed [3]. The incidence of iatrogenic ureteral injury is reported to be 0.14%, and the average time between the procedure and recognition of injuries is 3.1 days [3]. The ureteral injury cases reported in the literature mainly occurred during the perioperative period [4]. In some cases, four months elapsed between surgery and the presentation of ureteral stricture injury [5]. Here the authors present a retrospective study of ureteral injuries with delayed diagnosis.

Materials and Methods

The medical records of 3,922 patients were reviewed, who underwent laparoscopic surgery in the Department of Gynecology and Obstetrics, Beijing Chaoyang Hospital between January 1st, 2008 and January 1st, 2013. Among them, 302 patients received laparoscopic surgery for the treatment of malignant tumors, and 153 underwent laparoscopic lymphadenectomy. Six cases of postoperative ureteral injuries occurred in patients after gynecological laparoscopic surgery in the present hospital and the incidence of ureteral injury was 0.14%. Three patients with ureteral injuries were delayed diagnosed. All of these cases were patients with gynecological malignant tumors; two patients underwent lymphadenectomy. The incidence of delayed ureteral injury in patients who underwent laparoscopic surgery for the treatment of gynecological malignant tumors was 0.99%; in particular, the incidence in patients who underwent lymphadenectomy was 1.3%.

Demographic data and surgical information

The three patients who presented with ureteral injuries had an average age of 47.7 years (41, 42, and 60 years old, respectively); they were diagnosed with Stage Ia1 squamous cell carcinoma (SCC) of the uterine cervix, Stage Ib1 SCC of the uterine cervix, and Stage IA endometrial carcinoma, respectively (Table 1). None of the patients had a history of pelvic surgery or endometriosis. Furthermore, none of them presented with severe pelvic adhesion intraoperatively. The surgical protocols for these three patients were laparoscopic-assisted extrafascial hysterectomy, laparoscopic radical hysterectomy with pelvic lymphadenec-
tomy, and laparoscopic extraperitoneal hysterectomy with pelvic lymphadenectomy, respectively. All surgical procedures were performed successfully without the occurrence of severe intraoperative pelvic adhesion. The surgeries were all performed by veteran chief gynecologists who were qualified and had experience performing laparoscopic surgeries. Abdominal drainage was placed postoperatively in the two patients who underwent laparoscopic lymphadenectomy.

Delayed diagnosis of ureteral injuries

The three patients were in good condition during the perioperative period; they had normal temperature, a well-balanced daily water intake and excretion, gradually reduced drainage, no urinary fistula, and urination restored after urinary catheter removal. The average course of postoperative in-hospital observation was 13.3 days. The three patients presented with urinary fistulae on days 10, 16, and 63 after undergoing surgery, respectively; the average time from surgery to onset of injury was 29.7 days. All three patients had large amount of vaginal discharge. Vaginal speculum examination revealed visible discharge of pale yellow fluids from the vagina but no obvious fistula in the vaginal orifice. The laboratory assay results showed that the urea nitrogen content in the intra-vaginal fluid was similar to that in urine, suggesting the occurrence of a urinary fistula. The negative results on bladder methylene blue tests obtained from all three patients on admission led to the elimination of the occurrence of vesicovaginal fistula and suggested ureterovaginal fistula. Ultrasonographic scans performed after the patients’ admission revealed right ureteral dilation and hydronephrosis. Intravenous pyelography (IVP) findings suggested the presence of lower ureteral stricture in the right ureter (Figure 1). Cystoscopy showed no urine spouting from the right ureteral meatus in the contracted bladder. Retrograde catheterization of the right ureter was performed under the guidance of cystoscopy; however, the catheter was unable to be inserted into the right ureter in all three patients. In one patient, the inserted ureteral catheter returned to the vagina via the ureterovaginal fistula during catheterization. In the three patients, urinary fistula occurred for at least ten days after surgery.

Table 1. — Clinical data of the patients with delayed ureteral injuries.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Primary disease</th>
<th>Laparoscopic surgery</th>
<th>Duration of postoperative in-hospital observation</th>
<th>Time to the occurrence of urinary fistula</th>
<th>Diagnostic methods</th>
<th>Cause of injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>Stage Ia1 SCC of cervix</td>
<td>Laparoscopic-assisted extraperitoneal hysterectomy</td>
<td>4 days</td>
<td>16 days postoperatively</td>
<td>Intravenous pyelography + cystoscopic retrograde catheterization</td>
<td>Adhesion to surrounding tissues</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>Stage Ib1 SCC of cervix</td>
<td>Laparoscopic radical hysterectomy with pelvic lymphadenectomy</td>
<td>19 days</td>
<td>63 days postoperatively</td>
<td>Intravenous pyelography + cystoscopic retrograde catheterization</td>
<td>Adhesion in obturator foramen</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>Stage Ia endometrial carcinoma</td>
<td>Laparoscopic extraperitoneal hysterectomy with pelvic lymphadenectomy</td>
<td>17 days</td>
<td>10 days postoperatively</td>
<td>Intravenous pyelography + cystoscopic retrograde catheterization</td>
<td>Adhesion to iliac vessel</td>
</tr>
</tbody>
</table>

Figure 1. — An intravenous pyelogram showing injury to the right ureter.
tended to the obturator foramen. The ureteral tension in the adhered site was increased, which generated a traction force that caused trauma, resulting in the fistula. All three patients underwent ureteroneocystostomy, which aimed to re-implant the upper end of a transected ureter into the bladder. Double-J stents (D-J) were placed into the re-implanted ureters. After ureteroneocystostomy, none of the patients presented urinary fistula. The D-J catheters were removed three months later. The patients’ renal function returned to normal levels after surgery. Furthermore, the ureters exhibited normal morphology on ultrasonographic scans, indicating excellent prognosis.

Discussion

Incidence and time of onset of ureteral injuries following laparoscopic surgery

The reported incidence of ureteral injuries after gynecological laparoscopic surgery in the literature is < 1–2%; the leading cause of such injuries is reported to be laparoscopy-assisted vaginal hysterectomy (LAVH) [5]. A previous study [3] reports the incidence of ureteral injuries among patients who underwent gynecological laparoscopic surgery at the Peking Union Medical College Hospital to be 0.14% and an average time from operation to onset of injury of 3.1 days. A case report suggests the onset of ureteral stricture injury may occur as late as four months after gynecological laparoscopic surgery [6]. In the past five years, six cases of postoperative ureteral injuries occurred in patients after gynecological laparoscopic surgery in the present hospital. Two of these patients had ureteral injuries recognized intraoperatively, one was recognized within 24 hours, and three were recognized at least ten days after surgery. Therefore, the incidence of ureteral injury was 0.14%. In the present study, all the delayed injuries occurred in patients who underwent laparoscopic surgeries for the treatment of gynecological malignant tumors, especially in patients who underwent lymphadenectomy.

Causes of delayed ureteral injuries after laparoscopic surgery

The locations with the highest risks for laparoscopic surgery-associated ureteral injury include the pars pelvina of the ureter, areas near the uterine artery, the sacral ligament, and the ureterovesical junction [7]. The intraoperative injuries during laparoscopy-assisted surgery were mainly associated with the abnormal or disease-induced anatomic disruption of the ureteral course in the pelvis, including pelvic adhesion caused by endometriosis. The variations in the anatomic position of the ureter may affect surgical operations and result in ureteral injury. Intraoperative ureteral injuries normally occur earlier, and patients are likely to present certain situations that may cause operational difficulties, such as intraoperative adhesion and enlarged uterus. In some cases, dilatation or rupture of the ureter was discovered intraoperatively. Moreover, some patients may present with some perioperative manifestations, such as increased drainage, reduced urine, and lower back pain. In contrast, delayed postoperative ureteral injuries are more prevalent among patients who have undergone gynecological laparoscopic surgery for the treatment of malignant tumors, especially among those who have undergone lymphadenectomy. Patients with delayed injury usually have successful laparoscopic surgeries producing satisfactory outcome. No obvious adhesion is found intraoperatively, drainage and urination after surgery are normal, and the patients are likely to pass their perioperative period smoothly. The most common manifestation of delayed ureteral injury is urinary fistula taking place several days after surgery. Severe adhesion between the ureter and its surrounding tissues can be revealed by exploratory surgery. The adhesion imposes traction on the ureter, on which the fistula forms because of the increased tension.

There may be several causes of delayed ureteral injuries: First, the ureter can adhere to its surrounding body structures after laparoscopic surgery. Surgery for the dissection of malignant tumors requires the dissociation of the ureter, which affects a large area. In particular, lymphadenectomy involves the removal of a blend of soft tissues and lymph nodes between the ureter and pelvic vessels, resulting in an increased tendency of adhesion between the ureter and iliac vessels; such adhesion may even extend to the obturator foramen. Long-term adhesion-related traction increases the tension exerted on the ureter, leading to ureteral injuries. Second, thermal damage may occur during laparoscopic surgery [8]. During some laparoscopic surgeries, the techniques of electrocoagulation and electroincision may be applied to the uterine vessels and ligaments. For gynecological laparoscopic surgeries, especially lymphadenectomies, monopolar or dipolar electrical techniques are usually employed to dissociate the ureter. The current monopolar electrocoagulation and electroincision techniques produce thermal effects that can be transmitted at a distance of two cm. Such thermal transmission may alter the blood supply peripheral to the ureter, resulting in local ischemic necrosis of the ureter. Such indirect injuries to the ureter may not have significant manifestations during the perioperative period, but the consequences (e.g., urinary fistula) could manifest sometime after the surgery, thus resulting in delayed ureteral injuries. The third possible cause of delayed ureteral injury is technical operations. During the course of gynecological laparoscopic surgery, the surgery is performed under a two-dimensional view. The surgeon usually performs the procedure on the patient’s left side. Therefore, compared to the operation performed on the left side, procedures on the patient’s right side (e.g., uterine vessel processing and ureter dissociation) are more difficult, increasing the risk of thermal damage to the right ureter. The three patients with delayed ureteral injury all had lesions on the right ureter.
Diagnosis and treatment of delayed ureteral injuries

Ureteral injuries usually manifest as ureterostomia and ureterostenosis. Clinical symptoms include vaginal discharge (i.e., ureterovaginal fistula), backache, fever, and oliguria. Since symptoms of delayed ureteral injuries usually occur late, patients may be already discharged, signifying that symptoms of fistula may occur outside the hospital [9]. The diagnosis of ureteral injury can be confirmed on the basis of the following findings: (1) determination of creatinine and urea nitrogen levels in the fluids discharged from the vagina; if the contents have similar levels to that of urine, urinary fistula is suggested; (2) bladder methylene blue test positive results suggest bladder injury; otherwise, ureteral injury is more likely; (3) ultrasonographic examination of the dilation/stricture of the ureter; (4) IVP, which can be used to localize the lesion of the ureteral injury and estimate the severity of ureteral dilation/stricture; and (5) cystoscopy-guided retrograde ureteral catheterization. If the surgery is successful, the injury is expected to heal spontaneously; otherwise (i.e., catheterization failure), ipsilateral ureteral injury is suspicious and exploratory surgery is recommended.

Regarding the treatment of delayed ureteral injuries, if delayed ureteral injury occurs, the patient should be treated immediately to improve her quality of life. At present, surgical procedures are the primary therapy. The majority of patients undergo cystoscopy-guided retrograde ureteral catheterization, through which a D-J stent can be placed in the patient’s body for three months, allowing spontaneous healing of the fistula. However, as delayed ureteral injuries are often complicated with ureteral adhesion, stricture, or obstruction, retrograde catheterization is difficult. Hence, exploratory surgeries are often performed. The recommended procedure includes exploratory surgery on the ureter via an open and extraperitoneal approach, which should be performed by a veteran urologist. During the operation, ureteroneocystostomy (i.e., re-implantation of the ureter) can be conducted after the adhered ureter is carefully dissociated from the peripheral tissues. A D-J stent is subsequently placed ureterovesically for three months; the stent can be removed after three months via a cystoscopic approach. Early diagnosis and management of delayed ureteral injury can have excellent prognosis.

Prevention of delayed ureteral injury

Considering the specific causes of delayed ureteral injury, the following steps should be performed to prevent ureteral injuries. First, anti-adhesion measures should be taken after the ureter dissociates from the surrounding tissues. Application of anti-adhesion films to the surrounding areas is considered an effective measure for preventing adhesion. Second, the use of mono- or dipolar electrocoagulation and electrocision should be reduced in order to process the peripheral tissues in a “cold-management” manner. Some specially designed metal or plastic clamps can be used to process the peripheral tissues, reducing the thermal damage to the ureter during laparoscopic surgery. Finally, since gynecological surgery is the leading cause of iatrogenic ureteral injuries, surgeons may consider placing a D-J stent preoperatively for patients receiving a complex surgical protocol, which requires a large operation area, or in those with have a higher risk of postoperative adhesion at the preoperative assessment.

Conclusion

Ureteral injury is a severe complication that can occur during or after gynecological surgery, even two months later. The main way to prevent ureteral injuries is to avoid adhesion between the ureter and surrounding tissues, as well as to eliminate electrothermal trauma during laparoscopic operations. Surgical repair is effective for such patients.

References

Isthmocele in a retroflexed uterus: a report of an unrecognized case

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Summary
The term “isthmocele” refers to a niche on the anterior wall of the uterine isthmus or of the cervical canal at the site of a previous cesarean delivery scar. Such anatomic defect can cause many gynecologic sequelae that only recently have being identified and described. Hysteroscopy is commonly considered the gold standard for the diagnosis and also for the treatment, at least in the case of defects of small size. The authors described the case of a 37-year-old woman who underwent a cesarean section (CS) seven years before, with a long lasting history of menstrual irregularities, and pelvic pain increasing during menstruation at the hypogastric level. Magnetic resonance imaging (MRI) showed an exceptionally large isthmocele on the anterior wall of a retroflexed uterus which was otherwise misinterpreted as the uterine cavity filled with menstrual blood during a previous hysteroscopy (HSC). Although exceptional, this case highlights the possibility that a large sized isthmocele in a retroflexed uterus could be misinterpreted as the uterine cavity filled by menstrual blood at HSC. In this case MRI definitely clarified the diagnosis.

Key words: Isthmocele; Magnetic resonance imaging; Retroflexed uterus; Hysteroscopy.

Introduction
According to the latest global estimates, approximately 15% of deliveries take place by cesarian section (CS), with about 20 million CS deliveries occurring each year worldwide [1]. Such proportion has steadily increased in almost all middle- and high-income countries over the last three decades. Latest estimates indicate that in 2009, 39% of all women in Italy delivered by CS [2], making it the European country with the highest rate of CS.

Nearly half of the women may incur a poor uterine scar healing after CS which can cause anatomic defects of the uterine cavity of various degree [3]. Such defect may lead to gynecologic sequelae that only recently have being identified and described. Besides the most feared complications of cesarean scar pregnancy and uterine rupture during subsequent pregnancy and labor, also abnormal bleeding, pelvic pain, and infertility, have all been described as possible consequences [4]. Such anatomic defects can have different shapes such as thin linear defect, focal saccular outpouching, unilateral or bilateral diverticula and fistula, and different locations such as the uterine body, lower uterine segment, uterine isthmus and the upper endocervical canal [5]. Particularly the term “isthmocele” refers to a niche on the anterior wall of the uterine isthmus or of the cervical canal at the site of a previous cesarean delivery scar.

The authors report the case of a woman with a large hystmocele found on magnetic resonance imaging (MRI) which was otherwise undiagnosed during a previous hysteroscopy (HSC).

Case Report
In November 2013, a 37-year-old woman came to the Department of Gynecology and Obstetrics of the present Institute. She was complaining of menstrual irregularities and intense pelvic pain increasing during menstruation at the hypogastric level. These symptoms began about four years before.

The obstetric history of the patient revealed that she underwent dilatation and curettage under the diagnosis of missed abortion at seven weeks, in March 2006. In October 2007, the patient underwent CS for breech presentation. In August 2010 a HSC was performed due to an episode of vaginal hemorrhage. Hematometra was found and endometrial ablation was performed. In October 2012, a second episode of vaginal bleeding occurred, and once again hematometra was found at HSC. Also a thickening of the endometrium of the posterior wall of the uterine body was described. Endometrial ablation was again performed and a histopathologic diagnosis of disordered proliferative endometrium was made. Despite such intervention of endometrial curettage, the patient never experienced a relief of the symptoms.

In November 2013 a transvaginal-ultrasound (TVUS) was performed. The exam was partially hampered by the clinical condition of the patient and the intestinal bloating. It showed an inhomogeneous, mainly hypo-echoic, and ill-defined area, suggesting the presence of a partially organized fluid collection within the pelvis. Because of the results of previous HSC, the unclear findings on TVUS, and the refuse of the patient to undergo further endocavitary examinations due to the pelvic pain, she was...
referred to the authors’ Department of Diagnostic Imaging for a pelvic MRI.

MRI was performed on 1.5 T scanner using a surface coil (phased array pelvic coil). MRI was performed during the third day of the menstrual cycle. The authors’ imaging protocol consisted of oblique-axial (perpendicular to the major axis of the uterus) T1-weighted imaging (TR 487 ms/TE 10 ms, four-mm section thickness, 0.4-mm gap, 484 x 420 matrix, 41 cm FOV, and two signal averages), T1-weighted spectral fat saturation inversion recovery (SPIR) obtained in oblique-axial (TR 912 ms, TE 15 ms, four-mm section thickness, 0.3-mm gap, 484 x 420 matrix, 41 cm FOV, and two signal averages) and sagittal planes (TR 908 ms, TE 15 ms, four-mm section thickness, 0.3-mm gap, 484 x 420 matrix, 28 cm FOV, and two signal averages), and T-2 weighted imaging obtained in oblique-axial (TR 3000 ms, TE 90 ms, four-mm section thickness, 0.4-mm gap, 41 cm FOV, and two signal averages) and sagittal plane (TR 3239 ms, TE 90 ms, 3.5 mm section thickness, 0.4-mm gap, 24 cm FOV, and four signal averages).

Sagittal images (Figures 1 and 3) clearly show a retroflexed uterus. A pouch within the anterior wall of the uterine isthmus, at the site of a previous cesarean delivery scar, is a pouch (35 mm deep). In this site the uterine wall is represented only by a very subtle layer of myometrium with a thin layer of endometrium lining the inner wall of the pouch. The saccular pouch appeared in continuity with the endometrial line by a neck of 15 mm.

Discussion

As the incidence of CS is on the increase worldwide, the complications associated with them are becoming more common. Therefore the effect of postcesarean complications on women’s health is a focus of increasing attention.
Here the authors described the features on MRIs of a very large isthmocele (35 mm deep) in a woman with a history of cesarean delivery seven years before, who complained of pelvic pain increasing during menstruation and intermenstrual bleeding. This case is particular not only for the impressive dimension of the niche, indeed in a series of 44 symptomatic women described by Monteagudo et al., the largest niche was 11.5 mm deep [6], but also because MRI allowed the diagnosis of the isthmocele which was otherwise unrecognized at HSC. It is widely accepted that HSC is the “gold standard” for cesarean scar defect (CSD) assessment, in fact it enables confirming diagnosis and assessing treatment [7, 8]. In this case the isthmocele was unrecognized at HSC. This was probably due to the retroflexed position of the uterus and the large size of the CSD. Indeed, due to its location within the anterior wall of the uterus, the pouch of the isthmocele was misinterpreted as the uterine cavity.

A recent systematic review by Roberge S et al. showed that hysteroigraphy, sonohysterography (SHG), or TVUS can all detect uterine scar defects, with SHG having the higher detection power [3]. With ultrasound, the thickness of the residual myometrium, the thickness of myometrium bordering the scar, and the depth of the filling defect in the scar can all be depicted [9]. However in this case not even the TVUS allowed to clarify the diagnosis, perhaps because of some conditions that hampered the examination, such as the presence of pelvic pain and the intestinal bloating.

Finally MRI made clear the diagnosis of hystmocele. MRI also depicted the presence of functioning endometrial tissue within the walls of the isthmocele, the hypertrophic fibrotic scar which hampered the outflow of the blood at the ostium of the niche, and the accumulation of blood within the pouch. Moreover MRI, allowing a panoramic view, provided a clear depiction of the relationship between the niche and surrounding organs.

A widely accepted approach for the treatment of CSD has not been developed. Surgical techniques for repair of CSD include laparoscopic excision, resectoscopic treatment, vaginal revision, and endometrial ablation. The treatment should be individualized according to the symptoms, the sac size, and the patient’s desire for future fertility. Hysteroscopic surgical procedures are aimed at resecting the fibrotic tissue that sometimes hangs below the scar, thereby improving menstrual drainage and avoiding blood accumulation. Furthermore HSC enables the fulguration of superficial dilated blood vessels or endometrium-like glands inside the diverticulum to avoid the in situ production of fluid or blood. The procedure had to be performed with special care to prevent perforation of the isthmus [10]. Such risk is expected to be particularly high when the defects is deep with only a thin layer of residual myometrium, particularly when fulguration procedures are needed to destroy endometrial tissue within the wall of the pouch. In such cases surgical procedures may be preferable. MRI can be especially useful when surgery must be planned, because the correct topography of the lesion is clearly depicted together with possible associated changes in the surrounding tissue and structures.

In a recent review Tower et al. observed that CSD are an under-recognized cause of abnormal uterine bleeding and other gynecologic complications [4], and this case confirms such remark.

Although exceptional, this case highlights the possibility that a large sized isthmocele in a retroflexed uterus could be misinterpreted as the uterine cavity filled by menstrual blood at HSC. In this case, MRI definitely clarified the diagnosis.

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Recurrent multiple endometrial polyposis in patient treated by antipsychotic drugs

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Summary
Irregular uterine bleeding and profuse menstrual bleeding often occur in patients treated by antipsychotics, antiepileptics, and some antihypertensive drugs. Such bleedings represent an important problem in clinical practice, especially when related to antipsychotic treatment. Nonetheless, this problem has not been often analyzed in references. This paper describes a recurrent multiple endometrial polyposis accompanied by profuse menstrual bleeding in a patient undergoing a multi-year treatment of bipolar affective disorder by antipsychotics and discusses the possibilities of prevention of irregular and profuse menstrual bleeding in patients that must use antipsychotic therapy in order to treat a psychiatric illness.

Key words: Abnormal uterine bleeding; Endometrial polyps; Antipsychotics.

Introduction
Bleeding disorders are some of the most frequent gynecological problems. There are many causes of bleeding disorders. The most frequent causes are hormonal disturbances, which account for up to 90% of cases, followed by organic changes in the uterus such as myomas, adenomyosis uteri, or endometrial polyps, in up to 70% of causes [1]. Irregular and profuse menstrual bleedings in patients treated by antipsychotics, antiepileptics, and some antihypertension represent a separate issue because irregular bleedings occur as side effects of application of these drugs. According to reference data, some antihypertensive drugs, such as, for example, angiotensin-converting-enzyme (ACE) inhibitors and angiotensin receptor blockers could promote the onset of endometrial polyps in hypertensive women and cause irregular and/or profuse uterine bleeding as a side effect of drug application [2]. Concerning antipsychotics, basic antipsychotic effect of neuroleptics is related to blocking of dopamine receptors, thus causing the side effects of these drugs to be related to blocking of dopamine receptors in different parts of the central nervous system as well. Following this mechanism, neuroleptics acting upon D2 receptors, cause undesired endocrine effects such as hyperprolactinemia and galactorrhea which are caused by their action in the tuberoinfundibular region. Hyperprolactinemia is an unwanted adverse effect present in several typical and atypical antipsychotics [3, 4]. Increased serum prolactin levels result in decreased kisspeptin expression in Kiss1 neurons in both the hypothalamic arcuate and anteroventral periventricular nuclei, mediated by prolactin receptors expressed on both populations of Kiss1 neurons. Suppression of kisspeptin, in turn, reduces gonadotropin-releasing hormone (GnRH) release and results in loss of the ovulatory GnRH surge [5]. This leads to reduced pituitary gonadotropin (luteinizing hormone, LH and follicle stimulating hormone, FSH) secretion and loss of adequate ovarian stimulation, which results in hormonal changes causing prolonged estrogen stimulation during the entire menstrual cycle. Prolonged estrogen stimulation leads to changes in receptor concentration on endometrial cells, as well as changes in cell proliferation marker concentrations and apoptosis (Ki 67 and Bcl-2) in cells [6]. Concentration of estrogen receptor on endometrial cells increases while cell apoptosis marker concentration Bcl-2 declines. This opens up the possibility for development of organic lesions in the endometrium such as endometrial polyps and endometrial hyperplasia resulting in irregular uterine bleedings.

Case Report
This paper describes a case of recurrent multiple endometrial polyposis in premenopausal patient treated against bipolar affective disorder by antipsychotics. Endometrial polyposis always developed after an episode of exacerbation of bipolar affective disorder which was treated by high doses of neuroleptics.

Patient’s gynecological history showed that the patient was 12 years of age when she got her first period. Menstruations were regular, characterized by 28-day cycles, normal considering quantity and intensity of bleeding, and lasting around four days, until her twenties when treatment of bipolar affective disorder started. Several months after the introduction of neuroleptics in therapy, patient started to complain about more profuse menstrual bleedings. That is when the first complete gynecological examination was performed including colposcopy with cervical cytology and transvaginal ultrasonographic exam. Gynecological findings were normal. Nevertheless, gynecological discomforts patient com-
plained about continued. Menstrual cycles became more and more profuse, especially during the first two days, additionally disturbing her mental state. Therapy by levonorgestrel five mg tablets twice a day during the second half of the cycle was suggested. Patient accepted this therapy and took levonorgestrel (together with previously prescribed antipsychotics) for two or three years. Her menstrual cycles were still profuse, especially during the first two days, but pain intensity reduced. Although it was suggested to the patient to repeat for a gynecological exam after the third luteal phase, patient failed to do so. After some three to four years, a progression of affective bipolar disorder occurred, causing patient to be hospitalized and treated by high doses of neuroleptics. Even though patient reacted well to prescribed therapy, after discharge from psychiatric institution, profuse bleeding started, lasting around ten days, forcing the patient to come in for a gynecological exam. After a conservative treatment during which bleeding was stopped by medications, contrast sonohysteroscopy was indicated, showing endometrial polypsis inside the uterine cavity. Patient refused the recommended hysteroscopy, instead of which explorative curettage monitored by ultrasonography was accepted by the patient and performed. During the course of the intervention, more than 30 small endometrial polyps, averaging one cm in size, were evacuated from the uterine cavity. Histopathological examination showed that these were endometrial polyps without signs of cellular atypia. A thin endometrium was verified by the ultrasonographic exam performed after the intervention. Over the next three years, the patient did not show up for gynecological exams. No discomforts were present and menstrual cycles were regular. After this period, an exacerbation episode of the underlying psychiatric disease occurred, followed by an increase in the dose of neuroleptics after which illness again came to a remission phase. Then the patient reported again for a gynecological exam because of the same discomforts – prolonged and painful menstrual bleeding. Due to the fact that patient came in for a gynecological exam on the third day of the menstrual cycle while profuse bleeding was still present, a transvaginal ultrasonographic examination was performed and endometrial polypsis was visualized in the uterine cavity; polyps were made clearly visible by the presence of blood in the uterine cavity. Explorative curettage monitored by ultrasonography was performed again. In the curettage material, obtained from the cavity, a large number of small endometrial polyps (over 20) was found and subjected to histopathological analysis. Histopathological diagnosis once again confirmed the case of endometrial polyps without cellular atypia. Ultrasonographic exam performed after explorative curettage once again verified a normal, thin endometrium. Significance of regular six-month gynecological checkups was explained to the patient so she began to report regularly for gynecological control exams. No discomforts were present and transvaginal ultrasonographic exams confirmed normal gynecological ultrasonographic findings. Finally, after another exacerbation episode of the psychosis, about four to five years after the last explorative curettage, patient showed up with the same gynecological discomforts – profuse, painful, and prolonged menstrual bleeding, calling for the third explorative curettage monitored by ultrasound, once again yielding more than 30 endometrial polyps subsequently verified histopathologically too. Patient age 35, after the third explorative curettage was 41, patient gave no births and had no miscarriages, and was of normal osteomuscular constitution and normal nutritional status. Given that the patient was monitored gynecologically before the increase in the dose of neuroleptics, that it was confirmed that her cycles were mostly anovulatory, that the hormonal status verified a mild hyperprolactinemia during the entire course of the menstrual cycle and that the gynecological discomforts, accompanied by ultrasonographic image of nonhomogeneous thickened endometrium with subendometrial vascularization consistent with endometrial polypsis, appeared after the increase of the dose of neuroleptics over a four to six month period, the question arose whether neuroleptics do influence the occurrence of endometrial polyps.

Discussion

Psychotropic medications, particulary select antipsychotics, are a common cause of drug – induced hyperprolactinemia [7]. Numerous studies discuss the consequences of psychotropic-induced hyperprolactinemia, but data on endometrial polypsis in patients subjected to antipsychotic treatment, such as the case of recurrent multiple endometrial polypsis described in this paper, is very scarce [7-9]. According to data from references, the most frequent undesired effects caused by hyperprolactinemia in premenopausal patients using antipsychotic therapy are menstrual irregularities, galactorrhea, and infertility.

Monitoring of the clinical symptomatology of menstrual irregularities in patients on antipsychotic therapy is important, not only for the prevention of profuse uterine bleeding, but for prevention of organic lesions in the endometrium such as, for example, endometrial polyps. Reference data clearly show the efficiency of progestagen therapy such as, for example, levonorgestrel intrauterine system or oral progestagen therapy in patients who suffered from endometrial polypsis [10].

Some authors believe that baseline prolactin level should be determined at the beginning of the antipsychotic therapy and that serum prolactin levels should be tested every three months. Furthermore, each patient should be subjected to a thorough gynecological examination at the beginning of administration of antipsychotic therapy. In cases of anovulatory cycles, that are a common finding, hormonal therapy should be introduced in order to prevent profuse, prolonged, and irregular uterine bleeding, as well as to prevent the occurrence of organic lesions in the endometrium such as, for example, above described endometrial polyps i.e. multiple endometrial polypsis.

References

Recurrent multiple endometrial polyps in patient treated by antipsychotic drugs


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Prenatal diagnosis of lipomyelomeningocele by ultrasound and magnetic resonance imaging (MRI)

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Summary
Objective: The authors report a case of a lipomyelomeningocele with tethered cord, revealed on prenatal ultrasonography and confirmed by fetal magnetic resonance imaging (MRI).

Materials and Methods: A 32-year-old woman, gravida 1 para 1 underwent the routine second trimester prenatal ultrasound scan at 22\(^{+5}\) weeks of gestation at the present hospital. Results: The scan indicated an echoic semisolid subcutaneous mass covered by skin, posterior to the lumbosacral spinal canal of the fetus. Based on the findings indicating occult dysraphism, a fetal MRI examination was conducted, revealing that the mass was extending to the spinal cord, tethering the cauda equina. The diagnosis of lipomyelomeningocele was established. Conclusion: Lipomyelomeningocele is a form of closed neural tube defect with unclear predisposing factors. Its prevalence ranges between 0.3 and 0.6 per 10,000 live births. It leads to progressive conus tethering with associated neurological, urinary, and gastrointestinal deficits, demonstrating the importance of prenatal diagnosis.

Key words: Lipomyelomeningocele; Spinal dysraphism; Occult spina bifida; Tethered cord; Fetal MRI; Prenatal ultrasound.

Introduction
Spinal dysraphism is a medical term referring to a broad-spectrum group of congenital defects resulting from incomplete neural tube closure during the early stages of the developing fetus. The cause is multifactorial, involving both genetic and environmental factors [1]. These malformations can be categorized into open and occult forms [2]. The occult forms have normal skin cover in contrast with open ones that are associated with skin defects [2, 3]. Their clinical presentation varies from asymptomatic to causing serious disabilities to the patient with lethal complications [4].

Embryologically, the expansion of the neural tube and subsequent closure is completed by day 28. Open defects occur when the caudal neuropore fails to close [2]. The secondary neurulation sets the spinal cord formation [2]. Several types of closed spinal dysraphisms result from embryological abnormalities during this phase [2, 5]. Those that arise from premature disjunction result in fusion of the spinal cord with fatty elements, the most common of which is a lipomyelomeningocele [5].

Lipomyelomeningocele is a rare but complicated defect, lying in the spectrum of occult neural tube defects. It is actually a form of occult spinal dysraphism in which a subcutaneous fibrofatty mass traverses the lumbodorsal fascia, causes a spinal laminar defect, displaces the dura, and infiltrates and tethers the spinal cord [6]. The prevalence of lipomyelomeningoceles ranges between 0.3 and 0.6 per 10,000 live births [5,7]. The general prevalence of spinal dysraphism has declined internationally in the last few decades due to the better nutrition of women, folic acid supplementation, and establishment of ultrasound scan and biochemical markers as routine prenatal screening [2, 5, 8]. However, rates of lipomyelomeningocele do not seem to have been reduced according to recent studies, suggesting that the pathogenesis of lipomyelomeningocele is fundamentally different from that of other neural tube defects [7, 9]. According to the modified Chapman classification, that takes into consideration the anatomical relation of the lipoma with the cord interface, lipomyelomeningocele is subdivided into dorsal, transitional, and caudal [10-12]. As the subcutaneous lipoma infiltrates the spinal cord through the defect in the lumbodorsal fascia, the upward movement of the conus medullaris during axial growth may be limited and thus may lead to progressive conus tethering with associated neurological, urinary, and gastrointestinal deficits- the sequelae of a tethered cord [13]. Bowel and bladder function usually deteriorate prior to motor function or sensation [14]. However 62.5% of patients with lipomyelomeningocele have been found to be neurologically asymptomatic prior to six months of age, while only 29.3% are asymptomatic after six months of age [15]. No affected children remain asymptomatic after the age of five years [16]. The disease progression can result in frequent urinary tract infections and neurogenic bladder and bowel incontinence or constipation, as well as leg length discrepancy, foot deformities, gait abnormalities, scoliosis, spasticity, and back and leg pain [5, 14, 15]. Earlier intervention to correct a tethered cord increases the chance that its sequelae will be reversible [17]. These facts demonstrate the
importance of prenatal diagnosis of this rare medical entity, so that the parents decide after proper consultation on the preservation or termination of the current gestation and so that subsequently timely postnatal surgical intervention for the optimal treatment of the neonate is achieved. The authors describe a case of a lipomyelomeningocele with tethered cord, which was indicated on prenatal ultrasonography and confirmed by fetal magnetic resonance imaging (MRI).

Materials and Methods

A 32-year-old woman, gravida 1 para 1 underwent the routine second trimester prenatal ultrasound scan at 22+5 weeks of gestation at the Obstetrics’ Department of the present Hospital. The first trimester scan was normal with 1.7 mm measured nuchal translucency of a female fetus, the prenatal screen was unremarkable, and her pregnancy had been uneventful up to that point. Her medical and obstetric record was free and she had no family history of neural tube defects or other congenital malformations and no history of exposure to known teratogens. She had been on five mg of folic acid since the 5th week of gestation.

Results

The two-dimensional (2D) ultrasound examination that was performed showed normal amniotic fluid index (AFI) and single umbilical artery was noted. No cranial ultrasound abnormalities were identified- the posterior fossa and ventricles were normal, excluding anomalies like ventricular dilatation or Arnold-Chiari malformation. An echoic semisolid subcutaneous mass covered by skin was observed to protrude posterior to the lumbosacral spinal canal of the fetus (Figure 1). The color Doppler image revealed no blood flow in the lesion. The remaining fetal anatomy appeared normal. Maternal serum alpha-fetoprotein (AFP) level was not obtained. Based on the findings leading to a diagnosis of occult dysraphism, an ultrafast fetal MRI examination was conducted. The previously described lesion measured 24.5 x 10.8 x 14.5 mm. The additional information added by this examination was that the spinal cord was lying low and the subcutaneous mass was extending to the spinal cord, tethering the cauda equina (Figure 2). The diagnosis of lipomyelomeningocele was established. The parents consulted neurosurgeons and decided to terminate the pregnancy. The next gestation of the couple resulted in the birth of a healthy neonate with no neural tube defect.

Discussion

Lipomyelomeningocele is a form of closed neural tube defect with unclear predisposing factors [5]. Due to the risk of deteriorating neurological and urological function secondary to a tethered spinal cord, it is crucial to identify this condition prenatally for timely intervention. Familial forms of lipomyelomeningocele are rare [18, 19] but parents should be aware that these lesions could occur in subsequent pregnancies and thus that they should undergo early and detailed prenatal ultrasound examination in the next gestation.

The authors describe the prenatal diagnosis of a caudal-type lipomyelomeningocele. There are not many similar published case reports in the literature. It is important for the obstetrician to bear in mind that while most spinal dysraphisms are associated with abnormal ultrasound findings of the fetal brain, only 3.6% of the lipomyelomeningocele cases present with brain malformations [20]. As a result, in order to detect these occult anomalies, it is of great importance to completely scan the fetal spine, especially in the lumbosacral region, along with the fetal head [21]. MRI is indicated as the second-line prenatal diagnostic tool for a more detailed examination of the fetal anatomy when ultrasound indications for malformations of the central nerv-
ous system exist [22]. Its effectiveness in providing more accurate information is already well known. The Safety Committee of the Society for MRI has concluded that prenatal MRI is indicated when other non-ionizing methods are inadequate or when the MRI examinations will provide critical information that would otherwise require the use of ionizing radiation [23].

**Conclusion**

The existence of lipomyelomeningocele should be included in the differential diagnosis of cases of prenatally revealed sacral lesions accompanied with spinal dysraphism. Careful prenatal imaging may lead to the establishment of a prenatal diagnosis of this entity and contributes to discrimination between fetuses with malformations with a favorable prognosis from those with a poor one. As a result, parents are offered more accurate prenatal counseling from specialists regarding the clinical course and possible future disabilities of the offspring so that they can decide to preserve or terminate the current pregnancy, bearing in mind the possibility of recurrence in a subsequent pregnancy.

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Mature mesenteric teratoma in a child: a case report

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Summary

A 15-month-old girl with a palpable and mobile mass was misdiagnosed as an ovarian teratoma by computed tomography (CT). A midline laparotomy was performed and the pathology report identified that it was a mature mesenteric teratoma.

Key words: Mesenteric teratoma; Laparotomy.

Introduction

Mesenteric cysts are rare abdominal tumors in infants and children. Dermoid cysts of the mesentery in infants and children are rare and only few cases have been reported. Here the authors describe a 15-month-old girl with a palpable and mobile mass that was misdiagnosed as an ovarian teratoma by computed tomography (CT). A midline laparotomy was performed and the pathology report identified that it was a mature mesenteric teratoma.

Case Report

A 15-month-old girl was admitted to the present service as a case of progressive abdominal and pelvic distension, noticed initially at the age of one month with a palpable and mobile mass. She was, otherwise, asymptomatic. On examination, the pelvic and abdomen was massively distended with skin. A large cystic mass could be felt occupying the entire pelvic and abdomen (Figure 1). Bowel sound was normal. Her mother had a history of schizophrenia. CT scan confirmed the presence of a cyst with solid components, pressing the organs including stomach, intestine, pancreas, left kidney, and liver and extending down to the pelvis (Figure 2). Exploration revealed the mass of roughly 13.5 x 10.0 x 15.0 cm3. The uterus, ovaries, and ascites were not detected. The mass was full of fibrous trabs, adipose, calcification, and hydatid liquid. The mass had sharpness of border. CT suspected it was an ovarian teratoma. Intravenous pyelography was not performed.

Laboratory tests revealed serum CA 125 levels of 32.7 U/ml, CA 19-9 levels of 174.83 U/ml, CA 153 levels of 13.2 U/ml, AFP 9.3 ng/ml, β-hCG 1.40 mIU/ml, and CEA levels of 1.1 U/ml. She had symptomatic anemia (hemoglobin, 104 g/L) and thrombocytosis (blood platelets 722x10⁹/L). Blood biochemistry, blood clotting test, and electrocardiogram were normal.

Under general anesthesia, a midline laparotomy was performed. At exploration, the uterine and ovaries were all normal (Figure 3). A round and smooth mass of nearly 18 cm in diameter was identified, residing in the left half of the transverse mesocolon and upper small intestine mesentery. It was confirmed as a mesenteric mass (Figure 4). In order to prevent acute heart failure, slow suction of hydatid fluid was performed first. In the process of tumor dissection, the authors found that the mass was adhered to the pancreas, stomach, and spleen. Fortunately, during the operation, no mesenteric artery was sutured. Mesenteric lymph nodes and other abdominal viscera were normal. The child had an uneventful recovery. The specimen was sent to the pathologist. The pathology report confirmed a “cystic mature teratoma.” The histology showed that it was composed of adipose tissue, bone, hair, and glial tissue. All elements were described as

Figure 1. — A large cystic mass which occupied the pelvis and abdomen could be felt.
mature (Figure 5). She was discharged on the fifth postoperative day, and found in good health two months later.

Discussion

Mesenteric cysts are rare abdominal tumors with an incidence of one per 105,000 - 250,000 hospitalized adult surgical patients [1]. However, it has no exact incidence in infants and children. Dermoid cysts of the mesentery in children are unusual cause of mesenteric cysts and only few cases have been reported [2, 3]. Parents often bring their infants and children to clinicians after noticing an abdominal mass. Teratomas have no pathognomonic signs or symptoms, and their clinical manifestation depends greatly on the size and location of the growth. MRI, ultrasonography, and CT are considered

Figure 2. — Computer tomography scan (CT) suggesting the presence of a cyst with solid components.

Figure 3. — The uterus and ovaries are both normal.

Figure 4. — A mesenteric mass is identified

Figure 5. — The postoperative specimen.
suited for the diagnostic evaluation of teratomas. However, preoperative diagnosis of the mesenteric teratoma may be difficult, especially its origin [4]. In the present case, the mass was misdiagnosed as an ovarian teratoma. It always requires surgery to diagnose.

In 2000, De Perrot et al., suggested a classification for mesenteric cysts based on histopathological features: (a) cysts of lymphatic origin (simple lymphatic cyst and lymphangioma); (b) cysts of mesoenterial origin (simple mesothelial cyst, benign cystic mesothelioma, and malignant cystic mesothelioma); (c) cysts of enteric origin (enteric cyst and enteric duplication cyst); (d) cysts of urogenital origin; (e) mature cystic teratoma (dermoid cysts), and (f) pseudocysts (infectious and traumatic cysts) [5]. In the present case, it was a mature cystic teratoma.

The diagnosis of a teratoma has to be confirmed by the histology of the excised tumour. Complete tumour resection is sufficient to cure a benign teratoma. A significant portion of benign tumors have immature elements that may undergo malignant degeneration; hence the presence of malignancy or high-grade immature elements is an indication for adjuvant cisplatin-based chemotherapy after resection [6-8]. As with teratomas at other sites, complete tumor resection via laparotomy or laparoscopy is the definitive treatment of choice and outcome is generally favorable [8]. However, in the present case, the authors did not identify the origin and nature of the mass and they chose laparotomy with a longitudinal incision.

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