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Prevention of first-trimester miscarriage with dextroamphetamine sulfate treatment in women with recurrent miscarriage following embryo transfer - case report

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Summary

Purpose: To present a novel approach to prevent miscarriage by treatment with sympathomimetic amines. Materials and Methods: Two women undergoing in vitro fertilization-embryo transfer (IVF-ET) with a history of recurrent miscarriage even in IVF-ET cycles were treated with dextroamphetamine sulfate prior to their next IVF-ET cycles. Results: Both women successfully completed their first trimester. One woman delivered a live baby and one had neonatal death related to prematurity secondary to severe pre-eclampsia. Conclusions: Sympathomimetic amines therapy may prove to be an effective therapy to prevent recurrent miscarriage especially in women who have failed despite progesterone therapy, and where no other etiologic factors have been determined.

Key words: Embryo transfer; Sympathomimetic amines; Recurrent miscarriage; Dextroamphetamine sulfate.

Introduction

There is a wide variety of chronic disorders described involving multiple physiological systems that are refractory to “standard” therapies, but respond quickly and effectively to treatment with sympathomimetic amines [1, 2]. These disorders include marked relief of chronic pelvic pain, whether it is of bladder origin as in interstitial cystitis or chronic pelvic pain or dysmenorrhea as seen in endometriosis [3-5].

Interstitial cystitis can be diagnosed prior to development of inflammatory changes that can be detected by cystoscopy by performing a potassium sensitivity test [6]. Installation of a potassium solution into the bladder in a person without this disorder will not evoke pain but severe burning pain ensues in a person with interstitial cystitis because the bladder mucosa no longer prevents an effective barrier to inhibit the absorption of potassium into the bladder wall [6].

One of the important functions of the sympathetic nervous system is to diminish cellular permeability [2]. Thus it is the authors’ belief that the etiology for the vast variety of pain syndromes in different areas of the body, i.e., headaches, backaches, fibromyalgia, gastrointestinal system, not to mention the pelvis, and dramatic relief of these syndromes by treating these disorders with dextroamphetamine sulfate, is by correcting the cellular permeability defect and thus inhibiting the absorption of chemical toxins into the tissues which causes the pain [2, 5].

The possibility exists that increased cellular permeability may allow the absorption of chemical toxins into the endometrium which could impair implantation even following in vitro fertilization-embryo transfer (IVF-ET).

The authors describe two cases that had failed to successfully conceive following several IVF-ET cycles that were finally successful when sympathomimetic amine therapy was added.

Case Report

Case 1

The woman first presented to this reproductive endocrine practice for infertility at age 40. She had a history of one previous pregnancy with a different male partner at age 25 but had a miscarriage. She had been trying to conceive with her present husband for 3.5 years. She had failed to conceive at another infertility center after three cycles of follicle-maturing drugs and intrauterine insemination (IUI) and two cycles of IVF-ET. Her menstrual cycles were regular, her fallopian tubes were patent, and her husband had a perfectly normal semen analysis.

With her first IVF-ET cycle at our institution, she had 25 oocytes retrieved. Twenty-two were metaphase II and 17 fertilized. Three day three embryos [6, 9, 10] with very little fragmentation were transferred on day 3. Thirteen embryos were frozen (nine at the 2 pronuclear stage and four multi-cell ones). A pregnancy was achieved but she had a first-trimester spontaneous abortion related to a triploidy.

There were 15 oocytes retrieved on her second cycle and 14 were metaphase II. She fertilized 13 oocytes although six were allowed to cleave to day 3, there was only one with six blastomeres and the other two had four cells. She conceived and again had a first-trimester miscarriage.

She next had a frozen ET. This resulted in a pregnancy and the beta-human chorionic gonadotropin level doubled appropriately to 1,303 mIU/ml, but three days later only reached 1,739
mIU/ml. Ultrasound showed an anembryonic gestational sac. She had transferred three embryos—one 8-cell and two 5-cell embryos.

She then had her third IVF-ET cycle with our group at age 41.6 and conceived. However, she had another first-trimester miscarriage. Chromosome analysis of the aborted fetus found a normal male.

Following the miscarriage of a chromosomally normal fetus, despite aggressive progesterone therapy, and the unavailability of lymphocyte immunotherapy, the authors provided the option of sympathomimetic amine therapy to accompany her next frozen ET.

She was started on dextroamphetamine sulfate extended release capsule daily and conceived again following her next frozen ET. She successfully completed her second trimester. Unfortunately she developed severe pre-eclampsia in her last trimester and delivered preterm and the baby subsequently died. She had continued the sympathomimetic amine therapy and progesterone.

Case 2

A couple had ten years of unprotected intercourse and no live babies. Once they sought the opinion of an infertility specialist because of their difficulty in conceiving, the problem was thought to be secondary to severe oligoasthenozoospermia.

When they failed to conceive after 12 cycles of IUI, they decided to do IVF-ET at another IVF-ET center. She conceived three times and had first-trimester spontaneous miscarriages each time. They could no longer afford IVF-ET, so they opted for insemination with donor sperm. She conceived three more times but also had three more first-trimester losses.

The couple came to this infertility center to consider another IVF-ET cycle with her husband’s sperm using intracytoplasmic sperm injection (ICSI). However, they especially consulted the authors for a possible new consideration on how to prevent another miscarriage (i.e., so far six pregnancies and six first-trimester miscarriages. All the standard tests for recurrent miscarriage had been performed, e.g., thyroid tests and tests for coagulation disorders and infections. She was offered sympathomimetic amine therapy.

She started dextroamphetamine sulfate extended release capsules 15 mg daily. She proceeded with another IVF-ET cycle with intracytoplasmic sperm injection (ICSI). She had transferred three embryos—one 8-cell and two 5-cell embryos. Nevertheless considering the pregnancy were the result of the treatment with dextroamphetamine sulfate. Nevertheless considering the many pregnancy losses of these two women and the clear-cut benefit of this therapy for various pain syndromes, it seems probable that it could have prevented first-trimester miscarriage. These case reports should hopefully stimulate controlled prospective studies to evaluate the potential of this novel therapy. Dextroamphetamine sulfate in normal pharmacologic dosage is not considered to be a human teratogen [7–9].

Case 1 was age 42 and was nulliparous so that she was at greater risk for pre-eclampsia. However, women with this sympathetic nervous system hypofunction defect are more prone to edema related to the inability to compensate for the increase in hydrostatic pressure by diminishing capillary permeability leading to transudation from intravascular to extravascular space [10, 11]. Thus it is possible that women who are more prone to miscarriage because of sympathetic nervous system hypofunction allow the absorption of toxic material into the endometrium. It remains to be seen in further studies if this therapy allows progression to the last trimester and if pre-eclampsia will be more frequent.

References


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Secondary amenorrhea despite normal endometrial development with secretory changes and absence of uterine synechiae – a second case of the endometrial compaction – apoptosis syndrome

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Summary

Purpose: To report the second case of amenorrhea related to endometrial compaction apoptosis syndrome. Materials and Methods: A female with secondary amenorrhea was evaluated with sonography, hysteroscopy, serum estradiol and progesterone levels, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and endometrial biopsy. Results: Initially she was found to be ovulatory. However she did not menstruate despite the development of adequate endometrial thickness and a normal secretory endometrial biopsy. Hysterosalpingogram failed to detect synechial. Subsequently she developed hypogonadotropic hypogonadism, but she still failed to menstruate despite estrogen followed by progesterone. Conclusions: Amenorrhea can occur despite secretory endometrial changes without a uterine abnormality.

Key words: Amenorrhea; Normal uterine cavity; Endometrial compaction; Endometrial apoptosis.

Introduction

Amenorrhea in the presence of normal estrogen, either with normal ovulation or failure to menstruate despite withdrawal of exogenous progesterone, is usually secondary to endometrial synechiae, i.e., Asherman’s syndrome.

However, there are rare cases in humans where despite the production of adequate estrogen without evidence of uterine synechiae, menstruation does not occur [1]. In the aforementioned case, the woman ovulated as evidenced by a rise in serum progesterone and even had a normal luteal phase endometrial biopsy, but no menses. Hysteroscopy was normal [1].

Some animals, such as rabbits, sheep, and hamsters have hypertrophy and degeneration of uterine luminal epithelium in response to estrogen and progestins; however they do not menstruate but undergo a process of cell destruction by apoptosis [2]. These animals lack the spiral arterioles that are responsible for menstrual flow in primates [2]. Thus the aforementioned case may have a situation analogous to rabbits, sheep, and hamsters.

Indeed histological studies in the human species concluded that the marked reduction in endometrial thickness from the immediate pre-ovulation state to shortly post-menstruation may be primarily due to loss of fluid and the result of apoptosis of the spongy layer [3]. Another study in humans concluded that in most cases, an appreciable fraction of the stratum spongiosum actually disintegrates but endometrial tissue superficial to the basal layer remains in situ at the end of menstruation [4]. Very heavy vs. very light menses (or no menses) in ovulating women may be thus related to the extent of endometrial shedding [4].

A review of the literature found no new articles with similar findings (normal ovulation but amenorrhea without a known uterine factor e.g., obstruction to outflow or intrauterine adhesions). Another case of apparent endometrial apoptosis or compaction without shedding is now reported.

Case Report

A 22-year-old female consulted us because of a history of primary amenorrhea, despite normal sexual development at the appropriate age. Amenorrhea occurred despite documented normal ovulation at the age of 17, as evidenced by both serum progesterone and endometrial biopsy. Ultrasounds showed a normal uterine cavity with endometrial thickness reaching 10-12 mm.

More evidence of folliculogenesis was the fact that she had a tendency to develop ovarian cysts and had five laparoscopies to remove ovarian cysts. When she presented at the age of 22, she wanted to know the nature of her problem and to determine if pregnancy was possible. She added that a recent attempt to stimulate her to ovulate with gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH) combination failed to stimulate folliculogenesis.

The following serum studies were obtained: low estradiol - < 10 pg/ml, low FSH of < 0.7 mIU/ml, low LH < 0.2 mIU/ml, cor-

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tisol 25.3 mcg/dl (normal 4.0-22.0 mcg/dl), dehydroepiandrosterone sulfate – 185 mcg/dl (normal 45-320 mcg/dl), free thyroxine 1.0 ng/dl (normal 0.8-1.8 ng/dl), thyroid stimulating hormone 2.36 mIU/l (normal < 2.5 mIU/l), and prolactin 20.9 ng/ml (normal 2-20.0 ng/ml).

A pelvic sonogram revealed the right ovary to measure 16 x 17 x 16 mm and the left one to measure 21 x 19 x 22 mm. No antral sized follicles were seen and only a few pre-antral sized ones of two to three mm were noted.

With six mg/day of estradiol for 18 days, she developed a 14-mm endometrial thickness. She continued the estradiol while adding 10 mg medroxyprogesterone acetate for 14 days, however menses did not ensue.

Her endometrial echo pattern immediately prior to starting progesterone was triple-line and one week later on progesterone converted to the appropriate homogeneous hyperechogenic pattern [5, 6].

Discussion

Though her estrogen deficiency related to her apparent isolated gonadotropin deficiency (but not related to significant hyperprolactinemia) would result in amenorrhea, her development of secondary amenorrhea despite previous ovulation with no apparent uterine synechiae is consistent with the diagnosis of endometrial compaction – apoptosis syndrome that has only been reported once before [1]. Further confirmation was her failure to menstruate despite high-dosage estrogen followed by progestins which allowed endometrial proliferation but no shedding. Evidence that this problem is not related to progesterone receptor deficiency or inadequacy was excluded by the development of a secretory endometrium.

Her failure to ovulate despite a course of exogenous gonadotropins including LH and FSH could have two possible explanations. Sometimes, hypogonadotropic hypogonadism needs a prolonged course of exposure to gonadotropins in high-dosage before a response is seen even with estrogen priming. With no insurance coverage for these expensive drugs and failure to show a typical response to a moderate dosage, the therapy was discontinued. Sometimes this resistance may be related to associated growth hormone deficiency and the addition of growth hormone can allow response to less gonadotropins, but eventually with a high enough dosage and time of exposure, one will typically see a response [7]. Unfortunately though the young woman wanted to conceive, she would have to wait until she acquired the needed funds or the needed insurance coverage.

The question arises as to whether conception is even possible (the first case report chose not to try to conceive since her husband had a vasectomy). This author has seen one previous case of secondary amenorrhea related to endometrial compaction – apoptosis syndrome (unreported) and she did in fact have a successful pregnancy.

In the present case, it is possible that the multiple ovarian surgeries have damaged the ovaries and she would have shown an increased serum FSH related to diminished oocyte reserve, if there had not developed an independent hypothalamic pituitary problem. Thus, the frustrating thing for the patient without insurance is that there is no guarantee that following high-dose exogenous gonadotropins that she will even respond. It is interesting that in another case of amenorrhea related to a uterine defect, i.e., congenital absence of the uterus, which is usually associated with normal estrogen and ovulation, she also had accompanying hypogonadotropic hypogonadism [8].

References


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Human spermatozoa antigens in unexplained infertility

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Summary

Objective: To determine and compare the immunolocalization of functionally important antigens in human spermatozoa in an unexplained infertility (UI) group. Materials and Methods: In this study, the sperm samples of 20 patients undergoing evaluation belonging to normozoospermic group, whose primary reason of infertility was under investigation for this purpose, were screened. CD46, CD55 and CD52, CD69, CD98, fMLP, HI307, and 80280 were stained on the spermatozoa through indirect immunofluorescence technique. Results: In addition to CD46, CD55, and CD52 antigens, which are known to be localized on human spermatozoa, significant immunolocalization of several novel antigens including: CD52, CD69, CD98, fMLP, HI307, and 80280 were determined on the spermatozoa of the unexplained infertility group, possibly reflecting important roles in the pathophysiology of such unresolved clinical situations. Conclusion: Identification and characterization of antigens present on sperm cells is crucial for understanding of the diagnosis and treatment of unexplained infertility. Further studies were conducted to evaluate a possible correlation between the expression of these antigens and clinical outcomes in different well-defined infertility groups.

Key words: Spermatozoa; Surface antigens; Unexplained infertility; Immunofluorescence.

Introduction

Fertilization is a complex process involving numerous molecules, cell-cell, and cell-matrix interactions. For successful fertilization, the spermatozoa must undergo a cascade of events including capacitation, hyperactivation, acrosome reaction, binding to the zona pellucida, penetration through the zona pellucida, and fusion with the plasma membrane of oocytes [1]. Several families of molecules such as complement regulatory proteins, tetraspans, ADAM proteins, integrins, and others have been shown to be involved in this process [2]. Most of these molecules are not restricted to the reproductive system, but also play essential roles in a variety of immune reactions. Thus the function of these molecules is still unclear and the mechanisms controlling this complex event is not yet completely understood. Chemotaxis and activation of reactive oxygen intermediates (ROI) are also important components of the fertilization process; consequently, chemotactic factors and their receptors on spermatozoa are under intensive investigation [3-5].

Unexplained infertility (UI) refers to a diagnosis made in couples where standard investigations including semen analysis, tests of ovulation, and tubal patency are normal. UI still accounts for some 10% to 25% of all cases of infertility. The pathophysiology of unexplained infertility is still poorly understood, and various diagnostic tests are unable to determine the underlying cause of sperm dysfunction. Most possible causes of UI seems to be any disorder in the molecular interactions between sperm and oocyte in the reproductive environment [6, 7]. Thus any information on these molecules and/or their functions is of critical importance. The authors attempted to determine the antigenic profile of spermatozoa of normal individuals and UI patients at the light-microscopy level using several monoclonal antibodies (mAbs), some of which are reactive with previously reported antigens, while some others are introduced in the present study. The aim of this study was to determine and compare the immunolocalization of these antigens in normal and UI groups. In the future, the authors intend to extend these studies to the ultrastructural level for more precise localization.

Materials and Methods

The human semen samples were collected in sterile plastic containers through masturbation by unexplained infertile patients (n = 20) each with three consecutive conception failures on intrauterine insemination (IUI) attending the ART clinic in Hacettepe University Medical School, as well as from healthy proven-fertile donors (n = 6) after an abstinence of three to five days. The ejaculates were allowed to liquefy for 30 minutes, and semen parameters were analyzed according to World Health Organization (WHO) guidelines [8].

Sperm counts of UI subjects were similar to those of men of the proven-fertile group. It was ensured that each subject in both groups was married and lived with his spouse for two or more years without any recorded conception. All spouses were found normal after strict gynecological assessment. The controls had at least one child and had routine semen analysis within the normal range, according to WHO 1999 guidelines. Necessary approval was given by the institutional review board to perform the study.

After initial wash with human tubal fluid (HTF) medium, the spermatozoa were smeared onto a clean glass slide coated with gelatine and the smear was allowed to dry at room temperature. Slides were fixed in methanol for ten minutes and air-dried for at least 30 minutes. Slides were then incubated for 60 minutes

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with primary mAbs (Table 1). After washing in 0.01M phosphate buffered saline (PBS) pH 7.4, the slides were covered with mouse immunoglobulins/FITC labelled secondary antibody, except for CD52 monoclonal antibody for 30 minutes, washed in PBS 3 for ten minutes and covered by one drop of propidium iodide/antifade solution. Anti-rabbit IgG-FITCH secondary antibody was used for CD52 monoclonal antibody. Immunofluorescent labelled sections were then examined and photographed using a microscope.

Results

Complement regulatory/related proteins

**CD46**

Membrane cofactor protein (MCP; CD46) represented one of the most strongly-expressed antigens in human sperm. Extensive expression of this antigen in both groups provided a positive control for this technique. The main site of localization of the antigen was the acrosomal compartment of the sperm head (Figures 1A, B). In the control (normal) group a similar reaction was present. In the spermatozoa exhibiting abnormal head morphology (swollen or irregularly enlarged), a crescent-shaped reaction was confined to the tip of sperm head possibly representing an abnormal acrosome (Figures 1C-E).

**CD52**

CAMPATH-1 antigen exhibited a unique expression on the post-acrosomal membrane region in the UI group (Figures 2A, B). A similar but weaker reaction was observed in the control group (Figure 2C). There was also a weak reaction on the midpiece and initial segment of sperm tail in some spermatozoa of the control group (Figures 2D, E).

**CD55**

Decay accelerating factor (DAF) was expressed in the acrosomal region, midpiece, and tail in the UI group, being stronger in the equatorial segment and midpiece (Figures 3A, B). There was a restricted reaction in the midpiece in some samples of the control group (Figure 3C). A weaker reaction was present in the acrosomal region in the control group (Figures 3D, E).

**CD69**

Activation inducer molecule (AIM) was expressed in the acrosomal region, equatorial segment, midpiece, and tail in the UI group, being stronger in the equatorial segment and midpiece (Figures 4A, B). There was no significant reaction in the control group (Figure 4C).

**CD98**

Activation antigen 4F2 was expressed in the acrosomal region, midpiece, and tail in the majority of the spermatozoa in the UI group (Figure 5A). Both diffuse and patchy reaction patterns were present in the acrosomal region (Figures 5B, C). However different staining patterns were also observed in this group. In some of the spermatozoa, the reaction was confined to the midpiece and tail regions and absent in the acrosome (Figure 5D). No significant reaction was observed in the control group (Figure 5E).

**Novel mAbs from human leukocyte differentiation antigens (HLDA) 7th and 8th Workshop blind panels**

**5F1(fMLP)**

This antigen was another example of a very unique expression in the UI group. The reaction was present on the equatorial segment, being stronger at both edges, and in the midpiece resembling the corners of a triangle (Figures 6A, B). In some spermatozoa, a patchy reaction was present also in the acrosomal region (Figures 6B, C). However different staining patterns were also observed in this group. In some of the spermatozoa, the reaction was confined to the midpiece and tail regions and absent in the acrosome (Figure 6D). There was a moderate reaction in the tail as well. No significant reaction was observed in the control group (Figure 6E).

**80280**

In the UI group, the acrosomal region was diffusely stained. There was also a moderate reaction in the tail (Figure 7). No significant reaction was seen in the control group.

**HI307**

The main reactive site for this antigen in the UI group was the midpiece and the tail (Figure 8A). Reaction intensity in the midpiece was quite strong (Figure 8B). No significant reaction was seen in the control group.

Discussion

Characterization of cell differentiation and maturation relies on structural observations and/or cell specific expression of specific transmembrane or cytoplasmic antigens. However data arising from recent studies revealed that different cell types share a number of antigens which have recently been classified into several families of proteins according to their molecular structures and/or functions. Thus investigators work on anti-
Human spermatozoa antigens in unexplained infertility

gens on the cell groups of their interest for two main goals: (i) determination of antigens which are specific to a cell reflecting their differentiation/maturation state for their characterization; (ii) determination of antigens with known functions in other systems of the organism to obtain evidence of a similar function in the cells of inter-

Figure 1. — Localization of CD46. a, b: CD46 localization on the acrosomal compartment of the spermatozoa head in the UI group; c, d, e: acrosomal localization of CD46 in the control group.

Figure 2. — Localization of CD52. a, b: CD52 antigen localization on the post-acrosomal membrane region in the UI group; c: post-acrosomal membrane CD52 expression in the control group; d, e: weak CD52 reaction on the midpiece and initial segment of tail in some spermatozoa of the control group.

Figure 3. — Localization of CD55. a: CD55 localization on the acrosomal region, midpiece, and tail of the spermatozoa in the UI group; b: strong reactivity with CD55 in the midpiece of the spermatozoa in the UI group; c: restricted CD55 reaction on the midpiece in some samples of the control group; d, e: weaker CD 55 reaction on the acrosomal region in the control group.
Human spermatozoa antigens in unexplained infertility

Regarding the yet unsettled mechanisms of the complex reproduction process, the authors studied the antigenic profile of spermatozoa belonging to fertile and unexplained infertility groups to obtain some evidence to direct further studies. For this purpose, they used both monoclonal antibodies to known antigens and some others which have not been studied on spermatozoa previously and obtained valuable data. Following a screening study using large numbers of monoclonal antibodies, only those of interest which provide initial findings to explain some of the mechanisms leading to IU are presented in this paper.

Expression of complement regulatory proteins CD46, CD55, and CD59 on inner acrosomal membrane of spermatozoa has been previously reported [9-15]. The authors studied CD46 and CD55 expression together with CD52, a GPI-anchored surface glycoprotein, which is also known to be expressed on spermatozoa for comparison of spermatozoa from fertile and IU groups, also serving as a positive control. Both CD46 and CD55 were expressed on the acrosome in control and IU groups, however the intensity of CD55 expression in the fertile group is relatively weaker. CD46 is strongly expressed also on the spermatozoa with structural abnormalities, reflecting the structural deformities of the acrosomal vesicle. Thus CD46 antigen can be considered as a constitutive antigen being present in the spermatozoa, also providing a positive control for the technique used. CD55 expression shared variations, especially in the control group as a sign of maturational change reflecting the heterogeneity of the spermatozoa population in the smears. In the control group, reaction intensity was weaker in the acrosomal region, being the strongest in the midpiece in both groups. This observation leads to a conclusion that strong expression of CD55 on acrosome may be involved in a mechanism leading to IU, which should be confirmed.

CD52 (CAMPATH-1) antigen is known as an antigen exclusively expressed by immune system cells and epididymal cells transferred on spermatozoa [16-21]. Recently, this antigen was also shown on the mature cumulus cell mass [22]. In the present study, CD52 was shown on the post-acrosomal region of the spermatozoa in both groups. A significant expression on the midpiece in some spermatozoa of the control group was also evident. The localization of CD52 antigen strongly suggests a specific role for this molecule in sperm-oocyte contact, especially through their glycan moieties. Further ultrastructural studies should provide added evidence for this suggestion.

CD69 (activation inducer molecule) is a type II transmembrane glycoprotein with a lectin domain being mainly expressed on activated immune system cells, similar to CD52 [23, 24]. Expression of this antigen on spermatozoa, functioning as a signal transmitter on spermatozoa, has not previously been reported. This antigen was expressed on the acrosome, equatorial segment, midpiece, and tail of the spermatozoa in the IU group while no significant expression was determined in the control group. Thus, CD69 is another candidate molecule leading to signals initiating some mechanisms that result in IU.

CD98 (4F2), another activation antigen, was also broadly expressed in the spermatozoa of the IU group, however its expression was extensively variable when compared to the other antigens examined. It is reported to be expressed by a number of activated cells including neoplastic ones [25-28]. It is also expressed by trophoblastic lineage (the authors’ unpublished observations). The function of this molecule is not entirely known, however it is believed to serve as an amino acid transporter in some cells. Expression of this antigen in the IU group, but not in the fertile group, apparently reflects a deviation in sperm activation leading to IU.

Another antigen with a unique expression on the spermatozoa of the IU group, which has not previously been reported was fMLP. The fMLP receptor family represents a group of molecules that receive recently chemotactic signals from bacteria and mitochondria [29, 30]. Although it is postulated that members of this receptor family direct leukocyte traffic, their physiological role is poorly understood.

Presence of such receptors on sperm is not previously reported. The authors determined a unique expression of this antigen on the spermatozoa of the IU group, however no significant reaction was determined in spermatozoa of the fertile-normal group. This finding apparently reflects
a targeting mechanism for the spermatozoa of IU patients leading to a decreased number of normal spermatozoa incapable of fertilization.

Another novel mAbs from HLDA (human leukocyte differentiation antigens) 7th and 8th Workshop blind panels was 80280. In the UI group the acrosomal region was diffusely stained. There was also a moderate reaction in the tail. No significant reaction was seen in the control group. Further studies on the characterization of this antigen recognized by this antibody need to be evaluated.

Human leukocyte antigens (HLA) coded by human major histocompatibility complex on chromosome 6 represents a group of transmembrane glycoproteins carrying out immunological recognition function [31]. Previous reports on studies in different species including humans, display controversial findings regarding their expression [32-35]. The authors detected a significant reaction with an anti-MHC Class II monoclonal antibody on the post-acrosomal zone, midpiece, and tail of the spermatozoa reflecting a possible non-immunological function for these molecules.

In conclusion, as discussed briefly above, most of the antigens the authors studied were related to the immune system, but were also present on spermatozoa. Though the function of reproductive and immune systems are separate, some overlapping molecular mechanisms for similar functions in the organism are not really surprising and has been demonstrated for the neuro-endocrine system. Information on such molecules will help to better understand their functions, assisting in revealing the physiological mechanisms in the complex process of both systems. The findings of the present study for CD52, CD69, CD98, 80280, and fMLP will lead to further studies including immuno-electron microscopy for the precise localisation of the antigens, comparison of patient groups of unexplained infertility, and some functional studies.

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The practical role of anti-Müllerian hormone in assisted reproduction

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Summary

The objective of this study was to offer a brief critical summary of the literature on the role of AMH in the subfertility work up and during ART, while exploring its role in predicting ART success.

Key words: IVF/ICSI outcome; Ovarian reserve; AMH/ART.

Introduction

The primary goal in assisted reproduction is the continuous improvement of the “take home baby” rate. It would be greatly aided by the ability to anticipate how a woman will respond to ovarian stimulation and to predict her chances of pregnancy. The ideal way to achieve this would be to acquire advanced knowledge, that is, to be able to predict the response before a woman enters the cycle of multiple assisted reproduction technology (ART) - especially in vitro fertilization (IVF) - attempts. A meticulous pre-treatment workup would help, but only if a prognostic marker were available. Despite extensive research in the area, such a marker remains elusive [1].

Over the last ten years or so, anti-Müllerian hormone (AMH), has being investigated as a putative marker [1,2]. AMH is a dimeric glycoprotein, acting on tissue growth and differentiation. AMH has shown great potential as a prognostic marker of ovarian reserve and the ability to identify both extremes of ovarian stimulation [2]. Theoretically, AMH could help to dynamically facilitate the planning of women’s reproductive life in addition to predicting for whom IVF treatment is more likely to work [2]. There is no reliable proof though that it can directly contribute to assisted reproduction’s primary aim, the “take home baby” rate, hence in this context it isn’t an efficient marker in its own right [3,4].

Current clinical value of AMH

In clinical practice, AMH is useful in the prediction of poor response and also of hyper-response during ART [2,3,5]. It can additionally provide useful information on the risk of pitfalls during ovarian stimulation for ART, thus saving couples time and heartache, and guiding them fast to the justified “next step” decision of acquiring oocyte donation or adoption.

Many researchers, using a variety of statistical methods, have attempted to determine significant AMH measurements cut points for pregnancy and live births:

Gleicher et al. [3] used receiver operating characteristic (ROC) curves and reported that a uniform cut-off value for significantly improved live-birth rates independent of age stands at AMH = 1.05 ng/ml, with values of AMH ≤ 0.04 and 0.41 - 1.05 ng/ml relating to very low and increased pregnancy potential, respectively. Crucially, the authors did not report on which day in the stimulation cycle was AMH measured. Kini et al. [4] instead of reporting cut points, compared retrospectively the median AMH levels between women who achieved cumulative ongoing pregnancy and those who did not. They found that in the former, the median AMH level at day 6 was significantly higher. Gnoth et al. [5] employed discriminated analyses and used a calculated cutoff point based on minimized false positive and false negative results, concluding that levels of ≤ 1.26 ng/ml were highly predictive of poor ovarian response. In patients with PCOS, Kaya et al. [6] reported that the best day-3 AMH cut-off values for fertilization and clinical pregnancy rates were reported at 3.01 and 3.20 ng/ml, respectively, with the sensitivity and specificity of the method exceeding 72% for both. However, the study included only 60 patients and the analysis had a priori divided the sample into three groups using the 25th, and 75th percentiles as cutpoints. Similarly, Xi et al. [7] used these cutpoints and proceeded to make group comparisons of reproductive outcomes in 164 polycystic ovarian syndrome (PCOS) patients.

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In terms of AMH’s power to qualitatively assess the response to ovarian stimulation and the outcomes of ART, the literature is contradictory. While a positive correlation between AMH levels in the serum (weaker) or follicular fluid (stronger) with oocyte quality and embryo morphology [2,8] has been reported, the relationship has not been confirmed by others [9].

In summary, the available data on the relationship between AMH and pregnancy prediction are of limited value. This is not surprising since, clinically, there is no known marker reflecting directly the oocyte quality and the ensuing embryo. It is not straightforward to delve into such a relationship as there are a number of parameters involved, the interplay of which is not yet fully understood. So far it can only be quantified retrospectively following a live birth. The clinical value of AMH is certainly getting stronger, but a clinical model based solely on AMH is unlikely to be developed. An ideal strategy would be a systematic review and meta-analysis of all prediction studies, but given the current variability in reporting, this does not seem feasible.

The power of AMH in predicting outcomes

From the hormonal tests, AMH’s assumed superiority lies on the fact that it directly reflects the number of pre-antral follicles and the earlier stages of follicle development [2,4,10]. Together with antral follicle count ( AFC ), AMH is considered as the marker with the highest biological plausibility for ovarian reserve [2,11] and demonstrates less individual intra- and inter-cycle variation. However, when predicting poor or high response and pregnancy rates, it has demonstrated a sensitivity of 76% and a specificity of 86% in sub-fertile couples [2,3].

Broekmans et al. [1] carried out a comprehensive systematic review of each available putative marker, both separately and as part of a model, with respect to three outcomes of interest: accuracy of poor response prediction, accuracy of non-pregnancy, and clinical value. They found that no marker was significantly better than another, and where models were involved it was not possible to calculate individual model summary statistics for meta-analysis as each model was constructed in a different way, and/or inadequate levels of sensitivity and specificity were chosen. The models, as always, were especially poor in predicting pregnancy.

AMH shows limited power in predicting pregnancy. Surprisingly, a recent retrospective analysis showed that with extremely low serum AMH levels, moderate, but reasonable pregnancy and live birth rates are still possible, indicating that even in the presence of extremely low AMH levels, ART should not be withheld [12].

The future role of AMH

The future role of AMH in individualization of ART stimulation protocols with or without modeling

With an increasing number of women delaying motherhood until their thirties, there is a growing need for simple, low-cost biological markers that can offer individual guidance on when is best to plan a family. The future clinical role of any of these markers may be found in the individualization of ART stimulation protocols [13,14]. It is behind this novel field of personalization of treatment that the desired rise in ART outcomes may be hidden. A prospective cohort study by Nelson et al. [13] demonstrated the capability of AMH alone in individualized treatment strategies for ovarian stimulation, resulting in reduced clinical risk, optimized treatment burden and maintained pregnancy rates. Similarly, a more recent retrospective study of 769 women receiving IVF, found that individualized protocols resulted in reduced adverse effects and costs [14]. In this respect, AMH appears to have an important role to play. This may even comprise a multitasking role, ranging from helping to discriminate between non- or hyper-response, cycle cancellation, and ovarian hyperstimulation syndrome, to regimen, dose and protocol formation, and possible alteration throughout cycles.

This individualized approach is perhaps a superior avenue not only for utilizing the maximum AMH’s characteristics, but also involving a number of other markers that hitherto proved inadequate prognosticators on their own; woman’s age, the hormone-based FSH blood test, estradiol and inhibin B, the ultrasound markers AFC, ovarian volume and blood flow, the clomiphene citrate challenge test, the exogenous FSH and the gonadotropin agonist test from stimulation tests [1]. This arsenal of ovarian reserve and outcome prediction tests, along with AMH has, without much success, been put through its paces using a variety of statistical techniques, often of questionable robustness, either in a univariate or a multivariable setting [2,15-18]. Especially, worrying is the use of a priori chosen cut points in ROC curves, multivariate analyses adjusting for a multitude of combinations of markers (from the list mentioned earlier), discriminant analysis, and adjusted logistic regression. However, there is extensive literature warning against adopting random categorizing levels, or those yielding the best p-value [19]. Hence, in this respect individualized models, evolved through a validated process, may well be the best both biologically and statistically.

Finally, construction of new mathematical architectures based on artificial neural networks seems promising. AMH could serve as one of the trustworthiest input factors to build the network, which after proper training could raise the predictive power of the whole model [20]. However, at the moment attempts to combine individual markers into suitable models with, or without AMH, have also proved inconclusive.

Treatment denial

There is a lack of adequate data in defining when and how women need to start worrying for their fecundity and runs in parallel to the uncertainty of whether and when medical staff should deny treatment based on AMH values. It has been proposed that AMH should be used only with
very low cut-off values in order to minimize the occurrence of false positive tests [4,13]; in addition, the added value of AMH assay to chronological age is minimal [2,3], although reports are relating it with diminished ovarian reserve in young women [21].

Conclusion
The current literature of prognostic factors in assisted reproduction is rather diverse and inconclusive. The study variability hence prevents the possibility of combining all prediction studies into a meta-analysis, leaving the data scattered and thus unusable. AMH has emerged as a relatively suitable marker for predicting ART outcomes. It has superseded other traditional tests, but it has definite limitations when used on its own. While acknowledging the limitations is the first step, the combination of AMH with other known prognostic markers, such as woman’s age and AFC, into models, preferably individualized, provides a clear direction for the future. There are however certain caveats though that should be adhered to; the hypotheses should be verified through well-designed prospective studies, validated and robust statistical methods should be used for the construction of the models, and a consenting attempt to homogenize the reporting mechanisms of such studies should be promoted.

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Role of exclusive breastfeeding in energy balance and weight loss during the first six months postpartum

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Summary

Purpose: To investigate the energy intake (EI), energy expenditure (EE), and body weight changes of solely breastfeeding women during the first six months postpartum. Materials and Methods: This is a prospective observational study of lactating women (n = 64). Three-day dietary records were filled in to assess EI. EE was calculated with a short physical activity questionnaire. Energy cost of milk production was not included in EE estimation. Results: Daily EI and EE for the six-month period was 2,000 Kcal and 1,870 Kcal, respectively. Women had a positive energy balance throughout the study period. Nevertheless, they had a significant weight loss of 0.7 kg/month by the first trimester of lactation, but a non-significant weight loss of 0.5 kg/month by the second trimester. Overall, women lost 86% of the weight gained during pregnancy. Conclusion: Exclusively breastfeeding women manage to lose weight during the first six months postpartum as part of the normal process of energy cost of lactation.

Key words: Energy intake; Weight change; Lactation.

Introduction

The period of breastfeeding is the stage in a woman’s life with the greatest energy demands, even greater than those during pregnancy [1]. The production of milk just up to the fourth month of lactation represents a sum of energy equal to the total energy cost of the nine months of pregnancy [2]. Lactation requires an increased intake of nutrients and excess fat gained throughout pregnancy is generally considered to be the main supply of extra energy needed for lactation. After delivery, many women although willing to lose weight, fear that restricting their dietary intake can lead to a reduction in their milk’s volume and quality [3]. This is why they may choose to increase their energy intake more than recommended during the lactation period [3].

This study was designed to assess the energy intake, energy expenditure, and weight changes of Greek mothers who exclusively breastfeed their offspring for the first six months postpartum. It is the first study to research and describe the Greek data in this field.

Materials and Methods

Inclusion, exclusion criteria, and outcomes

This was a prospective observational study with a cohort of n = 64 pregnant women delivering healthy full-term neonates (> 37 weeks, weight > 2.5 Kg) in private maternity hospitals of Athens, Greece. All participants stated their intention to exclusively breastfeed their infants for up to six months and were followed up until the sixth month of lactation. Mothers who were following specific diet because of diabetes or hypertension, or were taking medicines known to influence their appetite were excluded.

Main outcome measures were to assess the lactating mothers’ energy intake (EI), energy expenditure (EE), energy balance (EB), as well as body weight changes at first, third, and sixth month of lactation. Secondary outcome was to evaluate any possible correlations of these with maternal characteristics.

Study protocol

Data collection

At the initial meeting, study requirements were clearly explained to the participants and an information sheet was given describing the goals of the study. They were asked to sign a written informed consent form. Ethical approval was obtained by Harokopio University Ethics Committee.

Participants were asked to fill in a questionnaire with demographic, socio-economic, and obstetric data. Three home visits during the morning hours, were made by a member of the research group at first month (i.e.: 25-30 days postpartum) and at the beginning of the third and sixth month of lactation. Weight and height were measured with subjects wearing only underwear and using a digital electronic balance (range 0.1 - 150 Kg) and a tape measure (range 0 - 200 cm). Body mass index (BMI) (kg/m²) was thus calculated.

Energy intake was assessed at first, third, and sixth month of lactation by giving lactating mothers a three-day dietary record to complete. Prior to diet-record keeping, the mothers were thoroughly instructed on how to fill in their food consumption, how to measure portions of food, and how important it was not to miss out any food or snack. They were also advised not to change their habitual diet during the three days of recording. Mothers recorded the type and amount of food and beverages consumed for two consecutive weekdays and one weekend day.
using standard household measures (cups, tablespoons, etc). On site, a member of the research team reviewed the records with the respondent to clarify entries, number and size of servings, and forgotten foods. Clarification of foods involved the use of food models, pictures, and measuring devices.

Energy expenditure was assessed at the above time points by asking women in this study to fill in a short physical activity questionnaire (Harokopio physical activity questionnaire-HAPAQ). HAPAQ is a questionnaire that consists of 22 items, which examine physical activity of the respondent and is based on previous work done by Ainsworth et al. [4]. HAPAQ has been validated for both men and women by comparing its outcomes against the activity monitored by an accelerometer [5].

Data processing

Energy intake was estimated by using an appropriate diet analysis software to assess food intake data for their energy and macro-nutrient intake. Traditional Greek foods were also included in the food database. Energy expenditure as estimated by HAPAQ was the sum of basal metabolic rate and physical activity cost. The energy cost for milk production was not included in the estimations of energy expenditure, due to great variations in women’s reports regarding the duration of breastfeeding during the day. Energy intake and energy expenditure were both adjusted for body weight in order to evaluate their correlation with body weight changes.

Energy balance was determined by energy intake and energy expenditure, as defined previously without the energy cost for milk production. If EB is positive, this indicates that energy intake is greater than energy expenditure. If EB is positive and there is an established body weight loss of women during lactation, then this finding should be attributed to the energy cost for milk production, although it was not calculated and included in the energy expenditure estimation.

Pre-pregnancy weight (PPW) was derived from women’s medical records and kept at the maternity hospitals where they delivered. Permission to access those records was secured from the clinic’s executive board. PPW was taken as a baseline in order to estimate body weight changes at first, third, and sixth month of lactation. In addition, the rate of body weight change between the first and third and third and sixth month of lactation was calculated to assess body weight loss during lactation.

Statistical analysis

Descriptive characteristics of investigated variables were expressed as mean ± standard deviation. Correlation between EI and body weight changes with parameters of interest was evaluated by computing Spearman’s correlation coefficient. Evaluation of body weight changes, EI, EE, and EB changes were calculated using paired-samples t-test and applying Bonferroni corrections to reduce the possibility of type II error. Comparisons were done in pairs because sample sizes were unequal at the three time points of measurement. Equality of means within the three measurements (first, third, and sixth months) for the parameters of interest was tested with repeated measures analysis of variance (ANOVA). The level of significance was defined at p < 0.05. Statistical analysis was performed using SPSS version 17.0 software.

Results

Population characteristics—EI and EE

Lactating mothers’ mean age was 32.5 ± 3.1 years (25-39 years) and 78.1% were nulliparous. All subjects were married and almost all were employed (93.7%), while two-thirds (65.6%) had a university degree. Mothers’ mean pre-pregnancy BMI (ppBMI) was 22.2 ± 4.1 kg/m² and 10/64 (15.6%) were classified as overweight or obese (BMI > 25).

From the 64 mothers who entered the study, 39 (60.9%) continued to exclusively breastfeed up to the third month and only 24 (37.5%) up to the sixth month postpartum. Lactating mothers’ mean daily energy intake during the first, third, and sixth month of lactation was 1999.8 ± 452.3 kcal, 2031.7 ± 464.7 kcal, and 2048.7 ± 558.8 kcal, respectively. Energy intake did not show any statistically significant difference among the three time points measured. The three-day dietary records indicated that protein contributed an average of 14.9%-16.2%, while lipids provided 36.5%-38.5% of the daily EI, with 16% being monounsaturated fat. Daily energy expenditure did not differ significantly among the three time points of the study (Table 1).

Body weight changes during lactation

The 64 women that were recruited for the study had a mean PPW of B pp = 62.2 ± 11.5 kg (45 - 106). Mean weight increase during pregnancy was 15 ± 5.9 kg (0 -

### Table 1. — Energy intake, energy expenditure, and energy balance at first, third, and sixth month of lactation (results obtained from repeated measures ANOVA). The energy cost of lactation was not included.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>1st month (n = 64)</th>
<th>3rd month (n = 39)</th>
<th>6th month (n = 24)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>1,999.8 ± 452.3</td>
<td>2,031.7 ± 464.7</td>
<td>2,048.7 ± 558.8</td>
<td>NS</td>
</tr>
<tr>
<td>Energy expenditure (kcal)</td>
<td>1,865.7 ± 315.8</td>
<td>1,866.8 ± 375.1</td>
<td>1,882.8 ± 326.8</td>
<td>NS</td>
</tr>
<tr>
<td>Energy balance (kcal)</td>
<td>134.1 ± 548.3</td>
<td>164.9 ± 480.2</td>
<td>165.9 ± 583.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS = Non significant (p > 0.05).

### Table 2. — Body weight changes at first (n = 64), third (n = 39), and sixth (n = 24) month of lactation.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>1st month (B1)</th>
<th>3rd month</th>
<th>6th month</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>68.6 ± 12.5</td>
<td>67.2 ± 12.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Weight-PPW (kg)</td>
<td>5.7 ± 5.1</td>
<td>5.3 ± 4.7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Weight-Weight at delivery (kg)</td>
<td>-8.4 ± 2.6</td>
<td>-9.8 ± 3.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>EI (kcal)</td>
<td>2,023.9 ± 402.8</td>
<td>2,031.7 ± 464.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>EE (kcal)</td>
<td>1,863.1 ± 343.9</td>
<td>1,866.8 ± 375.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>EB (kcal)</td>
<td>160.8 ± 508.6</td>
<td>164.9 ± 480.2</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*aNS = Non significant (p > 0.05).*
30). The mean weight increase between the first month postpartum and their PPW was 6.6 ± 4.9 kg \((p < 0.001)\). The 39 mothers who continued to breastfeed until the third month postpartum had a mean PPW of \(B_{pp} = 62.9 \pm 13.2\) kg \((45 - 106)\). The mean weight increase between the third month and PPW was 5.3 ± 4.7 kg \((p < 0.001)\). By the third month mothers were weighing an average of 1.5 ± 2.4 kg less than during the first month of lactation \((p = 0.004)\). In other words, weight loss for women who continued to breastfeed \((n = 39)\) during the first trimester was significant and was estimated to be 0.7 kg/month. Finally, the 24 mothers who continued to breastfeed their babies for six months had a mean PPW of \(B_{pp} = 63.5 \pm 13.1\) kg \((47 - 106)\). The mean weight increase between the sixth month of lactation and PPW was 2.8 ± 4.9 kg \((p = 0.02)\). At six months mothers were weighing an average of 1.3 ± 2.5 kg less than during the third month of lactation \((NS: p = 0.06)\). This signifies that weight loss during the second trimester postpartum for women who continued to breastfeed \((n = 24)\) until the sixth month was non-statistically significant and was shown to be 0.5 kg/month. Mothers’ BMI at first, third, and sixth month postpartum was 24.6 ± 4 kg/m², 24.2 ± 4.5 kg/m², and 23.5 ± 3.5 kg/m², respectively. It is noteworthy that BMI changes are also significant during the first three months of lactation \((p < 0.001)\), whereas they do not manage to gain statistical significance over the second trimester. During the first six months postpartum, women managed to lose an average of 85.6% of the weight gained during pregnancy.

Correlations

Spearman’s correlation coefficients were used to correlate energy intake and body weight changes with maternal characteristics. Energy intake was correlated positively at first month of lactation with parity and negatively with the weight increase during pregnancy. There were no significant correlations with age, educational level, number of cigarettes smoked, and ppBMI. Weight change at the end of the first, third, and sixth month of lactation in comparison to PPW had a significant positive correlation with the number of cigarettes smoked per day. There was also a significant negative correlation with PPW and ppBMI, and finally a significant positive correlation with the weight increase during pregnancy. There were no significant correlations with age, educational level, and number of children.

Discussion

This study was conducted in a sample of 64 mothers, who were exclusively breastfeeding their infants for a time period of six months. In this group, 60.9% (39/64) continued to exclusively breastfeed up to the third month and 37.5% (24/64) up to the sixth month postpartum. Samples of similar size have also been reported by other researchers in the past for the same follow-up period of six months \([6, 7]\).

This study is one of very few studies designed to assess the EI, EE, and weight changes of south-Mediterranean lactating mothers. Specifically, daily EI was found to be an average of 1,970 - 2,100 kcal \((28 - 31\) kcal/kg) similar to the EI mentioned in studies from other countries \([6, 8-10]\). Maternal EI well-covered what is considered to be the energy requirements during exclusive breastfeeding \([11-13]\). It was also noted that mothers had a relatively high daily fat intake of 36.5% - 38.5% of EI, while 16% was monounsaturated fat, probably due to the variety of foods consumed by the mothers of the sample, which were rich in monounsaturated and total fat. These findings are in accordance with literature concerning other south European populations’ habitual diets \([14]\). The mean daily EE (energy cost of milk production not included) was approximately 1,870 kcal during the first six months of lactation, and did not differ significantly throughout the study period. In other studies as well, EE was also similar throughout the entire period of lactation \([8]\).

Results show that over the six-month period, mothers of the sample had a positive energy balance. Nevertheless, a significant weight loss was indeed achieved at the end of the six-month period of 11.1 ± 4.1 kg in comparison to the body weight women had at their delivery (Table 2). During the first six months postpartum, it was estimated that women managed to lose an average of 85.6% of the weight gained during pregnancy. However, at the end of the six months women retained an average of 2.8 ± 4.9 kg in comparison to their pp weight. This finding is in accordance with other reports, which indicates that mothers do return to their pp weight after longer than six month periods of observation \([9, 12, or 18 \text{ months postpartum}]\) \([15-17]\). Statistical analysis showed that women had a significant weight loss of 0.7 kg/month during the first trimester of lactation, which was followed by a non-significant weight loss of 0.5 kg/month during the second trimester of lactation. This degree of weight loss is also in accordance with previous findings \([18]\). Weight loss of ~0.5 kg/month during lactation is considered to be common and safe \([19]\). Furthermore, a review of 17 studies has shown that well-nourished mothers lose weight with a rate of 0.8 kg/month, while undernourished ones with a rate of only 0.1 kg/month \([20]\). In literature, mothers lose more weight during the second trimester of lactation and not during the first trimester as the present study showed \([15, 21]\). In those reports however, larger cohort samples were used. Perhaps if a larger number of women had continued to breastfeed (> 24/64) beyond the third month postpartum in this study, then statistical significance might have also been achieved for weight loss in the second trimester.

The fact that mean energy balance was kept positive throughout the entire study period, but at the same time women were losing weight, leads to the conclusion that this weight loss was probably due to the energy cost of lactation, which was not measured in this protocol. On review of literature, during the first six months of exclusive breastfeeding, mean daily energy cost for milk production is estimated to be approximately 2,800 KJ (or 675 kcal) \([22, 23]\) and mean daily breast milk production is considered similar among women of different cultural and socio-economic background \([6]\).
The present study bears some limitations and constraints that need to be addressed. Firstly, a random sample was used which was restrained to women who gave birth at the area of the capital, Athens. Secondly, an additional limitation was the small sample size. The study initially recruited 64 women, however only 24 of them continued breastfeeding and hence remained in the study until the end. Other similar studies, which also did not use a control group and followed up mothers for ≤ six months, had larger sample sizes [16, 18, 24]. Next, the energy expenditure was not measured by experimental methods, as in previous studies, but with use of physical activity questionnaires, where energy cost of lactation was not measured. Concerning the use of three-day dietary records, it is generally highly-regarded for its validity by numerous researchers that have used them for similar studies [10, 25]. However, there is always the risk of under-reporting foods with a low nutrient density and over-reporting “healthy” food groups, especially by women who are overweight [23]. Such discrepancies together with the large number of tests carried out and the small sample size may have resulted in type I error and findings that may not be entirely applicable to a representative population [26].

On literature review and to the best of the authors’ knowledge, this study is the first to assess the EI, EE, and weight changes of Greek mothers who exclusively breastfed for the first six months postpartum. Therefore it provides additional knowledge with regards to the changes of EI throughout the lactation period, an issue that was not fully investigated by previous research. This study has shown that in exclusively breastfeeding women with usual physical activity postpartum, normal energy intake, and without basal metabolic rate disorders, EE comprising of basal metabolic rate and physical activity almost fully compensates EI. The authors can presume therefore that weight loss recorded postpartum in exclusive breastfeeding women can be attributed to the energy cost of lactation.

The practical implications of this study includes the fact that health professionals have additional data to properly counsel women to follow an appropriate diet without exaggerations in dietary EI and to perform normal physical activity. Hypocaloric diets and excessive physical activity may be well-avoided during exclusive breastfeeding, since they are not necessary for weight loss purposes. In this way mothers do not need to follow strict diets, the amount and quality of breast milk is not disrupted, and weight loss can be achieved as part of the natural process of energy cost of lactation.

References
Association of serum levels of vascular endothelial growth factor and early ectopic pregnancy

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Department of Obstetrics of the Universidade Federal de São Paulo, São Paulo (Brazil)

Summary

Background: This study evaluated serum vascular endothelial growth factor (VEGF) concentrations in women with ectopic pregnancy (EP), miscarriage, and normal pregnancy (NP). Materials and Methods: This was a case-control study comparing serum VEGF concentrations among 72 women with ectopic pregnancy (n = 35), miscarriage (n = 15), and normal pregnancy (n = 22) matched for gestational age. For the determination of serum VEGF concentration a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) was used. Patients were stratified according to serum VEGF above or below 200 pg/ml. Results: The serum level of VEGF was significantly higher in women with EP (median 211.1 pg/ml; range 5-1,017.0 pg/ml) than in women with normal pregnancy (median 5 pg/ml; range 5-310.6 pg/ml) p < 0.0001. Serum VEGF concentrations did not show any statistically significant difference between women with miscarriage (median 231.9 pg/ml; range 5-813.7 pg/ml) and EP (median 211.1 pg/ml; range 5-1,017.0 pg/ml). When threshold concentrations of serum VEGF level > 200 pg/ml were used, an EP could be distinguished from a normal pregnancy with a sensitivity of 51.4%, a specificity of 90.9%, and a positive predictive value of 90%. Between EP and miscarriage, the sensitivity was 51.4%, specificity 42.8%, and a positive predictive value of 69.2%. Conclusions: Serum VEGF could not distinguish an EP from a miscarriage. However, serum VEGF concentrations could discriminate a normal intrauterine pregnancy (IUP) from an unviable pregnancy (EP or miscarriage).

Key words: Ectopic pregnancy; Miscarriage; Normal pregnancy; VEGF.

Introduction

The incidence of ectopic pregnancy (EP) has dramatically increased over the last two decades and accounts for 1.5% - 2% of all pregnancies [1]. Although the mortality related to EP has decreased significantly, it is the most important cause of maternal death in the first trimester accounting for 9% - 13% of all pregnancy-related deaths [2-4].

Treatment of EP has changed over the years and a conservative approach (medical treatment with methotrexate, expectant management, and salpingostomy by laparoscopy) now predominates [5, 6]. Early diagnosis is important in order to allow conservative treatment options [6-8].

In spite of a high-resolution vaginal ultrasound and highly-sensitive quantitative beta-human chorionic gonadotropin (β-hCG) assays, at first presentation, an EP can be difficult to diagnose at an early stage; 36.4% of all cases do not exhibit adnexal tenderness, and nine percent report no pain [9]. For this reason, a serum biomarker of tubal implantation, which could accurately identify an EP at first presentation, would be a major clinical advance. Several markers have been investigated for early diagnosis of EP [10].

For the establishment of a viable pregnancy, implantation and placentation are the early and crucial processes, both accompanied by angiogenesis, for which vascular endothelial growth factor (VEGF) is mainly accountable and plays a key role [11]. Several authors hypothesized that implantation of the conceptus within the oviduct might increase VEGF production as a form of accommodation to the hypoxic unfavorable environment [4, 8, 12, 13]. Therefore, serum VEGF could distinguish an EP from a miscarriage [8, 12, 13].

The aim of the study was to determine the serum levels of VEGF and compare them in cases of EP, miscarriage, and normal pregnancy (NP).

Materials and Methods

Patients

The study group was comprised of 35 women with EP confirmed by transvaginal ultrasound (TVUS) or at surgery and gestational age under 7.5 weeks. The inclusion criteria were the presence at TVUS of an extra-ovarian adnexal mass in women with a suspected EP (amenorrhea, uterine bleeding, and pain) with positive β-hCG test. The exclusion criteria were non-tubal EP (intrauterine, cervical, cesarean scar, ovarian, interstitial, and abdominal) and the suspect cases of early EP not confirmed by TVUS.

The control group consisted of 15 women with miscarriage and gestational age under 7.5 weeks. The control group was composed of 22 women with NP and gestational age less than 7.5 weeks. The TVUS confirmed a viable intrauterine pregnancy.
In all groups, blood samples were collected as soon as amen
ness suggested a possible patient for the study. When there was
doubt in diagnosis of any patient, she was followed up with
TVUS and serial quantitative β-hCG, until the authors were
certain which group could match her.

The three groups: EP (n = 35), miscarriage (n = 15), and NP
(n = 22) were matched for gestational age (by date of last men
strual period and ultrasound findings).

This work has been approved by the Ethics Committee of the
Universidade Federal de São Paulo. All patients agreed with the
study and signed Informed consent.

Serum assay

All blood samples were collected, before treatment, by periph
eral venous puncture, and immediately centrifuged at 1,000 rpm
for ten minutes, and the supernatants were stored at –80°C until
assayed. For the determination of serum VEGF concentration, a
solid phase sandwich enzyme-linked immunosorbent assay
(ELISA) was used, which involved two kinds of highly specific
antibodies (human VEGF) specific for the human molecule.

Statistical analysis

Data are presented as median and range (minimum, maximum).
The three groups were compared using the Kruskal-Wallis test and the Mann-Whitney U test with Bonferroni’s correc
tion. Results were considered significant when p < 0.05. The statistical analysis was performed using SPSS r12.

Results

The mean (± SD) gestational age was similar in the three
groups of women: 47.6 ± 4.8 days, 48.3 ± 4.9 days, 49.7 ± 4.3 days for the EP, miscarriage and NP groups, respectively.

The serum level of VEGF was significantly higher in women with EP (median 211.1 pg/ml; range 5 – 1,017.0 pg/ml) than in women with NP (median 5 pg/ml; range 5 – 310.6 pg/ml) p < 0.0001 (Table 1).

In this study, the median VEGF level among women with EP (median 211.1 pg/ml; range 5 – 1,017 pg/ml) and miscarriage (median 231.9 pg/ml; range 5 – 813.7 pg/ml) was not statistically significant (Table 1).

When cut-off concentrations of 200 pg/ml for VEGF were used, EP could be distinguished from NP with a sen
sitivity of 51.4%, a specificity of 90.9%, and a positive pre
dictive value of 90%. Between EP and miscarriage, the sen
sitivity was 51.4%, specificity 42.8%, and positive pre
dictive value of 69.2%.

Discussion

The evidences found in the present study suggest that serum VEGF levels are higher in women with EP than in those with NP of comparable gestational age (p < 0.0001). The median of the VEGF serum values in EP was (211.1 pg/ml, n = 35) that is similar to the levels measured by Daniel et al. (226.8 pg/ml, n = 20), by Kucera-Sliutz et al. (211.2 pg/ml, n = 42), by Mueller et al. (203.6 pg/ml, n = 43), by Daponte et al. (227.2 pg/ml, n = 27) and differ from the study of Ugurlu et al. (55.2 pg/ml, n = 28).

The comparison of serum VEGF concentration between EP and NP demonstrated in several studies that the levels of VEGF are higher in EP [4, 8, 14] similarly to the present results. However, other authors showed no difference between both groups [3].

The current results support, that serum VEGF may dis
tinguish EP from NP. Therefore, early diagnosis of EP could be suspected in a high probability when the serum VEGF concentration is higher.

The crucial point is the discrimination between ectopic and abnormal intrauterine pregnancy. In this work, accordingly to previous studies, serum concentrations of VEGF in women with EP were higher than in those with miscarriage, but these concentrations did not show any statistically significant difference between the two [8, 12-15].

When threshold concentrations of a serum VEGF level > 200 pg/ml were used in previous studies, EP could be distinguished from a NP with a sensitivity of 88%, speci
ficiy of 100%, and a positive predictive value of 100% [8], however, in the current study, these corresponding values were 51.4%, 90.9%, and 90%, respectively. For the discrimi
nation between EP and miscarriage, Daniel et al. found a sensitivity of 60%, a specificity of 80%, and a positive predictive value of 86%, when a cut-off of 200 pg/ml of serum VEGF concentration was used [12]. Another study found a sensitivity of 87.5%, a specificity of 75%, and a positive predictive value of 77.8% [8]. The corresponding values of another study were 56.1%, 51.2%, and 53.5%, respectively [14]. For discrimination between EP and miscarriage the present authors found a sen
sitivity of 51.4%, a specificity of 42.8%, and a positive predic
tive value of 69.2%. On the other hand, serum VEGF levels can distinguish an EP from a NP with a sen
sitivity of 90.9% and a positive predictive value of 90%.

Serum VEGF initially seemed to be a very helpful ser
um marker for EP [8, 12, 13]. Furthermore, other re
ports showed the limitation of serum VEGF to distin
guish an EP from a miscarriage [14, 15].

Recently a study has shown that using a two-step algo
rithm with four markers (progesterone, VEGF, inhibin A, and activin A), it was possible to achieve 99% accuracy when diagnosing EP [16]. This suggests that even if VEGF is not important alone, it could be helpful in asso
ciation with other markers.

It is important to point out that TVUS used as a routine diagnosti
ic method for EP demonstrated to have a sensi-

| Table 1. — Serum VEGF concentrations in women with EP, abnormal IUP, and normal IUP. Values are mean ± SD and median values with ranges. |
|-----------------|-----------------|-----------------|
| VEGF (pg/ml)    | EP (n = 35)     | Abnormal IUP (n = 15) | Normal IUP (n = 22) |
| Mean            | 297.5           | 299.6            | 39.9 |
| Standard deviation | 259.4          | 278.3           | 91.4 |
| Median          | 211.1           | 231.9           | 5    |
| Min             | 5               | 5               | 5    |
| Max             | 1,017           | 813.7           | 310.6 |
| p < 0.0001 between normal IUP and the other two groups (EP and abnormal IUP). |
tivity and specificity to detect EP of 90.9% and 99.9%, with positive and negative predictive values of 93.5% and 99.8%, respectively [17].

In the present authors' point of view, serum VEGF measurement could be useful in the diagnosis of EP. In this way a single serum VEGF measure could discriminate a viable from an unviable pregnancy in early stages of gestation. In this phase a single \( \beta \)-hCG measurement could not discriminate an EP from a miscarriage and in this situation repeated \( \beta \)-hCG measurements with intervals of 48 hours are necessary. A single serum progesterone measurement could not discriminate between EP and miscarriage according to meta-analysis [18]. TVUS, sometimes, could not identify the exact site of the implantation in early stages of pregnancy. Despite the fact that serum VEGF concentration is not very specific in the early diagnosis of EP, it could discriminate the viable pregnancy from an unviable one. This aspect is very relevant since it helps the diagnosis of the cases with major risk of complication.

Conclusions

Accordingly to the present results, VEGF levels could not distinguish an EP from a miscarriage. However, serum VEGF concentrations could discriminate a normal from an unviable pregnancy (EP or miscarriage).

Acknowledgments

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References


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Chronic pelvic pain: evaluation of the epidemiology, baseline demographics, and clinical variables via a prospective and multidisciplinary approach

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Summary

Background: Chronic pelvic pain (CPP) is a common clinical condition with significant impact on quality of life. The etiology and pathogenesis of CPP is poorly understood. Materials and Methods: To examine the epidemiology, base line demographics, and clinical variables, women with CPP were prospectively analysed by an integrated and synchronised approach. Results: Of the 89 women with CPP analysed, the majority were assessed earlier, had a variety of surgical interventions and used pharmacological agents. Irritable bowel syndrome, dysfunction of the pelvic floor musculoskeletal system, and physical or sexual abuse were the most common diagnosed etiologies. Evaluation revealed an increased level of psychological impairment. Discussion: CPP is a debilitating clinical condition and a result of complex interaction between different contributing factors. Patients will benefit from an orchestrated, multidisciplinary, and synchronized approach with attention paid to the different domains of pain. Treatment is mostly not curative; avoiding profound suffering despite persisting pain should be the goal.

Key words: Chronic pelvic pain; Diagnosis; Risk factors; Evaluation; Treatment; Therapy.

Introduction

Chronic pelvic pain (CPP) is a frequent and widespread disorder. The estimated prevalence in the general female population is 15%, with the highest prevalence up to 24% in women of reproductive age [1-3]. The most used clinical definition is a continuous or intermittent, non-menstrual and non-cyclic pelvic pain, lasting for at least six months. The pain is of sufficient severity or intensity to interfere with daily activities and is often unresponsive to regular treatment [4-7].

The aetiology and pathogenesis of CPP is poorly understood and as a result, effective diagnostic evaluation and interventions remain scarce [8]. About 60% of women never receive a specific diagnosis for their pain [9, 10]. Any abdominal-pelvic structure may be involved, especially organs of the genital tract, blood vessels, muscle and fasciae of the abdominal wall, pelvic floor, and gastrointestinal tract [8].

Women with CPP have a great tendency to utilize healthcare resources and undergo exhausting diagnostic evaluations without revealing an obvious cause [5]. Even if abnormalities are detected, they are mostly coincidental and not causative [11]. Forty to 50 percent of performed gynaecological laparoscopies and 12% of hysterectomies are performed because of CPP [1, 12-15].

There is a lack of published data evaluating the epidemiology of women with CPP; there are no guidelines for evaluation and treatment. The authors present an extensive description of the evaluation of women with CPP who consulted their multidisciplinary team.

Materials and Methods

Since 2007, a multidisciplinary chronic pelvic pain team (CPP team) is active at the gynaecological outpatient department of the Sint Lucas Andreas Hospital. The aim of the team was to analyse, evaluate, and advise women with CPP, while avoiding prolonged suffering and hopefully reducing the number of undue surgical interventions. Because of its observational and anonymous character, this study was exempted from approval by the Institutional Review Board. The CPP team consisted of an urologist, gynaecologist, gastro-enterologist, psychologist-sexologist, and physical therapist as permanent members with experience in treating women with CPP.

After referral, but before consultation, women were asked to complete questionnaires; women had to be capable to read and understand the Dutch language. The self-administered questionnaire was the first step in the analysis and consisted of different parts. The general part covers baseline demographic characteristics and socio-economic status. The medical part covers clinical and obstetric history, previous operations, current and past treatment, and medication use. Pain-related variables included onset, intensity, duration, association, character, and modifying factors. Pain characteristics were measured by a composed questionnaire and by the McGill Pain Questionnaire Dutch Language Version (MPQ-DLV), which is a validated self-questionnaire for measuring sensory and affective components of pain [16, 17].

The Dutch language version of the Symptom Checklist-Revised (SCL-90-R) was used to assess physical and psy-
chopathological symptoms [18, 19]. The SCL-90-R is a validated 90-item multidimensional self-report symptom inventory using a five point rating scale. The statements are assigned to eight different dimensions: somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. The degree of psychological distress/impairment is reported by the Global Severity Index (GSI): the value of all 90 items (range 90-450). Sub-scales of the the SCL-90-R and the GSI were compared with the reference score of a normal female and a chronic pain population using the unpaired t-test. Statistical significance was determined at \( p < 0.05 \).

Inventarisation and treatment

In the inventarisation phase, all women were individually evaluated by each team member. A thorough exploration of the pain and restrictions was performed including medical, social, and cultural history. Physiological characteristics, including history of traumas, were obtained by the psychologist-sexologist. This was done through a semi-structured interview with a fairly open framework, which allowed for focused conversational two-way communication. Subsequent investigations, such as ultrasonography of the abdomen, sigmoidoscopy, and/or colonoscopy, radiography, gastroscopy, cystoscopy, and/or urodynamic study were performed if necessary.

The work-up of women with CPP consisted of complete blood count, serum chemistry, sedimentation rate, urine microscopy, and culture. A bladder diary was required, including frequency-volume chart. Vaginal and endocervical swabs for culture and chlamydia trachomatis PCR were taken. Transvaginal ultrasonography (TVUS) for screening of the vagina, tubes, uterus, and ovaries was performed. Uroflowmetry was performed and the post-voided residue was estimated by a bladderscan.

After all CPP-team members reviewed each woman, a final multidisciplinary meeting was arranged to review and generate multi-disciplinary diagnosis, advice, and treatment proposal. If necessary, women were referred for additional analysis and treatment. Otherwise, the advice and treatment was directed at pain control and reassurance. In a last visit, the results of the evaluation were thoroughly discussed and explained to the women by the gynaecologist.

Results

From January 2007 to January 2009, 108 women were referred to the outpatient department for evaluation. Nineteen women had to be excluded from this analysis; two women did not meet the definition of CPP, whereas multidisciplinary advice could not be provided to 17 women due to incomplete evaluation. Finally, 89 women with CPP were included.

The mean age was 37.5 year (SD 10.1), ranging from 17 to 61 years. The majority, 68 women (76%) had Dutch nationality, although 45 women (51%) were first-generation and 16 (18%) second-generation immigrants. Twenty-nine women (33%) were nulliparous and 73 (82%) were premenopausal.

The characteristics of the women are shown in Table 1.

Seventy-five women (84%) used pharmacological agents before consultation, including laxatives in 64 women (72%) and analgesics (opiates and non-opiates) in 63 women (71%).

Sixty-six women (74%) were previously evaluated in secondary or tertiary care because of CPP and only 26 women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Percentage (%)</th>
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<tr>
<td><strong>Age (years)</strong></td>
<td></td>
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<tr>
<td>&lt; 25</td>
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<td>15.7</td>
</tr>
<tr>
<td>26 – 35</td>
<td>23</td>
<td>25.8</td>
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<tr>
<td>36 – 45</td>
<td>35</td>
<td>39.3</td>
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<tr>
<td>46 – 55</td>
<td>13</td>
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<td>Married / living together</td>
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<td>Separated / divorced</td>
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<tr>
<td>Alone with children</td>
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<tr>
<td>With spouse (and children)</td>
<td>46</td>
<td>67.8</td>
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<td>With parents</td>
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<td>Other</td>
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<td>23</td>
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</tr>
<tr>
<td>&gt; 2</td>
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<td>22.5</td>
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<tr>
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<td>4.5</td>
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<td>Social security</td>
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<td>Part-time</td>
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<td>Unemployment</td>
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<td>Other</td>
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<tr>
<td><strong>Stages of reproductivity</strong></td>
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<td></td>
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<tr>
<td>Premenopausal</td>
<td>73</td>
<td>82.0</td>
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<td>Postmenopausal</td>
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<td><strong>Medication</strong></td>
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<td></td>
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<tr>
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<tr>
<td>Laxative</td>
<td>64</td>
<td>71.9</td>
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<tr>
<td>Analgesic (including opiates)</td>
<td>63</td>
<td>70.8</td>
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<tr>
<td>Paracetamol</td>
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<td>44.9</td>
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<td>Non-steroidal anti-inflammatory drugs</td>
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<td>Opiates</td>
<td>11</td>
<td>12.4</td>
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<td>Hormonal / contraceptives</td>
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<td>20.2</td>
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<td>Antidepressants</td>
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<td>Benzodiazepines</td>
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<td>13.5</td>
</tr>
<tr>
<td>Antacids / H2-receptor antagonists</td>
<td>7</td>
<td>7.9</td>
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</table>

Table 1. — Baseline characteristics of the 89 women analysed by the chronic pelvic pain team.
Chronic pelvic pain: evaluation of the epidemiology, baseline demographics, and clinical variables via a prospective and etc.

(29%) had no prior surgery. Laparoscopy was the most performed procedure in 49 women (55%); in 23 (26%) within 24 months before evaluation and in eight (9%) repeatedly. However, it was not completely clear whether all surgical interventions were only indicated because of CPP. In the majority of procedures, no abnormalities were detected; adhesions were detected in 15 cases (17%), endometriosis in nine (10%), myoma uteri in three, and benign ovarian cyst in two cases. Irritable bowel syndrome (IBS) was the most diagnosed etiology in 24 women (27%); adhesions, endometriosis, and myoma uteri in 15 (17%), 10 (11%), and seven (8%) women respectively.

Evaluation

In the work-up, 11 women (13%) had an elevated sedimentation rate, without signs of a clinical infection. The median duration of pain was 36 months, interquartile range 16-96 months. Thirty-eight women (43%) reported pain duration of more than four years. Seventy-two respondents (81%) had pain for at least three days a week and 45 (51%) had daily pain. The pain had a varying course in 39 women (44%) and was moderate to severe in 82 (81%), as measured by MPQ-DLV. The pain characteristics and details are presented in Table 2.

Forty-six women (52%) required additional investigation to rule out somatic disorders. Seventy-four procedures were performed. Ultrasonography of the abdomen was the most performed examination in 22 women (23%); the other investigations were performed in 23 women (25%). In the 67 women previously evaluated, 62 abnormalities were detected in 38 women (57%); in the 22 women not previously analysed, 12 abnormalities were detected in seven (27%). The examinations performed, as well as the detected abnormalities, are shown in Table 3.

Table 1. — Baseline characteristics of the 89 women analysed by the chronic pelvic pain team.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td><strong>Prior surgery</strong></td>
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<td></td>
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<tr>
<td>None</td>
<td>26</td>
<td>29.2</td>
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<tr>
<td>Appendectomy</td>
<td>12</td>
<td>13.5</td>
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<tr>
<td>Laparoscopy</td>
<td>49</td>
<td>55.1</td>
</tr>
<tr>
<td>No anomalies</td>
<td>24</td>
<td>26.9</td>
</tr>
<tr>
<td>Adhesions</td>
<td>9</td>
<td>18.4</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>4</td>
<td>8.2</td>
</tr>
<tr>
<td>Uterine fibroids</td>
<td>3</td>
<td>6.1</td>
</tr>
<tr>
<td>Benign ovarian cyst</td>
<td>2</td>
<td>4.1</td>
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<tr>
<td>Unknown</td>
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<td>14.3</td>
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<tr>
<td>Hysterecstomy</td>
<td>10</td>
<td>11.2</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>12</td>
<td>13.5</td>
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<tr>
<td>Miscarriage</td>
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<td>6.7</td>
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<tr>
<td>Induced abortion</td>
<td>12</td>
<td>13.5</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
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<td></td>
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<tr>
<td>Irritable bowel syndrome (IBS)</td>
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<td>27</td>
</tr>
<tr>
<td>Adhesions</td>
<td>15</td>
<td>16.9</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>10</td>
<td>11.2</td>
</tr>
<tr>
<td>Myoma uteri</td>
<td>7</td>
<td>7.9</td>
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</table>

* All used medication were registered, mostly more than one medication was used.

<table>
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<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Percentage (%)</th>
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<tr>
<td><strong>Duration of pain (in years)</strong></td>
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<td>&lt; 1</td>
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<td>22.5</td>
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<tr>
<td>1 to &lt; 2</td>
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<td>2 to &lt; 4</td>
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<td>15.7</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>38</td>
<td>42.7</td>
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<td><strong>Pain description</strong></td>
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<tr>
<td>Non-continuous</td>
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<td>37.1</td>
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<tr>
<td><strong>Pain localisation</strong></td>
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<td></td>
</tr>
<tr>
<td>Left lower abdomen</td>
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<td>19.1</td>
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<td>Right lower abdomen</td>
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<tr>
<td>Left and right lower abdomen</td>
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<tr>
<td>Other</td>
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<tr>
<td><strong>Pain type</strong></td>
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<tr>
<td>Boring</td>
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<td>Cutting</td>
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<tr>
<td>Cramping</td>
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<td>Burning</td>
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<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>No correlation</td>
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<td>Menstruation</td>
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<td>Meal</td>
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<tr>
<td>Exertion</td>
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<tr>
<td>Voiding</td>
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<tr>
<td>Defecation</td>
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<td>10.1</td>
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<tr>
<td>Stress/tension</td>
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<td>6.7</td>
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<tr>
<td>Other</td>
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<td>42.7</td>
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<tr>
<td><strong>Pain onset</strong></td>
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<td></td>
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<tr>
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<td>45.5</td>
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<tr>
<td>Gradual</td>
<td>44</td>
<td>49.4</td>
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<tr>
<td>Other</td>
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<td><strong>Pain course</strong></td>
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<td>Identical</td>
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<td>7.9</td>
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<tr>
<td>Moderate</td>
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<td>44.9</td>
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<tr>
<td>Severe</td>
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<tr>
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<td>11.2</td>
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<td><strong>Pain frequency</strong></td>
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<td>5 to 6 days / week</td>
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<tr>
<td>&lt; 2 days / week</td>
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</tr>
<tr>
<td>Unknown</td>
<td>14</td>
<td>15.7</td>
</tr>
</tbody>
</table>

* More than one correlation could be present.

Urology

Urine analysis, including urine culture of all women, revealed no abnormalities. Twenty-four women (27%) had a
A sense of urgency when needing to urinate; 15 (17%) had urge-incontinence. A sense of hesitation was reported by 16 women (18%) and dysuria by 22 (25%). Recurrent bladder infection was reported by 28 women (32%). The frequency-volume chart showed an abnormal urine volume in 23 women; 45 (51%) reported a urine frequency of at least eight times/day. In 58 women (65%) no abnormalities could be detected during urological evaluation. Dysfunction of the musculoskeletal pelvic floor was the most diagnosed etiology in 24 women (27%) based on uroflowmetry; a combination of urine flow, a striking abnormal flow pattern, and volume. Other detected urologic abnormalities included overactive bladder (n = 3).

**Gynaecology**

Dyspareunia was the most reported abnormality by 48 respondents (54%) and dysmenorrhea by 24 (27%). One woman had a positive culture for chlamydia trachomatis, while two had a candida infection, and all were treated. In 36 women (40%), no abnormalities were detected during gynaecological evaluation, while musculoskeletal pelvic floor dysfunction and provoked vulvodynia were diagnosed in 29 (33%) and 19 women (21%), respectively. Other gynaecological abnormalities included endometriosis (n = 4), myoma uteri (n = 4), adenomyosis (n = 3), and other abnormalities (n = 2).

**Gastro-enterology**

Constipation was reported by 58 women (65%), followed by nausea, diarrhea, and heartburn in 21, 20, and 13 women, respectively. In 13 women (15%) no abnormalities could be detected during evaluation. Fifty-one women (57%) were diagnosed with IBS according to the Rome II criteria [20]. Other detected pathology included peptic ulcer (n = 6), diverticulosis (n = 2), inflammatory bowel disease (n = 1), and colorectal cancer (n = 1).
Psycho-sexology

A history of sexual and/or physical abuse was reported by 50 women (56%); 28 (32%) reported affective deprivation, physical/verbal abuse or neglect, and 18 women (20%) reported domestic violence or assault, while 11 (12%) reported both. A history of childhood or adult sexual abuse was reported by 38 women (43%). Rape of violation was reported by 31 women (35%). The combination of sexual abuse and physical or emotional abuse was reported by 20 women (23%). Support, counselling, and therapy were provided to 50 women (56%).

Dyspareunia was reported by 73 women (82%); profound, superficial, and combined in respectively 63, 59, and 49 women. Vulvodynia, based on characteristic findings in history and gynaecological examination was diagnosed in 29 women (33%). Thirteen women (15%) reported to have no sexual relations. Decreased desire for sexual activity was reported by 43 women (57%) and decreased or impaired excitement by 38 (50%) women. Pelvic pain after or during intercourse was reported by 48 women (54%); after orgasm by 40 (45%) women.

The SCL-90-R scores of 82 women (92%) of this study group are presented in Table 4. All the dimensions of the SCL-90 and the GSI, the degree of psychological distress impairment, were all significantly elevated compared to a general female and chronic pain population.

Treatment proposal

The multi-disciplinary diagnosed etiologies were IBS in 51 women (57%), followed by pelvic floor musculoskeletal dysfunction in 50 (56%), and physical and/or sexual abuse in 50 women (56%). The other etiologies are presented in Table 5. Fourteen women (15.7%) were referred for further analysis or surgical treatment.

The majority of women, 51 (57%), received a combination therapy, 29 (33%) received mono-therapy, while five (6%) women were considered untreatable. The most provided treatment proposal included counselling or psychotherapy in 52 women (58%), followed by pelvic floor physiotherapy in 51 (57%), and pharmacotherapy in 50 (56%). The other proposals are shown in Table 5.

Discussion

This prospective study reports the epidemiology of women with CPP, concentrating on the baseline demographic and clinical variables, evaluated by a pragmatic and clinically-fixed protocol. Questionnaires were the first step in the evaluation.

The median pain duration was 36 months while 43% had pain for at least four years and 81% at least three days a week, a group with long lasting discomfort. Before consultation, 74% of the women were evaluated because of CPP, while 71% underwent a variety of surgical interventions without revealing a definitive cause for their pain.

When pain is long-lasting, it becomes a disease with its own physiopathology, involving multiple systems, leading to psychological impairment [8]. A thorough evaluation is advised as unrecognized or undetected abnormalities can be present, even in women previously evaluated. However, abnormalities may be coincidental rather than causal or secondary.

The final multidisciplinary diagnosis and treatment advice was generated, based on detailed evaluation of the patient and identification of all possible factors. The most diagnosed etiologies were IBS in 57%, pelvic floor musculoskeletal disorders in 56%, and psychosexual dysfunction in 56%. Treatment aims to stop or reduce the severity of pain and exacerbations. Opioid analgesics should generally be discouraged due to the risk of dependence. Other pharmacological agents include (combined) oral contraceptives, laxatives, and anti-depressants.

Surgery can be used as a diagnostic tool but only after consultation and evaluation by different specialists [21-23]. Laparoscopy does not appear to affect either pain symptoms or quality of life at long term [23, 24]. There is still no consensus in the role of adhesions in generating CPP; they constitute a very common finding [25]. Hysterectomy is often performed but almost 40% will have persistent and three to five percent worsening of pain [3]. Treatment of anxiety and depression in women with CPP improves the quality of life [26]. Pelvic floor training is effective, resulting in significant relief and improvement [27, 28].

CPP is not a diagnosis but a description of a long-lasting condition; the single most common indication for referral to the gynaecologist [3, 11, 21]. The reported prevalence of CPP varies according to several variables, but the rate is similar to that of asthma, migraine headaches, and chronic back pain [1, 29, 30]. Women with CPP are mostly managed by primary care physicians and only 30%-40% are referred for further evaluation [1, 9, 11].

Women were individually analysed by all team members for several reasons. First, exploration of the medical history is crucial and of the upmost importance, mostly being more indicative than several diagnostic investigations [11]. Second, the etiology of CPP is often complex with presence of associated disorders. The combination of medical history combined with multidisciplinary examination rules out gross pathology and can prevent unnecessary diagnostic and invasive interventions [31]. Finally, the physician-patient relationship is positively influenced, which encourages advice and treatment compliance.

The diagnostic label a women receives depends on various factors, including age, symptoms, tract involvement, presentation, result of performed evaluation, and investigations [21]. A complex interaction between different factors exists and treatment of only some of them will lead to incomplete relief and frustration of both patient and clinician [11, 32]. In line with other reports, the most frequently reported etiologies in the present cohort were non-gynaecologic, while most women were referred to a gynaecologist for evaluation [5, 11, 31]. The results obtained by a multidisciplinary approach are significantly better compared to traditional treatment by a gynaecologist alone [33].

CPP is related to low-self-esteem, physical, sexual, and emotional abuse, domestic violence, low marital satisfaction, anxiety, depression, and somatic symptoms with a
high correlation between anxiety and depression in the same woman [5, 26, 34, 35]. It is unclear whether pain, depression, and anxiety are related to the specific diagnosis of CPP or if they better correlate to the presence of a chronic secondary illness.

Women with CPP have an increased level of psychological impairment/distress as shown by SCL-90 and by the GSI, and compared to a normal female and chronic pain population, the degree of psychological suffering is significant elevated (Table 4). Medical specialist cannot be expected to conduct a thorough psychological evaluation. However, they have an important role in identifying women who may benefit from psychological assessment and treatment [11].

Endometriosis, generally associated with cyclic symptoms, is considered a different entity with specific diagnostic and therapeutic strategies, although it was diagnosed in four women. Interstitial cystitis has intentionally not been diagnosed, as it is a syndrome of unknown etiology without pathognomonic diagnostic findings [36, 37]. A systematic review did not demonstrate apparent differences between multi-treatment modalities and placebo [38]. As such, this diagnosis is not particularly helpful in women with CPP.

Women with CPP are generally recognized as difficult to evaluate, diagnose, and treat, mainly because of the complexity and the different components of the condition [23]. Women are often referred because they are dissatisfied with provided care and feel dismissed [21, 39-41]. CPP is a costly condition; in addition to the frequent use of healthcare resources, 15% of women report absence from work, while 45% report decreased productivity [1]. The treatment of women with CPP should focus towards restoring normal function and control of pain, minimizing disability and enhancing quality of life [31, 40].

The present study has several strengths and from a clinical point of view, important implications. This is the first prospective study in which the epidemiology of women with CPP is systematically reported. Potential components, including psycho-social ones related to the onset, maintenance, and clinical course of CPP were analysed in the evaluation with validated instruments. However, interpretations of these findings cannot be generalised to all women with CPP because the study was conducted in a highly-selected population.

Conclusion

CPP is a debilitating condition among women with a considerable impact on quality of life and is a result of a complex interaction between multiple factors. Individuals with CPP have a long history of pain, psychiatric suffering, decreased productivity, and diagnostic evaluations. Identification of relevant components of CPP by an integrated approach leads to a better evaluation compared to analysis by individual specialists alone. Treatment is mostly not curative and achievement of a higher quality of life despite persisting pain should be the goal; managing rather than curing. Further research is necessary to establish the relationship between demographic, clinical, and pain variables and long-term outcome.

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References

Chronic pelvic pain: evaluation of the epidemiology, baseline demographics, and clinical variables via a prospective and etc.


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Comparison of the classic TVT and TVT-Secur

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Summary

Background and aims: Tension-free vaginal tape (TVT) is a well-established surgical procedure for the treatment of female stress urinary incontinence (SUI) and TVT-Secur was designed to reduce the undesired complications and to minimize the operative procedure as much as possible. Aim: To present the authors’ experience in using the classic TVT and TVT-Secur and to evaluate and compare complications and short- and long-term results. Materials and Methods: A retrospective study and analysis of 230 patients presented with SUI at King Abdulaziz University Hospital (KAUH) and United Doctor Hospital (UDH) from March 1, 2007 until July 3, 2010. Classical TVT and TVT-Secur with or without associated operation were performed. All patients were controlled at six months and complications, as well as objective results, have been reported. The study was approved by ethical committee of KAUH. Results: All patients with SUI admitted to KAUH and UDH for sub-urethral tape were analyzed (230 patients); 149 had classical TVT and 81 had TVT-Secur. Their age ranged from 30 years to 73 years with a mean of 49.8 years and std of 9.4. Their parity ranged from two to 15 with a mean of 6.2 and std of 2.4. One hundred eighty patients had SUI and 50 patients had mixed incontinence. The type of anesthesia used was general anesthesia in 69.6% (160) of cases and regional anesthesia in form of epidural or spine in 30.4% (70) of cases. Operative complications revealed a bladder perforation in 3.5% (eight) of cases and 2.2% had bleeding of more than 200 ml, and 53 patients which contribute to 23% had retention and need for catheterization. Conclusion: The classical TVT and TVT-Secur were found to be very effective, easy, and safe procedures and with excellent results.

Key words: TVT; TVT; Secure urinary stress incontinence; Sling procedures.

Introduction

Urinary incontinence is involuntary leakage of urine [1]. It is a common problem among adult women; the overall prevalence of 40% and between six to ten percent of women with severe incontinence. It is well-known that urinary incontinence is more common in women than in men [2]. Stress urinary incontinence (SUI), being the most common type of urinary incontinence in women, is due to insufficient strength of the pelvic floor muscles. It is defined as the complaint of involuntary leakage of small amount of urine as a result of increased intra-abdominal pressure and thus increased pressure on the bladder due to effort, exertion, sneezing or coughing [1-3].

Tension-free vaginal tape (TVT) is a well-established surgical procedure for the treatment of female SUI. It was first described by Ulmsten in 1996, which is based on a mid-urethral tape support, which is accepted as effective and safe surgical technique [4-6].

Bladder penetration, urinary outlet obstruction, potential bowel penetration, intraoperative bleeding, and postoperative infections are known complications of the classical TVT [5-13]. TVT-Secur was designed to reduce the undesired complications and to minimize the operative procedure as much as possible. This device is composed of an eight-cm long polypropylene mesh and is introduced by a metallic inserter, while no exit skin cuts are required [5].

The aim is to present the authors’ experience of using a minimally invasive sub-urethral tape in form of either the classic TVT, and TVT-Secur and to evaluate and compare complications and short- and long-term results.

Materials and Methods

A retrospective study of 230 patients suffering from SUI had TVT or TVT-Secur procedures performed at King Abdulaziz University Hospital (KAUH) and United Doctor Hospital (UDH) from March 1, 2007 until July 3, 2010 were analyzed.

Inclusion criteria were: urinary incontinence symptoms with no intrinsic sphincteric deficiency, based on subjective complaints, objective clinical signs, and confirmed in some cases with urodynamic diagnosis including cystometry, uroflowmetry, and stress test. An age of at least 30 years and patients desiring surgical correction of SUI. The exclusion criteria were: postvoid residual volume > 100 cc and desired future childbearing. History of bleeding diathesis or current anti-coagulation therapy, current genitourinary fistula or urethral diverticulum, reversible cause of incontinence (i.e. drug effect), and contraindication to surgery.

All the procedures were performed after receiving consents from the patients, inform them that tape would be positioned to elevate the bladder. The type of operation and whether TVT or TVT-Secur was to be utilized were selected according to the surgeon’s preference and experience. All patients were given prophylactic antibiotics and were subjected to an iodine antisep tic vaginal wash prior to commencement of the operation.

The mode of anesthesia depended on patient request and the surgeon’s preference. Foley catheter was placed in all cases.
and cystoscopy was performed in all patients that underwent classical TVT but not TVT-Secur. Patients presenting with significant cystocele or rectocele were managed with anterior or posterior colporrhaphies (anterior and posterior) as required.

Intraoperative and postoperative complications were recorded. All patients were personally contacted through interview after six months of the operation and then before writing this paper. Failure was defined as persistent complaints of SUI reported by the patients and then clinically confirmed that it conditioned the quality of life. Minimal residual leakage, not deteriorating the patient’s quality of life as reported by the patients, was not considered as therapeutic failure. The study was approved by ethical committee of KAUH.

The Statistical Package for the Social Sciences (SPSS) 15.0 software was used to analyze data using t-test, chi-square test, and K independent sample (Kruskal-Wallis Test) were used when appropriate. A p value of < 0.05 was considered to be statistically significant.

Results

Out of 230 patients, 149 had classical TVT and 81 had TVT-Secur and their age ranged from 30 to 73 years, with a mean of 49.8 years and std of 9.4. Their parity ranged from two to 15 with a mean of 6.2 and std of 2.4.

One hundred eighty patients had SUI and 50 had mixed incontinence. Thirty percent of patients had past medical history in form of hypertension and diabetes and 23.5% had past surgical history. Fifty-eight patients (25.2%) had urinary tract infection treated with antibiotics. Out of 230 patients 175 patients (76.1%) had a confirmed diagnosis of either SUI or mixed by urodynaminc testing.

Table 1 shows the comparisons of the age in years and parity which was not statistically significant. The operative time in minutes, hospital stay in days, and the number of days needed to keep the catheter between the two groups of patients who had classical TVT and those who had TVT-Secur using t-test were statistically significant (p = 0.001).

One hundred forty-eight patients had either classical TVT or TVT-Secur without concomitent gynecological surgery and 54 patients underwent anterior and posterior repair and only 28 patients underwent posterior repair Table 2.

Out of 230 patients, 161 had no complications and 53 patients had voiding difficulties that required prolonged catheterization; most of this type of complication occurred in the group who had classical TVT. The frequency of complications were not statistically significant with (p = value 0.05) between the group who had Classical TVT and TVT-Secur but the type of complication were different (Table 3).

The complications were more common in patients who had concomitant surgery than patients who had TVT alone and this was statistically significant (p = 0.001) Table 4.

Discussion

The mid-urethral slings like TVT, TVT-Obturator (TVT-O), and TVT-Secur became very popular procedures among surgeons specializing in female pelvic reconstructive techniques and had gained experience in treating SUI. These procedures are simple and have excellent results.
The experience at the present institution suggest that TVT And TVT-Secur are easy to master and minimally invasive with respect to tissue handling. It had been reported by Rackley et al., that complications and surgical outcomes were similar to the present results and found that patient selection was important to minimize the potential morbidity, avoid patient’s mortality, and produce a high-rate of durable success [14].

Neuman reported the complications and early follow-up of TVT and TVT-Secur. TVT-Secur was associated with early safety and efficacy problems. Intraoperative complications associated with the TVT, such as bladder penetration and postoperative complications, such as thigh pain and bladder outlet obstruction, may be reduced with TVT-Secur [15]. He also reported a comparison of two anti-incontinence operations: TVT and the TVT-O. The surgeons’ learning curves of these two minimally invasive surgical procedures for the treatment of female SUI was comparable. The safety and cost-effectiveness of TVT are well-established. TVT-O, was designed to overcome some of TVT-related operative complications. TVT-O patients seem to have less intraoperative and postoperative complications than the TVT patients. However, long-term comparative data collection is required prior to drawing solid conclusions concerning the superiority of one of these two operative techniques [16].

Tommaselli et al., in their study to reduce complications of transobturator TVT, single-incision devices were introduced in the last years. A comparison between TVT-O and TVT-Secur techniques in terms of efficacy and safety, showed no differences in terms of cure rate between the two groups (81.6% vs 83.8%). Complication rate in the TVT-secur group was lower (8.1%) than in the TVT-O group (15.8%), but not significant. So both techniques seem to be effective and safe, with a low incidence of complications in both groups [17].

In another study by Oliveira et al. to evaluate the short-term surgical complications and results of a TVT system and TVT-Secur, in the treatment of SUI, it concluded that TVT-Secur is a simple and safe treatment for female SUI, but before recommending this sling as a first choice for treating SUI, TVT-Secur must pass the test of time and comparative studies with conventional slings [18].

Conclusion

The classical TVT and TVT Secur were found to be very effective, easy, and safe procedures and with excellent results. The complications were found to be more in patients who had concomitant surgery of the TVT.

References


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Expression of regulatory T and helper T cells in peripheral blood of patients with pregnancy-induced hypertension

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Summary

Objective: To analyze the expression of regulatory T cells and helper T cells in peripheral blood of patients with pregnancy-induced hypertension (PIH). Materials and Methods: Twenty-seven patients hospitalized with PIH were consecutively collected for detection of regulatory T cells (CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+ Treg) and helper T (CD+3, CD+4, CD+8, CD+4/CD+8) cells in peripheral blood. Meanwhile, 20 normal hospitalized pregnant women served as the control group. Results: In the comparison of regulatory T cells, the level of serum CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+ Treg in PIH group was significantly lower than control group (all p < 0.05). In the comparison of help T cells, the expression level of serum CD+4/CD+8 in PIH group was obviously higher than control group, while the expression level of CD+8 was significantly lower than control group (all p < 0.05). Conclusions: There were obvious abnormal expressions of regulatory T cell and helper T cells in peripheral blood of patients with PIH.

Key words: Pregnancy-induced hypertension; Regulatory T cells/peripheral blood; Helper T cells/peripheral blood.

Introduction

Pregnancy-induced hypertension (PIH) is a disease occurring in late pregnancy. In severe cases, there may be fetal growth retardation, maternal placental abruption, premature birth, and postpartum hemorrhage, which is one of the main causes leading to the death of pregnant women and perinatals in China. Immune factors have a close relation to the onset of PIH. In recent years, according to the study involving maternal immune process in gestation, regulatory T cells were gradually recognized as the regulator of Th1 and Th2 cells [1-4]. CD4+ CD25+ Treg is a unique subtype of CD4+ T [5], of which the main function is to inhibit the autoreactive T cells from immune response, the activation of conventional T cells, and to promote the secretion of inhibitory cytokine, as well as to preserve the homeostasis of the body and induce tolerance to grafts. CD4+ CD25+ Foxp3+ Treg are the Foxp3 transcription-factor of X chromosome which is necessary for the development, growth, and function of CD4+ CD25+ Treg. Expression of CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+ Treg in the maternal peripheral blood during various stages of pregnancy plays an inhibitory effect on maternal immunological rejection to a semi-allogeneic fetus during the dominant control of fetal-maternal immune. Under normal conditions, the absolute number of CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+ Treg in peripheral blood during pregnancy increases and dynamically changes. The preservation of normal pregnancy depends on the stability of the immune balance, which once has been broken, pathological pregnancy will occur. According to recent findings [6, 7], the onset of PIH was closely-related to the imbalance of maternal immune, although there are few researches or reports addressing regulatory T cells in peripheral blood of PIH patients. In this research, the expression of regulatory T cells and helper T cells in the peripheral blood was studied to determine the possible immune mechanism in PIH.

Materials and Methods

Twenty-seven patients with a systolic blood pressure ≥ 140 mm Hg and a diastolic blood pressure ≥ 90 mm Hg or urine protein from - to ++++ after 20 weeks gestation in the present obstetrics department from January 2009 to December 2009 were consecutively selected for PIH. This study was conducted in accordance with the declaration of Helsinki and approved from the Ethics Committee of the Fourth Affiliated Hospital of China Medical University. Written informed consent was obtained from all participants. Exclusion criteria included: patients recently suffering from acute and/or chronic infectious diseases, patients with autoimmune diseases, patients with reproductive tract infections which was confirmed by TORCH, chlamydia and mycoplasma examination, and patients suffering from liver, kidney, and systemic blood diseases. Twenty healthy pregnant women hospitalized simultaneously were selected as the control group. Descriptive statistics about patients and control groups were summarized in Table 1 with similar mean age and mean gestational age (all p > 0.05).

Fasting cubital venous blood was obtained, centrifuged at 2,500 r/min for ten min to separate the serum and stored at -70°C. Type FC-500-MPL of flow cytometry was utilized to detect regulatory T cells (CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+ Treg). Indirect immunofluorescence was used to determine the level of regulatory T cells in peripheral blood (CD3+, CD4+, CD8+, and CD4+/CD8+). SPSS 10.0 was adopted for data analysis. Data were expressed as mean ± SD. T-test was used for comparison between groups. A p < 0.05 was considered statistically significant.
Table 1. — General information of the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age in years</th>
<th>Mean age in years</th>
<th>Gestational age in weeks</th>
<th>Mean gestational age in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHG (n = 27)</td>
<td>25 - 39</td>
<td>29.12 ± 5.39</td>
<td>36 - 40</td>
<td>37.91 ± 3.66</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>24 - 37</td>
<td>28.64 ± 4.72</td>
<td>35 - 39</td>
<td>38.23 ± 3.25</td>
</tr>
</tbody>
</table>

Table 2. — The comparison of the expression levels of peripheral blood CD3, CD4, CD8, and CD4/CD8 between the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHG (n = 27)</td>
<td>66.28 ± 9.34</td>
<td>35.62 ± 4.53</td>
<td>21.80 ± 3.25</td>
<td>1.78 ± 0.25</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>67.79 ± 10.55</td>
<td>36.17 ± 4.88</td>
<td>27.72 ± 3.64</td>
<td>1.39 ± 0.17</td>
</tr>
</tbody>
</table>

Table 3. — The comparison of the expression levels of peripheral blood CD4+CD25+, Treg, and CD4+CD25+Foxp3+Treg between the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4+CD25+ Treg</th>
<th>CD4+CD25+Foxp3+Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHG (n = 27)</td>
<td>9.06 ± 2.56</td>
<td>2.27 ± 0.85</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>14.82 ± 3.35</td>
<td>3.98 ± 1.26</td>
</tr>
</tbody>
</table>

Results

Expression levels of peripheral blood

The comparison of the expression levels of peripheral blood CD3, CD4, CD8, and CD4/CD8 between two groups: the results suggested that the expression level of peripheral blood CD4/CD8 in PHG group was higher than control group, while the expression level of CD8 was lower than control group (all p < 0.05) (Table 2).

Expression levels of peripheral blood

The comparison of the expression levels of peripheral blood CD4+CD25+ Treg, and CD4+CD25+Foxp3+Treg between two groups: the expression levels of peripheral blood CD4+CD25+ Treg and CD4+CD25+Foxp3+Treg were significantly lower than control group (all p < 0.01) (Table 3).

Discussion

Immune factors have a close relation to the onset of PHG. According to the recent findings [8-10], regulatory T cells played an important role in the balance between the regulation of human peripheral immune tolerance and response to the immunological stress caused by infection. It is well known that pregnancy induces enhancement of immunosuppression to ensure the stable growth of the fetus. Numerous Foxp3 related factors played an important role as immunosuppression factor [11-13]. According to other studies, CD4+CD25+ Treg played an important role in pregnancy maintenance [14, 15]. It was also confirmed that its expression was enhanced during normal pregnancy implying the immunosuppressive effect of T cells for the preservation of pregnancy [16-18]. Therefore, it was proposed that the onset of PHG was closely related to the disruption of maternal immune balance during pregnancy. The results in this study showed that the expression level of serum CD4+CD25+ Treg, CD4+CD25+Foxp3+Treg and CD8 was significantly decreased while the expression level of CD4/CD8 significantly increased in PHG group in line with other reports [6, 9]. Treg cells suppress the response of immune system to its own and foreign antigens mainly through the “active” way, but the amount and functional changes of CD4+CD25+ Treg in patients with PHG still remain unknown. Previous studies [19, 20] reported that the number of CD4+CD25+ Treg cells in peripheral blood of PHG patients was significantly decreased when compared with normal pregnancy or normal non-pregnant women, suggesting that the decreased expression of Foxp3 in PHG women was probably related to the reduction of CD4+CD25+ Treg cells’ number. After further analysis, it was found that T lymphocytes cells of these patients that were activated, followed the lack of regulatory cells, especially reducing Treg cells leading to maternal immune rejection towards the fetus. It is believed that the significant decrease of Treg cells in PHG patients affecting the immunomodulatory in the third trimester, prompted a shift in the Th1/Th2 balance from Th2 to Th1 and disrupted maternal-fetal immune tolerance, resulting in decreased immunosuppressive protection from embryonic antigen and embryonic susceptibility to immune attack. Therefore, a series of pathophysiological changes occurred including the onset and progression of PHG.

In conclusion, Treg cells, which are important immunoregulatory cells, have the effect of inducing maternal immune tolerance and preserving internal environment stability. There are significantly lower expressions and absolute amounts of CD4+CD25+ Treg in peripheral blood in pregnant women, which might be one of the causes of PHG. It is believed that producing more CD4+CD25+ Treg cells via different ways and the balance between regulatory T cells and effector T cells may become a new option for the treatment of PHG.

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Expression of regulatory T and helper T cells in peripheral blood of patients with pregnancy-induced hypertension


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Semi-automatic Sono T measurement of nuchal translucency

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Summary

A prospective study of 63 singleton pregnancies between 11 + 0 and 13 + 6 weeks gestation underwent semi-automatic nuchal translucency (NT) measurement and were compared with two-dimensional ultrasonography (2D US). Inter-observer variation and the repeatability were evaluated. Sono T automatically achieves mid-sagittal plane views and measures the maximum NT thickness. Measurements have less inter-observer variation (CI = -0.13, -0.04) when compared with 2D measurements (CI = -0.45, 0.28). It is reproducible and comparable to conventional 2D US technique for NT measurement. However, incorporating Sono T into routine practice requires further program refinements in order to reduce erroneous NT measurements.

Key words: 2D/3D; HDlive US; Semi-automatic Sono T; Nuchal translucency measurements.

Introduction

Nuchal translucency (NT) measurement, detection of presence or absence of nasal bone, and evaluation of the characteristics of vascular flow in the ductus venosus, are highly sensitive screening tools for trisomy 21, for other major chromosomal defects, for congenital structural anomalies, for heart defects, and for adverse pregnancy outcome that results from other etiologies [1].

Using properly-measured NT alone allows prenatal detection of over 70% of cases of trisomy 21. Using NT in combination with maternal serum alpha-fetoprotein (AFP), pregnancy-associated plasma protein A (PAPP-A), and free beta-human chorionic gonadotropin β-hCG, provides efficient Down’s syndrome risk assessment, with a detection rate of 80%-87% (five percent false-positive rate), and also allows earlier diagnosis of fetal aneuploidies [2, 3].

NT measurement is well-standardized for two-dimensional ultrasonography (2D US) [2, 4]. Errors in measurement may have a significant effect on risk assessment.

To improve reliability and to avoid errors, new US measurement modes such as:

– three/four dimensional (3D/4D) surface [5-18],
– volume calculation with virtual organ computer-aided analysis (VOCAL),
– automated volume count (AVC) [19, 20],
– semi-automatic systems [1, 21-28] and
– HDlive [29, 30] (Figure 1) have been tested.

Volume measurement of the nuchal area has been reported [19, 20] and provides more detailed information when the shape of a target object, such as an hygroma colli, is irregular on a 2D image [20].

Only a small number of studies [1, 8, 24, 25, 27, 28] have been reported on the potential benefits of using a semi-automated approach in NT measurement. Six of the references are scientific papers, all with a small sample size, and one is an editorial [26]. All of them indicate that the experience is too small and it is not possible to recommend its use.

The aims of this study were: to evaluate the clinical usefulness of semi-automated distances using a 3D Sono T software and to establish if the measurements using either 2D or Sono T have significant differences, in order to justify a high-economic inversion with the new software.

Materials and Methods

2D and 3D NT mid-sagittal measurements were performed in 63 patients with normal singleton pregnancies at gestational ages between 11 weeks and 13 weeks + six days. 2D US and Sono T software were then employed to calculate the maximum NT width. All measurements were acquired trans-abdominally.

Although the sample size is small (as the other publications), it is mathematically sufficient. This investigation obtained the approval from the Ethics Committee from the “Fundación para la Investigación del Hospital Clínico Universitario de Valencia, (Spain)”. All patients signed informed consent.

Semi-automatic measurements were performed using the Sono NT function in a mid-sagittal section determined by conventional 2D US. The operator placed the region of interest (ROI) in the most representative section of the nuchal area. The upper calliper was located on the inner border of the upper echogenic line and the lower calliper was placed on the inner border of the lower echogenic line (on-to-on measurement). The maximum vertical distance was automatically selected (Figure 1) [25].

Abnormal fetuses with enlarged NT and fetuses in the prone position were excluded from the initial enrolment.

In each one of these, the authors measured NT in mm by one operator, using 2D (NT1) and Sono T software (NT2). Manual measurement of NT was performed according to the Fetal Medicine Foundation (FMF) guidelines [4].

Statistical analysis

The repeatability of the observations provided by both operators was compared by calculating the 95% ranges of agreement over the differences [31]. This measurement is used by the British Standards Institution [32] to define the repeatability coefficient. Likewise, the point estimate of this difference and the 95% con-
confidence interval was calculated. This method was applied for NT measurements (NT1 vs NT2). Measurements were compared with values of the FMF. All calculations were made with the Statistics R, version 2.12.2 software [33].

Results

Two measurements, one of the NT in mm using 2D (NT1), and one using Sono T software (NT2), were carried out from observations on 63 patients.

Figure 2 shows NT1 (2D) and NT2 (Sono T) measurements with a confidence interval of 90%, according to the values of the FMF [4].

Figure 3 shows the differences between NT1 (2D) and NT2 (Sono T) with respect to the percentile 50 of FMF. The authors conclude that both technique measurements are not significantly different from percentile 50 of FMF.

As can be seen in Figure 3, there is an association between the two measurements since Pearson’s correlation is r = 0.9. The measurement of differences between the techniques was a calculation of a range where disagreements occurred in 95% ranges of agreement [27, 28]. With more than 50 observations, it was based on the mean of the observed differences (d) and the standard deviation of these differences (s_diff). Defined as d ± 1.96·s_diff. In this case, the interval obtained [-0.45, 0.28] indicates no significant differences between two measurements.

The confidence interval for the values (d, [d ± s_diff/√(n)]) is [-0.13, -0.04], which indicates that there is a bias in the measurements of both operators. This means that a 2D technique with an interval of -0.024 provides values that are significantly lower than the values obtained with Sono T, with a +0.06 interval.

Discussion

Unfortunately, fetuses are not always properly positioned for technically adequate NT measurements (only
Semi-automatic Sono T measurement of nuchal translucency

10%-20% with the standard 2D abdominal or vaginal, approaches) [6]. Sonographers spend valuable time waiting (often unsuccessfully) for the fetus to move into an optimal position [19]. Moreover, when measurements obtained with 2D/3D have been compared, it has been observed that the 2D observations were often not realized in the optimal plane [6, 9].

In order to improve NT measurements, other technologies have been used:

The introduction of 3D US measurements created high expectations. Data from two decades were used for differential diagnoses between NT and hygroma colli [10, 11]. Later on, measurements between 2D and 3D were compared and values were attached to inter- and intra-observer visualization and reproducibility [11].

Referring to the semi-automatic methods, they have also been reported years ago [21, 22] and were not incorporated in the software of ultrasound machines. These methods are based on tracing the inner borders of the nuchal membrane, and consequently, they do not avoid the problem of underestimation of NT width associated with increased image magnification.

There are six recent reports similar to these in studies that used Sono T software [1, 8, 24, 25, 27, 28]. There is also one update, a state of the art report that raises many questions [26]. They all suggest that fetal NT measurement might afford some benefits.

Some like Moratalla et al. [1] compare the inter- and intra-observer variability with traditional measurement. Both variables were reduced with the automatic method. The standard deviation of measurement was ten times lower using a semi-automatic compared with a manual method (0.0149 mm vs 0.109 mm), and the semi-automatic method had an extremely high intra-class correlation coefficient of 0.98 mm. Others like Abele et al. [25] conclude that results are much better when obtained by “experts.” They conclude that there is little evidence of any benefit in terms of measurement error variability when compared with manual methods.

A third group, Grangé et al. [24], suggests, curiously, that the only benefit would be obtained when this technology is used by less experienced operators and when they work with images of poorer quality.

Finally, a fourth group [8] comparing the differences between “experts” and “beginners” observed that the differences with 2D were significant but were not with Sono T.
measurements. They recommend, as the present authors do, that Sono T be employed when experienced operators are not available.

Crude errors are generated in these measurements if the ROI box encompasses more of the nuchal area than strictly the margins of NT. It therefore remains operator-dependent [25].

Automatic measurement failed in 18.4% cases (the program was unable to acquire the correct mid-sagittal plane in 13.1% of cases or the caliper was misplaced in 5.3% of cases). [27, 28].

Manual skills are sufficient for reliable and reproducible NT measurements until proven otherwise with other clinical studies.

Widespread use of semi-automatic NT measurements, which is only now taking off as part of many national healthcare guidelines, could also lead to confusion at this critical time, thereby undermining 19 years of effort, exemplary teaching programs, and quality assessment projects [26]. Whether the new technologies Sono NT [24-26], AVC, and VOCAL [27, 28] can replace the current manual 2D methods, and whether the minimal tenths and hundredths of a mm differences in measurements are of interest, are yet to be determined.

Perhaps the new semi-automatic systems that evaluate the maximum distance over a 3D volume will be able to solve this problem [8, 27, 28]. However, the authors have not been able to see any evidence that this will be the case. At this time, these inconveniences stand in the way of universal unanimity in the use of these new 3D modes, since data are not available for them as is the case with 2D methods.

Conclusions

This work supports normal measurements between the gestational ages of 11 and 13 weeks + six days for Sono T as is the case with other reports [1, 8, 24, 25, 27, 28]. It is evident that semi-automatic measurements require further research [26] before definitive recommendations can be made [8, 27, 28]. The initial expectations for 3D US, AVC, and Sono T have yet to be fulfilled [1, 26-28].

References


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mtDNA^{4977} deletion is not a common feature in patients with premature ovarian failure and primary infertility

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Summary

The aim of the current study was to investigate the incidence of mtDNA^{4977} deletion in peripheral blood leukocytes of patients diagnosed with premature ovarian failure (POF) and primary infertility. The study group consisted of 17 patients with POF, 32 women with primary infertility, and 31 fertile women with the prevalence of the mtDNA^{4977} deletion using the reverse transcription-polymerase chain reaction (RT-PCR) based technology. None of the patients affected by POF revealed mtDNA^{4977} deletion. This deletion was detected only in one 26-year-old infertile patient. No significant difference in relation to mtDNA^{4977} deletion was reported between the affected by POF and primary infertility. The occurrence of mtDNA^{4977} deletion in women between 20 and 39 years of age may not increase with increasing patients’ age, independently of their fertility status.

Key words: mtDNA^{4977}; Premature ovarian failure; Primary infertility.

Introduction

In recent years more and more women decide to become pregnant after the age of 35 [1]. Postponing pregnancy causes difficulties in having offspring, mainly as a result of age-related disadvantageous changes. Furthermore, the capacity of oocyte fertilization and the endometrial receptivity decrease [2].

A significant effort has been made to “stop the biological clock” and to preserve fertility in older patients. Unfortunately, the results of these attempts have not yet been satisfactory. Moreover, premature ovarian failure (POF) constitutes nowadays a problem as serious as primary infertility [3].

Mitochondria take part in cellular respiration and their function has a significant influence on the normal functioning of the gamete [4, 5]. Mitochondrial DNA (mtDNA) is a double-stranded chain, which, in humans is 16.6 kb long. Almost each cell in the human body contains around 1,000 mitochondria, and every mitochondrion has two to ten copies of mtDNA. Studies on mtDNA conducted over the last 30 years, have led to the conclusion that anomalies within mtDNA are related with fertility disorders in women [6, 7]. It is possible, that POF may be associated with a decreased oxidative phosphorylation, which is observed in the majority of cells in an aging body [8].

Over 150 types of rearrangements have been found in human mtDNA. The most common deletions are the following deletions: mtDNA^{4977}, mtDNA^{7436}, and mtDNA^{10422} [9]. The mtDNA^{4977} deletion occurs within the limits of the so-called “hot spot” in 8,468 and 13,446 nucleotide positions and is also called “common deletion” [10].

The mtDNA^{4977} deletion causes removal of the following genes: Fo-F1-ATPase (ATPase 6 and 8), cytochrome oxidase (CO III), and oxidoreductase NADH-CoQ, which play a pivotal role in the oxidative phosphorylation and therefore mainly results from mitochondrial function [11]. It has been observed that in women over the age of 38 years, the granulosa cells within the follicles have a lower proportion of mitochondria with normal DNA [12]. It is possible that the age-related loss of mitochondrial function results from deletion or point mutations within mtDNA. Hsieh et al. [11, 13] suggested that some of the mutations within the mtDNA of an oocyte may be responsible for failures in oocyte fertilization. However, to the best of the authors’ knowledge, none of the researchers described the mtDNA^{4977} mutations in peripheral blood leukocytes of women suffering from POF and primary infertility.

The objective of the study was to investigate the incidence of mtDNA^{4977} deletion in peripheral blood leukocytes of patients diagnosed with POF and primary infertility.

Materials and Methods

The study subjects comprised of 17 patients with POF and 32 patients with primary infertility. The control group consisted of 31 age-matched fertile (confirmed by at least one pregnancy) individuals. All participants underwent a complete examination and history, including family diseases, at the Second Department of Gynecology of the Lublin Medical University in Lublin, (Poland). None of them mentioned fertility problems in family anamnesis. Among the fertile patients, 15 of them had one birth, seven had two births, five had three births, and four had a miscarriage. The study was approved by the Ethical Committee of the Medical University of Lublin. Informed consent was collected from all the persons enrolled.

Positive (endometrial cancer with mtDNA^{4977} deletion [14]) and negative (water instead of sample) controls were used in all experiments.
Blood in an amount of two ml was sampled from an antecubital vein in each of the study individuals in the morning after an overnight fasting and was quickly deposited into a plastic tube containing ethylenediaminetetraacetic acid (EDTA). Leukocytes were immediately separated from plasma as the buffy coat in a Ficoll gradient and were immediately forwarded to DNA isolation.

The use of polymerase chain reaction (PCR) Master Mix reduced tube-to-tube difference caused by differences in the amount of enzyme. Molecular probes were used (Table 1); the probe for mtDNA<sup>4977</sup> was labelled with reporter VIC. The probe for mtDNA<sup>6307</sup> was 6-carboxyfluorescein (FAM).

Total DNA from whole blood (5x10<sup>6</sup> leukocytes) was extracted. Following extraction, DNA was quantified and qualified by UV spectrophotometric analysis. Template DNA included 50-100 ng of DNA extracted from leukocytes. Reaction mixtures included 0.25µM forward and reverse primers, 200 nM probe, and 1x PCR Universal Master Mix for a final volume of 50 µl. All experiments were performed under “multiplex” conditions. Primers and probes for both mtDNA<sup>total</sup> and mtDNA<sup>4977</sup> were present in each reaction (Table 1). The real-time PCR reactions were run on a 7300 Real-Time PCR biosystem. Cycling temperatures and times were 50°C for two minutes, 95°C for ten minutes, 95°C for 15 seconds, and 60°C for one minute.

Data were collected and analyzed using 7300 Real-Time PCR System. Data were also normalized to mtDNA<sup>1307</sup> amplified from the cellular sample using the delta comparative threshold cycle (CT) method. The CT value is the parameter used for quantifying the amount of target template in the given reaction well. Delta CT (ΔCT) sample = CT (FAM) – CT (VIC). The PCR-products were purified and separated on a 1.2% agarose gel at 50 V in 1X TBE buffer, and the products were visualized by ethidium bromide staining and photographed. DNA bands of enzyme. Molecular probes were used (Table 1); the probe for mtDNA<sup>4977</sup> deletion using the PCR-based methodology. None of the patients affected by POF revealed pation was noted (p > 0.05) Figure 1 presents a graphical demonstration of real-time PCR data from 13 representative patients – 12 patients without mtDNA<sup>4977</sup> deletion (wells from one to 12), one patients (well 13) with deletion, and a negative control (well 14).

### Results

The current study investigated peripheral blood samples collected from 17 patients with POF, 32 individuals with primary infertility, and 31 fertile women. The characteristics of the study subjects are listed at Table 2. The age matched with no other health problems for the prevalence of the mtDNA<sup>4977</sup> deletion using the PCR-based methodology. None of the patients affected by POF revealed mtDNA<sup>4977</sup> deletion. This deletion was detected only in a 26-year-old infertile patient. No significant difference between groups investigated in relation to mtDNA<sup>4977</sup> deletion was noted (p > 0.05) Figure 1 presents a graphical demonstration of real-time PCR data from 13 representative patients – 12 patients without mtDNA<sup>4977</sup> deletion (wells from one to 12), one patients (well 13) with deletion, and a negative control (well 14).

### Table 1. — Primer sequences used in the experiments.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA1307FOR</td>
<td>5’-GTA CCC AGC TAA AGC CGT TAG G-3’</td>
<td>VIC</td>
</tr>
<tr>
<td>mtDNA1433REV</td>
<td>5’-TAC TGC TAA ATT CAC CTT CG-3’</td>
<td>VIC</td>
</tr>
<tr>
<td>mtDNAdel49778416</td>
<td>TGG CTT TGG AGT AGA AAC C-3’</td>
<td>FAM</td>
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<tr>
<td>mtDNAdel49778542</td>
<td>TGCG CGT AGT AGA AAC C-3’</td>
<td>VIC</td>
</tr>
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</table>

### Table 2. — Clinical characteristics of the patients with POF, primary infertility, and the control group.

<table>
<thead>
<tr>
<th>Number of individuals</th>
<th>Patients with POF</th>
<th>Patients with primary infertility</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Mean ± SD</td>
<td>30.14 ± 5.32</td>
<td>31.44 ± 3.89</td>
<td>31.52 ± 4.02</td>
</tr>
<tr>
<td>Median</td>
<td>31</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>Menarche (years) Mean ± SD</td>
<td>14.50 ± 1.09</td>
<td>13.85 ± 1.54</td>
<td>13.28 ± 1.17</td>
</tr>
<tr>
<td>Median</td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>13 – 16</td>
<td>10 – 17</td>
<td>11 – 16</td>
</tr>
<tr>
<td>Height (cm) Mean ± SD</td>
<td>163.13 ± 7.57</td>
<td>164.30 ± 6.36</td>
<td>165.57 ± 5.46</td>
</tr>
<tr>
<td>Median</td>
<td>163</td>
<td>164</td>
<td>164</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>154 – 171</td>
<td>150 – 179</td>
<td>156 – 177</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;) Mean ± SD</td>
<td>22.83 ± 4.12</td>
<td>22.18 ± 4.05</td>
<td>19.19 ± 1.83</td>
</tr>
<tr>
<td>Median</td>
<td>22.5</td>
<td>21.27</td>
<td>19.29</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>17.60 – 34.29</td>
<td>15.99 – 33.22</td>
<td>14.24 – 21.51</td>
</tr>
<tr>
<td>Plasma β-estradiol (pmol/l) Mean ± SD</td>
<td>32.02 ± 15.28</td>
<td>81.85 ± 53.95</td>
<td>63.31 ± 12.58</td>
</tr>
<tr>
<td>Median</td>
<td>20</td>
<td>81.85</td>
<td>38.40</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>1.87 – 66.20</td>
<td>43.70 – 120.00</td>
<td>20.00 – 52.90</td>
</tr>
<tr>
<td>FSH Mean ± SD (IU/l)</td>
<td>45.98 ± 28.82</td>
<td>6.59 ± 2.23</td>
<td>6.39 ± 1.84</td>
</tr>
<tr>
<td>Median</td>
<td>39.9</td>
<td>6.16</td>
<td>6.51</td>
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<tr>
<td>LH Mean ± SD (pmol/l)</td>
<td>20.91 ± 18.677</td>
<td>6.36 ± 3.59</td>
<td>5.99 ± 2.36#</td>
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<tr>
<td>Median</td>
<td>15.10</td>
<td>5.04</td>
<td>6.10</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>1.00 – 77.20</td>
<td>3.50 – 12.90</td>
<td>2.75 – 9.5</td>
</tr>
<tr>
<td>FSH/LH Mean ± SD</td>
<td>2.80 ± 1.94</td>
<td>1.08 ± 0.45</td>
<td>1.08 ± 0.58*</td>
</tr>
<tr>
<td>Median</td>
<td>2.15</td>
<td>1.09</td>
<td>0.84</td>
</tr>
<tr>
<td>Minimum – maximum</td>
<td>1.17 – 8.68</td>
<td>0.48 – 1.71</td>
<td>0.61 – 2.37</td>
</tr>
</tbody>
</table>

<sup>p < 0.05; p = 0.01; p = 0.001; ∗ measured at the second day of the follicular phase. BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone.</sup>

### Discussion

POF is a frequently occurring condition. The prevalence of POF in women below 40 years of age is one to two percent and in those below 30 years of age is 0.1%. It leads to the absence of menstrual period, hypoestrogenism, and elevated levels of gonadotropins. It has been observed that POF occurs in 10%–28% of women suffering from primary amenorrhea and in 4%–18% of those with its secondary form [15, 16]. It is worth mentioning that a major component of this disorder may remain unsolved as a result of low awareness among women who do not consider a loss of menstruation before the age of 40 to be a serious medical condition requiring gynecological consultation. Other causes of the lack of monthly menstruation, such as pregnancy, hyperpro-
mtDNA4977 deletion is not a common feature in patients with premature ovarian failure and primary infertility

Lactinemia occurring due to the drug-induced or spontaneous diminution of the dopaminergic hypothalamic activity, or because of adenomas of the pituitary gland, thyroid dysfunction, and POF, have to be excluded. Women should be checked for POF when amenorrhea persists for at least three to six months, and when the level of FSH exceeds 40 mIU/ml in at least two tests separated by at least a couple of months. Intermittent ovarian function must be excluded, as it gives similar symptoms, such as hypoestrogenism (less than 50 pg/ml) and high gonadotropins levels, along with the absence of follicles or loss of their function [16, 17]. The loss of the ability to conceive is mainly a result of the absence of ovarian follicles, or, less frequently, the fact that the existing follicles are unable to respond to stimulation.

The present study aimed to establish the frequency of mtDNA4977 deletion in patients with POF and primary infertility in comparison to healthy women. Furthermore, as the normal structure of the cell membrane is lost, the damaged mitochondria may release proteins that induce apoptosis, such as cytochrome C [18]. These phenomena have been proved in observations of a mouse model. By means of microinjection, normal mitochondria were inserted into mice’s oocytes, which prevented them from apoptosis [19]. Tsai et al. [20] presented the effects of mitochondrial DNA variations in cumulus cells upon in vitro fertilization and embryo transfer outcomes. Pregnancy tests were positively correlated with younger age, better-transferred embryo qualities, and lower dmtDNA-delta5Kb (mtDNA4977 deletion) ratios in cumulus cells. These authors concluded that mtDNA4977 status in granulosa cells might be a potential tool for oocyte evaluation and embryo selections during in vitro fertilization [21]. Although Keefe et al. [21] suggested that the common deletion may serve as a marker of oocyte senescence, others failed to confirm these observations [22, 23]. Most of the previous studies have shown that the incidence of 4977bp deletion was significantly higher in older women. This observation is in line with the hypothesis that there is an age-related accumulation of mtDNA rearrangements in human oocytes. However, none of the scientists checked if deletions occur in somatic cells, such as leukocytes of infertile patients. Unfortunately, the present data definitely reported that POF and primary infertility are not associated with the presence of deletion within mtDNA4977 in peripheral blood leukocytes. In findings among 80 patients, only one deletion revealed that the age-related effect on occurrence of the mtDNA4977 is not apparent between the ages of 20 to 39 and may be spontaneously present.

The objective of the study of Tong et al. [24] was to determine if mitochondrial DNA polymerase gamma deletions were associated with spontaneous 46,XX primary ovarian insufficiency. Among 201 examined women, they found only one case of heterozygosity for a polymerase gamma, suggesting that this was not a common genetic etiology for this form of infertility [24]. The present results confirm these observations.

The authors conducted this study on peripheral blood leukocytes with the use of highly-sensitive technique. To the best of their knowledge, there are only a few studies focused on the mentioned data. A significantly higher incidence of mtDNA4977 in peripheral blood leukocytes was observed in coronary artery disease patients with respect to healthy subjects; even the examined group was not so large as in this study (65 vs 80) [25]. Iwai et al. [26] examined the effect of green tea enriched with catechins on the presence of the mtDNA4977 deletion mutation in human leukocytes obtained from ten healthy young females (median age 20.8 years, similar to this study group). They found that mutation was present in nine participants before drinking the tea and after the experiment; the mutation was noticed in none of the participants. Perhaps the dietary habits and other yet unknown predictors are more connected with mtDNA state than other conditions, including fertility. Current study was performed on the second day of the follicular phase in all participants and subsequently further research is necessary to assess a possible relationship, if it exists, between mtDNA4977 state and menstrual cycle.

Figure 1. — Graphical demonstration of real-time PCR data from 13 representative patients [12 without mutation (wells from 1 to 12) and one patient (well 13) with mutation, well 14 – negative control]. A: In this view, normalized reporter (Rn) is graphed vs the cycle. B: ΔRn is Rn minus the baseline, graphed vs the cycle of PCR. C: Ct vs well position.
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References


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Summary

Purpose of investigation: Clinic visits during pregnancy and puerperium provide a unique opportunity to counsel women on contraception practices. With the aim of evaluating postpartum contraceptive attitudes among urban women attending an antenatal care center and delivering in the same facility, a structured questionnaire was administered to assess desired and received information on contraception in the postpartum period. Results: A total of 436 consecutive interviews were collected during the study period. Pregnancy was unplanned in 39% of the women interviewed. Overall, 269 women (61.7%) had decided to use a method of family planning during postpartum. Among the 112 women who stated they did not want to use a method during postpartum, almost 50% stated that they “did not think they needed it”, due to a perceived lack of real risk. Of the 436 women interviewed, only 5.5% acknowledged that they had received information on contraceptive use. Conclusion: The present study indicates a need for ante- and postpartum counseling of women even in urban areas of Italy.

Key words: Postpartum contraception; Contraception attitudes; Hormonal contraception; Intrauterine contraception; Contraceptive needs; Contraception unmet needs.

Introduction

Pregnancy and childbirth are fundamental events in the life of most women and in the majority of them these events change priorities, attitudes, and lifestyle. This is particularly true when dealing with future contraception. During pregnancy and in the postpartum period, women have been found to be more receptive to discussions with their care providers regarding the provision of methods capable of delaying or preventing altogether the occurrence of another pregnancy [1]. Thus, clinic visits during pregnancy and puerperium provide a unique opportunity to counsel women; this is indispensable since, in many cases, even if the woman has utilized a contraceptive method before a planned pregnancy, this previous method may no longer be desirable or ideal after childbirth [1, 2]. Unfortunately, despite the great opportunity to provide advice during antenatal care visits on postpartum contraception, caregivers often miss this opportunity. Even immediately after delivery the issue of future contraception is often neglected. For instance, a survey conducted some 15 years ago in Edinburgh showed that only 50% of new mothers received a contraceptive supply when leaving the hospital. The same study reported that only a scanty percent of women were given an opportunity to discuss postpartum contraception and this was usually a brief, limited encounter before leaving the hospital [3].

Even when postpartum programs are in place, their appropriateness has been questioned and, indeed, more attention needs to be given to this issue because postpartum contraception is vital to ensure adequate birth spacing, a major component of every effort to improve maternal and infant health. It has been estimated that globally implementing a two-year birth interval would avoid some 100,000 maternal deaths every year and also significantly reduce abortion rates [4].

Over ten years ago, in a large multinational survey of 27 countries, Ross and Winfrey [5] estimated that many postpartum women had unmet family planning needs, including a significant lack of information regarding postpartum contraception and optimal available methods. Obviously, this lack of information varies with geographical areas, education and social class, but substantial improvements are mandatory everywhere. For instance, in the Russian Federation, Vikhlyaeva et al. [6] have shown that a major improvement in counseling services for post-delivery contraception is necessary both in the maternity hospitals and in local family planning centers.

While many studies have evaluated patient satisfaction with specific contraceptive methods, few have focused on contraceptive needs of peripartum women [1, 2, 7, 8]. In a recent study, Glazer et al. [9] investigated 175 postpartum women attending an American University hospital out-patient clinic, asking whether contraceptive advice was offered either at ante- or postpartum. They found that three-quarters of the respondent (77%) had discussed future contraception before delivery and 87% did so during postpartum. Interestingly, 23% of the subjects would have elected immediate post-placental intrauterine device (IUD) placement if available, although at follow-up contacts four to six months after delivery, only five percent reported using an IUD, 29% were using no contraception, and 32% utilized a method which was not highly effective. This indicates that even in a tertiary urban hospital in the USA, there can be an unmet need for contraception, at least during postpartum.
Several investigations have been conducted on the delicate issue of counseling adolescents pre- and postpartum, as well as on reasons for contraceptive non-use among young women who have had a delivery; these investigations are important for a proper understanding of adolescent attitudes and for reducing teenage pregnancy [10-13]. In 2007, Lemay et al. investigated non-use of contraception prior to first pregnancy among adolescent mothers and listed as reasons: denial, not planning to have sex, not considering the consequences of unprotected sex, and wanting to become pregnant. They concluded that in the USA, adolescents favored routine discussions of the topic, parental involvement, exchange of information between young mothers and teenagers at risk, and media campaigns [14].

Recently, Lopez et al. [15] have conducted a Cochrane review of existing data on “education for contraceptive use by women after childbirth.” They found eight trials meeting their initial criteria for inclusion. On further analysis, there were only two studies evaluating short-term interventions with sufficient data and statistical power and both showed a positive effect on contraceptive use. They also analyzed four programs with multiple contacts: two showed more contraceptive use, fewer pregnancies or births among adolescents when there were enhanced services, and a structured home-visiting program. A group in Taiwan has now defined a “theory-based interactive postpartum sexual health education program” aimed at enhancing effective contraceptive behaviors in postpartum women with a follow-up over three months. They randomized 250 women into three groups. The first group received the full intervention program that utilized strategies matching participants’ learning preparedness, as determined by a “transtheoretical” model including health education. The second group received only a pamphlet and the third group (used as controls) received routine education. The study proved that this new approach was capable of enhancing postpartum contraceptive self-efficacy and effective contraceptive behavior in participating women.

In Italy in 1978, after the passing of legislation permitting voluntary pregnancy termination [16], the Ministry of Health has been mandated by Parliament to draw-up annual reports providing full information on legal abortions (e.g. number of abortions, abortions’ rates, and number of repeated abortions) and the most recent report, once again indicates that women who already had one pregnancy are at higher risk of a new pregnancy, thereby showing lack of postpartum counseling.

With the aim of evaluating postpartum contraceptive attitudes among urban women attending an antenatal care center and delivering in the same facility, a study was designed to assess desired and received information on contraception in the postpartum period.

Materials and Methods

All consenting pregnant or puerperal women admitted to the Department of Obstetrics, Gynecology and Urology at the Policlinico Umberto I° Hospital, of the “Sapienza”, University of Rome were interviewed during the period from January 2009 to December 2009.

The Ethics Committee of the Hospital approved the study and individual informed consent was obtained after study characteristics, and the questionnaire were verbally explained to prospective participants. General characteristics were recorded even for those who did not accept to participate to the study.

Considering an alpha level of 0.05 and a statistical power of 0.80, the minimum sample size required was 213. The sample size was then adjusted to compensate for a non-response rate of 20%. Thus a minimum final sample size of 256 was established. Statistical analysis was performed using SPSS (version 15), categorical variables were compared with chi-square test and Fisher exact test, as appropriate, while continuous variables were compared using t test. A p value of < 0.05 was considered significant.

A self-administered structured questionnaire with closed questions was utilized for the interviews. The questionnaire was organized in seven sections: general demographic characteristics; obstetric history and breastfeeding attitudes; previous contraceptive usage; intention to use a contraceptive method after delivery; knowledge of contraception in general and of specific postpartum contraceptive modalities; information received on postpartum contraception; factors that influenced their intentions, as well as their intended contraceptive choices. Additional information was obtained on whether their pregnancy was planned or not, whether the woman attended a hospital or a private clinic for antenatal care, and if she attended a preparatory course before delivery. The questionnaire was first administered in a pilot study and then validated.

The mean time for filling the questionnaire was estimated to be approximately 15 minutes.

Results

During the study period, 1,760 women gave birth at the Department Obstetrics, Gynecology and Urology. A total of 436 consecutive interviews were collected during the study period, 284 respondents were pregnant, while 152 were puerperal women. Of these, 36.9% has been followed during pregnancy by the outpatient obstetrics service of the Department, 30% by a private physician, 20% by public clinic, and the last 13.1% by the obstetric clinic of a different hospital.

The mean age of respondents was 31.7 ± 6.08 years (SD) with a range of 18-41 years.

Non-respondents were similar to respondents for general demographic characteristics and obstetrical history.

Overall, pregnancy was planned in 61% of the women interviewed, while in 39% it was unplanned (266 and 170, respectively).

As indicated in Figure 1, among women below 25 years of age, the vast majority of pregnancies (84.8%) were unplanned; this proportion decreased with age and reached a minimum (24.8%) among women aged 30-34 years, increasing again thereafter.

Table 1 shows that overall, 269 women (61.7%) had decided to use a method of family planning during the postpartum, with 112 (25.7%) opting or having opted for no contraception and 55 (12.6%) undecided. Of the three variables and many categories listed in Table 1, the only
ones that showed a significant association with the intention to use a contraceptive in postpartum were: previous contraceptive use ($p = 0.0001$), having received a higher education (diploma; $p = 0.04$), and paradoxically being of Catholic religion ($p = 0.05$). However, it must be pointed out that only some 12% of all participating women were non-Catholic, with 7.5% being Orthodox Christians.

The overwhelming majority of subjects wanting to use a method of contraception during the postpartum (220 or 81.8%) gave the need to achieve a proper “birth spacing” as the reason. Only 13 (4.8%) stated that they had completed their project for a family, with 36 (13.4%) being unable to provide any specific reason (Table 2).

An analysis of postpartum contraceptive choices made by women who wanted to use a method during postpartum, indicated that the vast majority (82%) preferred the use of combined hormonal contraceptives. Intrauterine contraception was selected by some eight percent of the subjects, while 4.8% stated that they would use a barrier method.

Among the 112 women who did not believe that a contraceptive method could be used during postpartum, as stated above, almost half felt that – at any rate – they were not at risk. Of the 436 women interviewed, four did not provide information on contraceptive methods to be used during the postpartum period. In addition, only 24 women (5.5% of the 432 that gave an answer) acknowledged that they had received information on contraceptive use; most of

### Table 1. — Association between variables (age, education religion) and intention to use contraception (n. 436).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intention to use a contraceptive</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
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<th>p value</th>
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<td>&lt; 25</td>
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<td>32.6</td>
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</table>

* Percentage of 436 women.
and parity.

likely to wanting to use contraception in postpartum (methods (use contraception', with a preference for hormonal methods (Table 5). All these subjects were among the 220 women who wanted to use hormonal contraception.

jects were among the 220 women who wanted to use hor-

useful information in the media (Table 5). All these sub-

them (21) from their obstetrician, with three who found useful information in the media (Table 5). All these subjects were among the 220 women who wanted to use hormonal contraception.

No statistically significant difference was observed between pregnant and puerperal subjects in 'intention to use contraception', with a preference for hormonal methods ($p = 0.02$).

Parity was significantly related to intention to use a contraceptive: women with a prior pregnancy being more likely to wanting to use contraception in postpartum ($p = 0.0048$) (Table 6).

Discussion

In the Industrial world, many believe that a pregnancy is the result of careful planning; yet, data from the Global Health Council indicate that, of the 205 million pregnancies occurring annually worldwide, between 60 and 80 million are unplanned. In addition, more than half of the millions of unwanted pregnancies are terminated by elective abortion, a high proportion of which, are performed in developing countries under unsafe conditions [17].

The present study found that, overall, pregnancy was unplanned in almost 39% of the women interviewed; this percentage rose to almost 85 among those below 25 years of age. Almost two-thirds of them opted for a method of family planning during the postpartum period, giving as the main reason the need to properly 'space' pregnancies. In their vast majority, these women preferred oral contraception. Almost half the women who did not want to use contraception during the postpartum believed that the risk of another pregnancy was negligible, although they were not even aware of the Lactational Amenorrhea Method (LAM). This was evidenced by the fact that among the 13% of women who knew that a method could be used during breastfeeding, not a single one mentioned LAM. Finally, only 15.8% of 152 women interviewed during postpartum had received information regarding contraceptive use during the ante- or postpartum periods, mostly from their obstetrician. This finding is particularly problematic when considering that the overwhelming majority of women interviewed (86.2%) stated that they would have appreciated receiving such information. Thus, the present study indicates a need for ante- and postpartum counseling of women even in urban areas of Italy.

It is also important to reflect on the high proportion (almost 50%) of women interviewed who did not believe that they needed contraception after birth of their baby, due to a lack of perception of risk of another pregnancy.

Many and diverse reasons have been given to explain the high rate of unintended pregnancies even in Western countries; they include: lack of patient education, ineffect-ive or inconsistent use of contraceptive methods, unplanned sexual activity, and contraceptive failure. In this connection, a paper just published attempted to assess in a sample of 248 women, their knowledge of health risks connected with pregnancy, and how such an evaluation compared to their estimates of the risks of oral contraceptives. This investigation found that over 75% of respondents rated oral contraceptives as more hazardous than pregnancy and, intriguingly, women with greater levels of education were more likely to believe that oral contraceptives were riskier than pregnancy [18]. The study did not address the question of whether these misconceptions would lead to non-use of contraception in the postpartum period, but the inference seems obvious.

One of the aforementioned reasons seems especially relevant for the postpartum period: lack of proper education and information. Back in 2003, a comparative study was performed in the USA on contraceptive information received after delivery. Whereas all women in the intervention group received an information booklet during their postpartum stay at the hospital, one-third of those in the control group reported having received some kind of written information. The study concluded that the simple distribution of written material about contraceptive options during postpartum increases the ability of a woman to make an informed decision regarding future pregnancies [19].

The already mentioned recent, careful review of the literature on this subject concluded that educating women during the postpartum period led to increased contraceptive use and fewer unplanned pregnancies. Interestingly, the review found that both short-term and multiple-contact interventions were effective; however, data on short-term intervention did not always show improvement. Longer-term actions seemed to hold promise and were not necessarily more costly, although – by definition – they were more complex and not ubiquitously applicable [15].

Several national studies have addressed the issue of providing postpartum contraception: in Finland a study found out that the most common contraceptive method recommended by physicians and nurses to breastfeeding women was the condom, followed by progestin-only pills and intrauterine contraception. Only a few health operators recommended LAM, and only some 10% inserted an IUD postpartum [20]. In Nigeria, a study found that more than 50% of the women surveyed intended to use contraception

<table>
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<th>N</th>
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<th>p value</th>
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Table 5. — Contraceptive counselling during pregnancy.

Table 6. — Relationship between intention to use contraception and parity.
after delivery. Their preference went to condoms (38.3%) followed by intrauterine devices (11.5%). Advanced age and high parity significantly predicted intention to use postpartum contraception. Also counseling by doctors and nurses increased the intention to use postpartum contraceptives, stressing – once again – the importance of family planning counseling and education [21].

In Turkey, after postpartum counseling, one-third of the women involved in a study decided to use intrauterine contraception, followed by condoms (16%), injectable progestins (11%), oral contraceptives (5%), and coitus interruptus (5%). However, one-fourth of the women still decided against the use of contraception during puerperium. Authors concluded that, in spite of postpartum contraception, followed by condoms (16%), injectable progestins (11%), oral contraceptives, stressing – once again – the importance of family planning counseling and education [21].

In conclusion, available evidence indicates that initiation of effective contraceptive methods is often delayed after childbirth. In order to promote better postpartum contraception practice, it is necessary to educate physicians, nurses and women. This can be better achieved through widespread distribution of updated evidence-based guidelines for health operators and of educational material for pregnant and postpartum women.

References

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Cerebral and renal abscess and retino-choroiditis secondary to candida albicans in preterm infants: eight case retrospective study

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1 Department of Pediatrics, First Hospital of Jilin University, Changchun (China)

Summary

Objectives: To assess the tissues and organs commonly involved and the clinical features in the invasive fungal infection (IFI) of candida albicans in the preterm infants. Materials and Methods: Eight preterm infants who developed IFI with positive blood culture for candida albicans were retrospectively studied. All infants received selected clinical and laboratory parameters evaluation, such as blood culture, cerebral magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) biochemical test, routine urine test, urine culture, renal ultrasonography, renal computer tomography (CT), and fundus examination. The re-examinations were performed after one to two months follow-up. Results: Cerebral abscesses were detected in six infants. Five cases developed renal systemic fungal infection, among which one had renal abscess. Three cases were complicated with fungal retino-choroiditis. Conclusions: Preterm infants, especially very-low-birth-weight (VLBW) and extremely-low-birth-weight (ELBW) infants are susceptible to fungi. The majority of preterm late-onset fungal infections are due to candida albicans. The organs commonly involved in the IFI of candida albicans are central nervous system (CNS), kidney and fundus, among which renal systemic fungal infection are prone to recur, calling for a prolonged anti-fungi treatment course.

Key words: Candida albicans; Preterm neonates; Invasive fungal infection; Cerebral abscess; Renal abscess; Retino-choroiditis.

Introduction

The neonatal intensive care unit (NICU) is rapidly developing. The application of mechanical ventilation, nutritional support through peripherally inserted central catheter (PICC), umbilical artery and vein catheters, and broad-spectrum antibiotics has increased the survival of the very-low-birth-weight (VLBW) and the extremely-low-birth-weight (ELBW) infants. However, fungi have become part of the major pathogens leading to the late-onset infection of VLBW and the ELBW infants. Genus candida accounts for the majority of invasive fungal infection (IFI). Since it is difficult to differentiate disseminated infection of candidemia from bacteremia, early diagnosis and prompt management of fungal infection are delayed. The delay and the properties of adherence and proliferation lead to the dissemination to multiple end organs like brain, kidney, lung, intestinal tract, heart, eye, liver and joints. To assess the tissues and organs commonly involved and the clinical features in the IFI of candida albicans in the preterm infants, the authors retrospectively studied a case series of eight preterm children who developed IFI with positive blood culture for candida albicans and complications of cerebral abscess, renal abscess or retino-choroiditis.

Materials and Methods

Patients

Retrospective studies were done in eight cases diagnosed as candida albicans IFI between January 2011 and February 2012 in the First Hospital of Jilin University. This study was conducted in accordance with the Declaration of Helsinki and was conducted with approval from the Ethics Committee of First Hospital of Jilin University. Written informed consent was also obtained from all participants. All cases were preterm infants, with the gestational age of 27 to 32 weeks, birth weight of 940 g to 2,200 g, and main pre-existing conditions of premature and respiratory distress syndrome (RDS). One case was ELBW infant, two cases were VLBW infants, and the other five were low-birth-weight (LBW) infants. Five cases required invasive mechanical ventilation. All infants received nutritional support through PICC for 15 to 53 days. Positive blood cultures for fungi occurred between days 7 to 40 after admission. The catheters were all removed as soon as possible after the positive culture. Table 1 summarizes the clinical data.

Imaging evaluation

Eight preterm infants who developed IFI with positive blood culture accepted selected imaging evaluation like cerebral magnetic resonance imaging (MRI), renal ultrasonography, renal computer tomography (CT), and indirect ophthalmoscopy examination. Cerebral abscesses were detected by cerebral MRI in six infants. The observations of the cerebral MRI: multiple punctate, relatively small, disseminated wide lesions performed higher signal in bilateral frontal, temporal, occipital, and parietal lobes (Figure 1). After the administration of fluconazole for four to six weeks, multiple cerebral abscesses disappeared after one to two months. Five cases developed renal systemic fungal infection, among which one had renal abscess. Kidney CT showed enlarged bilateral kidneys, with multiple well-defined, low-density parenchymal lesions. Renal Doppler ultrasonography showed multiple parenchymal echoless areas in bilateral kidneys. Punctate hyperechoic areas were detected in the renal pelvis (Figure 2). Three cases were complicated with fungal retino-choroiditis. Fluffy white retinal balls were detected by indirect ophthalmoscopy (Figure 3).
Table 1. — Clinical data of eight preterm infants.

<table>
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<th>Gestational age (weeks)</th>
<th>Birth weight (kg)</th>
<th>Pre-existing condition</th>
<th>Nutritional support through PICC (days)</th>
<th>Ventilation support (days)</th>
<th>Timing of positive blood culture (days)</th>
<th>Organs involved</th>
<th>Course of anti-fungi medication (days)</th>
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NOTE: RDS: respiratory distress syndrome; PDA: patent ductus arteriosus; BPD: bronchopulmonary dysplasia; Recurred cases: renal systemic infection recurred.

Figure 1. — Fungal cerebral abscesses. Case 7: T2-weighted dark flare cerebral MRI. A, B: the multiple punctate lesions performed higher signal in bilateral frontal, temporal, occipital, and parietal lobes. C, D: one month later, the multiple punctate higher signal lesions disappeared.

Figure 2. — Renal systemic fungal infection complicated with kidney abscess. Case 7: Kidney CT and renal Doppler ultrasonography. A, B: kidney CT showing enlarged bilateral kidneys, with multiple well-defined, low-density parenchymal lesions. High-density mass in the bilateral renal pelvis and upper nephritic ducts. C, D: renal Doppler ultrasonography showing multiple parenchymal echoless areas in bilateral kidneys. Punctate hyperechoic areas in the renal pelvis.
Results

Cerebral abscesses were detected in six infants (Figure 1). Five cases developed renal systemic fungal infection, among which one had renal abscess (Figure 2). Three cases were complicated with fungal retino-choroiditis (Figure 3).

Discussion

NICUs are rapidly developing. The application of mechanical ventilation, nutritional support through PICC, umbilical artery and vein catheters, and broad-spectrum antibiotics has increased the survival of the VLBW and the ELBW infants. However, fungi had become part of the major pathogens leading to the late-onset infection of VLBW and the ELBW infants. Genus candida accounts for the majority of IFI. Since it was difficult to differentiate disseminated infection of candidemia from bacteremia, early diagnosis and prompt management of fungal infection were delayed. The delay and the properties of adherence and proliferation lead to the dissemination to multiple end-organs like brain, kidney, lung, intestinal tract, heart, eye, liver and joints [1]. Course of anti-fungi medication were prolonged and outcomes were poor.

Candida albicans is considered an opportunistic pathogen. Whether people become ill or not depends on the immunity and the defense of the host, as well as the virulence of the pathogen. In normal conditions, candida albicans in the body is yeast-like and non-pathogenic. However, when the immunity and defense of the host decreases, candida albicans proliferates and transforms to an invasive, multicellular filamentous form (also called pseudohyphae) to infect the host tissue, thus people will become ill and clinical manifestations arise. candida albicans has several known virulence factors contributing to its pathogenicity: adherence to epithelial and endothelial cells: virulence is parallel with adherence and candida albicans adheres most strongly to epithelial cells among the genus candida. Pseudohyphae formation: When infection occurs, candida albicans is in the multicellular filamentous form, which is of greater virulence than the yeast-like morph. Toxin: the polycose toxin on the surface and another kind called ‘candida toxin’ may be the pathogenic factors. The components of the cell wall; extracellular membrane-damaging enzymes: candida albicans can excrete some species of enzymes like lysophospholipase, phospholipase, acid protease, etc, among which extracellular acid protease is the most important, which can hydrolyze not only protein, but also keratin and collagen, leading to the promotion of the ability of adherence of candida albicans.

The process of the candida albicans infection is as follows: The fungus adheres to the epithelial cells and forms infectious focus with the help of the aforementioned pathogenic factors. The process of adherence is accomplished by the combination of collagen and adherence acceptors, which are located on the surface of the candida albicans and the host cells respectively. The collagen widely distributes in vascular walls, inflammation and trauma, making the candida albicans adhere and invade the host’s tissues much more easily. Compared to other candida species, candida albicans demonstrates increased adherence and penetration of vascular endothelium, possibly accounting for its higher incidence as a cause of IFI. Since the kidney, ocular fundus and central nervous system are abundant in blood vessels, which are the destination of candida albicans’ adherence, these organs are prone to be involved.

What are the clinical features of preterm end-organ dissemination of candida albicans infection? The authors demonstrate the clinical data of the eight cases infected with candida albicans, with the involvement of central nervous system (CNS), kidney, choroidal and/or retina as follows.

CNS candida infection may involve disseminated minor abscesses (diameter < three mm), meningitis, ventriculitis, cerebral infarction, mycotic aneurysm, and subarachnoid hemorrhage [2]. In the present study, six of eight
cases had CNS infection, with the clinical manifestations of fever, decreased responsiveness, and apnea in all, convolution in only one case, CSF changes in three cases of increased of protein, and white blood cells and negative culture, and multiple minor abscesses in all the CNS involved cases’ MRI except for one who could not undergo the examination because of the severity of the disease. Thus, the CNS candida albicans infection cannot be excluded even the cerebral spinal fluid (CSF) is normal, and the infants with the clinical manifestations of IFI should routinely accept cerebral MRI screening. The present observations that foci of abscesses, numerous and relatively small, disseminate widely and coordinate well with Mueller’s study [3]. After the administration of fluconazole for four to six weeks, multiple cerebral abscesses disappeared after one to two months. Among the cases are a couple of twins who are nine-month-old now and normally developed their CNS, left with no sequelae.

Five cases during the study developed candida albicans infection in the urinary system, with positive urine culture in all the five cases, the same with the blood culture. Other auxiliary examinations included urine routine test, renal Doppler ultrasonography, and renal CT. White blood cells increased in the urine. With ultrasonography, multiple parenchymal echoless areas and hyperechogenic areas were detected respectively in parenchyma and renal pelvis bilaterally in several cases. Corresponding with CT, the renal abscesses appeared as parenchymal oval low-density lesions. Also, high-density masses appeared in the renal pelvis and upper renal duct. One case with renal abscesses developed renal dysfunction, and recovered after peritoneal dialysis, urinary tract flushing and anti-fungal medication. Candida albicans adheres easily to epithelial cells of vessels and other tracts because of the ability of adherence. Since the glomerulus and nephric tubules are abundant in vessels, candida albicans infection easily involves urinary system and forms abscess, which are difficult to eradicate. The clinical symptoms of fungal infection in urinary tract are always insidious, so it should be routine for the patient with candidemia to accept the urine test, urine culture, and image examination to clear whether the patient has fungal urinary infection [4-6]. It should also be noted that central venous catheters create a unique surface for proliferation of candida albicans, so the catheters should be removed for any preterm infant with candidemia. As for management, medicine-like fluconazole that has a high concentration in the urine system should be administrated. In case of recurrence, the course should be prolonged [7-8].

In the present study, the five cases accepted fluconazole for two to three weeks until the urinary culture turned negative. However, two of them relapsed after the drug withdrawal. The short course may account for the recurrence. So in case of recurrence, the course of urinary tract fungal infection should last for at least four to six weeks until the several negative urine culture results.

Three cases caused fungal retino-choroiditis with the white fluffy balls in the fundus examination. According to the reference, candida albicans infection, the main part of the endogenous endophthalmitis, may occur at any age, have no gender difference, and 70% of the patients develop the disease in binocular [9-11]. Fungal retino-choroiditis has the following characteristics: the infective process develops gradually. The posterior segment lesions are mainly caused by invasion via the choriocapillaries, crossing the pigment epithelium affecting the retina. If the organism penetrates the internal limiting membrane of retina, the lesions break free and disseminate to form ‘satellite foci’. If the fungus gains access to the vitreous cavity, multiple clumps may form within the vitreous. The multiple clumps in the vitreous are often connected by thread-like strands, thus their aspect is referred to as having ‘string of pearls appearance’ [12]. The posterior hyaloid fixed by inflammatory foci, around which granulation and organization form, results in the severe sequel of hemorrhage or traction retinal detachment [13]. The course of the disease can be divided into two phases [14]: retino-choroiditis phase and endophthalmitis phase involving vitreous and sometimes anterior uvea. Medical treatment varies according to the tissues involved in the candida albicans infection: systemic administrations through the venous route of antifungal agents like fluconazole or amphotericin B for retino-choroiditis; as for the endophthalmitis, injection of amphotericin B in the vitreous cavity or vitrectomy is performed, and the simultaneous administration of antifungal agents helps. Because of the insidious clinical symptoms and the severe sequel-like retinal necrosis, traction retinal detachment, bulbus oculi atrophy, and visual loss of the fungal retino-choroiditis [15-16], infants who are suspected to have fungal infection especially IFI, should accept routine screening through indirect ophthalmoscope after mydriasis [17-18]. Since the fungal infection can be detected in the retinal phase, endophthalmitis and the severe results may be prevented under proper and prompt treatment. The three cases were administrated with fluconazole for two to four weeks, resulting in the gradual disappearance of the white dots. No visual loss was detected during the follow-up.

Preterm infants, especially smaller and more immuno-compromised ones, are susceptible to fungal infection [19]. In the present study, fungal end-organ infection of cerebral abscess, urinary infection, and retino-choroiditis in the eight preterm infants with IFI have obvious and specific signs detected through imaging examination. Candida species can also cause fungal arthritis, dermatitis, cardiac valvulitis, and fungal abscesses may form in skin and liver, etc [20]. When the neonates develop candida albicans invasive infection, they should accept the auxiliary examination to identify whether they are complicated with end-organ infection in CNS, kidney, fundus, skin and joints, which are necessary for the determination of the management and the prediction of the prognosis.
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References


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New horizons in the non-invasive diagnosis of endometriosis

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Summary
Endometriosis is a chronic disorder, clinically associated with chronic pelvic pain, dyspareunia, dysmenorrhea, and infertility. Its socio-economic impact is extensive, given the large number of affected women in reproductive age, its symptomatology (that interferes with normal social life and the patient’s ability to work), and its frequent association with infertility. Nonetheless, the diagnosis of endometriosis is still difficult and late in the evolution of the disorder. The authors have used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria to make a systematic review of the literature of the last 28 years, seeking to identify potential biomarkers useful for a non-invasive diagnosis of endometriosis. The authors have highlighted more than 50 biomarkers in the studies included in the present report, but they have not succeeded in identifying a clinically useful non-invasive diagnostic biomarker or panel of biomarkers. More studies are needed before biomarkers can be introduced in clinical practice.

Key words: Endometriosis; Infertility; Peripheral biomarkers; Early diagnosis.

Introduction
Endometriosis is a chronic, estrogen-dependent disorder, characterized by the presence of endometrial glands and stroma in an ectopic site. It is clinically associated with chronic pelvic pain, dyspareunia, dysmenorrhea, and infertility. Endometriosis has a high socio-economic impact given the large number of affected women in reproductive age (10% - 15%); its symptomatology undermines normal family and social life and it interferes with the patient’s ability to work. The disorder is frequently associated with infertility. The partial understanding of the pathogenesis, its multifactorial nature, and the low specificity of its symptoms render the diagnosis of endometriosis difficult and late in the evolution of the disorder [1,2].

The scientific literature of recent years has shown a growing interest in the research on biomarkers and sets of biomarkers that could be useful in making an early and non-invasive diagnosis of endometriosis and in following-up treated patients and identifying relapses in their earliest stages.

The goal of the present study was to highlight all the biomarkers (plasma, serum, urinary, peritoneal, and endometrial biomarkers) proposed in the international scientific literature of the last 28 years and, through a meta-analytic reprocessing of the data, assess their clinical value (based on sensitivity and specificity) in making a non-invasive diagnosis of endometriosis.

Materials and Methods
The present work was divided into three stages: computer search throughout the scientific literature on this issue from January 1984 to January 2012, definition of the inclusion and exclusion criteria, analysis of the sensitivity (S), and specificity (Sp) of individual biomarkers and panels of biomarkers proposed by the authors.

The computer search envisaged the use of some online medical search engines (PUBMED, EMBASE, MEDLINE, CINHAL) and of the following keywords: endometriosis, plasma-serum-blood-urine-biological-tissue-endometrial biomarkers, cells, diagnosis, non invasive, and mass screening. Only publications in English that met the inclusion and exclusion criteria (Table 1) were taken into account. A further selection was then made using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria in the version modified by Whiting in 2003 (Table 2). Finally through the statistical processing of the data, the best potential biomarkers or panels of biomarkers (greater specificity and sensitivity) for a non-invasive diagnosis of endometriosis were identified.

Results
The computer search produced 11,665 total results; of these 11,488 were eliminated after evaluating the title, content of the abstract, and compliance with the Quality Assessment of Diagnostic Accuracy Studies inclusion and exclusion criteria and with the “QUADAS criteria”. In this way, a final number of 177 articles remained whose analysis highlighted many potential biomarkers and panels of biomarkers, that are listed below:

Cytokines
Interleukin 6 (IL-6): Six studies show a relationship between increased IL-6 serum levels and endometriosis [3-7]. In particular, in the study by Martinez et al. [7] high levels of IL-6 were found above all in women with a Stage I-II disease. With a threshold value of 25.75 pg/ml, a 75% sensitivity, and an 83.3% specificity were obtained. Badaawy et al. showed a sensitivity and specificity respectively of 90% and 67% with a threshold of two pg/ml [4]. On the contrary, other studies did not report a significant increase in IL-6 [8-12].
Interleukin 8 (IL-8): A study of 2003 showed increased serum levels especially for Stages I and II [13].

**TNF-α:** Various authors report particularly high serum and peritoneal levels in women with endometriosis in Stages III and IV [5, 13-18]. In the study by Bedaiwy et al., with a threshold of 15 pg/ml, a sensitivity and specificity of 100%, and 89% [4], respectively, are achieved when the cytokine assay was performed on the peritoneal fluid of affected women.

**Monocyte chemotactic protein 1 (MCP-1):** by using a threshold value of 100 pg/ml, a 65% sensitivity and a 61% specificity are obtained [19].

**Interferon-gamma (IFNγ):** In 2003, Darai et al. found an increase in the serum levels of IFNγ in women with endometriosis [6].

**Other cytokines:** Other interesting findings are the high levels of interleukin 1α (IL-1α) in the serum [20] and high levels of IL-12 and IL-18 in the peritoneal fluid of affected women [21-23].

### Inflammatory markers

**C-reactive protein and high-sensitivity C-reactive protein (CRP and hs-CRP):** The study carried out by Lermann shows higher CRP (3.54 mg/l) and higher hs-CRP (3.61 mg/l) average values in the group of patients with endometriosis (E-group), as compared to healthy controls (non-E group) (CRP = 2.88 mg/l; hs-CRP = 2.48 mg/l) [24]. Although there is a real difference in the concentration of molecules between the two study groups, the difference is not statistically significant. Hence, CRP and hs-CRP cannot be potential biomarkers.

### Antibodies (Ab)

**Anti-endometrium antibodies:** These have an 86% sensitivity and a 76% specificity in the diagnosis of endometriosis [25]. Sensitivity and specificity increase considerably, up to 87%, if used for the diagnosis in women with infertility, dysmenorrhea, and chronic pelvic pain [26]. IgG antibodies are those that appear to correlate most with endometriosis [27,28]. A recent study identified eight new antibodies against some endometrial antigens such as: tropomyosin 3 (TPM3), stomatin-like protein 2, (SLP2), and tropomodulin-3 (TMOD3). The following are respectively, the sensitivity and specificity of these antibodies in the early stages of the disease: Ac anti-TPM3α (61%, 93%), Ac anti-TPM3c (44%, 93%), Ac anti-TM3pd (78%, 89%), Ac anti-SLP2a (50%, 96%), Ac anti-SLP2c (61%, 93%), Ac anti-TM3D 3b (61%, 96%), Ac anti-TM3D3c (78%,93%), Ac anti-TM3D3d (78%, 96%) [29].

**Anti-carbonic anhydrase Ab:** Kiechle et al. have shown a sensitivity of 13% for type I and of 24% for type II [30].

**Anti-transferrin and anti-α2-HS glycoprotein Ab:** these present maximum sensitivity and specificity if assayed using the ELISA technique, reach values of 95% [31, 32].

**Ab against oxidative stress markers:** women with endometriosis present increased levels of Ac anti-lipid peroxide modified rabbit serum albumin, Ac anti-copper oxidized low-density lipoprotein, and Ac anti-malondialdehyde-modified low density lipoprotein [33].

**Anti-laminin Ab:** some authors have found high concentrations of these autoantibodies in patients with infertility (the cut-off of one U/ml has a sensitivity of 43% and a specificity of 89%) [34, 35].

**Anti-α enolase Ab:** have a sensitivity and specificity comparable with that of CA125 [36].

**Anti-PDIK1L (PD-interacting kinase 1 like) Ab:** PDIK1L is abundantly expressed by endometriotic cells. With a cut-off of 300 U/ml, the test provides a sensitivity of 59.4% and a specificity of 84.1%. Anti-PDIK1L autoantibodies are expressed in larger amounts in Stage I-II, therefore they could be of assistance in the early diagnosis of the disease [36].

**Anti-syntaxin 5 Ab:** at a cut-off of 400 U/ml shows a sensitivity of 53.6% and a specificity of 87.8% in a Stage II endometriosis [37].

**Anti-IGFII mRNA-binding protein1 (IMP1) and Anti-cyclin B1 Ab:** Yi et al. have reported for IMP1 a sensitivity of 85.7% and a specificity of 63.3% in women with en-
dometriomas. In combination with cyclin B2, it presents lower sensitivity (83.9%) but greater specificity (72.7%) [38].

**Glycoproteins**

*Cancer antigen-125 (CA-125):* This is the glycoprotein of great interest for endometriosis. Some recent studies show that CA125 is the most reliable glycoprotein in diagnosing Stage III-IV endometriosis [39, 40]. Xavier et al. show that the cut-off that provides the greatest sensitivity and specificity (86% and 91% respectively) is lower (22.6 IU/ml) than that reported in most of the literature (35 IU/ml) [41]. Various studies have established that the serum concentration of CA125 correlates with the severity of the disease [42] and tends to be higher in women with ovarian endometriosis (with a threshold of 30 IU/ml the sensitivity is 79% in women with endometrioma and drops to 44% for other sites) [43]. Finally, O’ Brien et al., have demonstrated that the technique used to assay CA-125 considerably influences its efficacy as clinical biomarker of endometriosis [44].

*Cancer antigen-19-9 (CA-19-9):* The threshold value of 5.4 IU/ml gives the best diagnostic performance [45, 46].

*Cancer antigen-15-3 (CA-15-3) and Cancer antigen-72 (CA-72):* Various authors have studied these glycoproteins but have obtained contrasting results [47-49].

*Haptoglobin:* Typically produced by endometriosis lesions. A selective increase in serum levels of the β isof orm in the follicular phase of the menstrual cycle has been found [50].

*Follistatin:* The serum concentrations of follistatin are raised in women with endometriomas compared to healthy controls [51].

*Gremlin-1:* This glycoprotein is hyperexpressed in the endometrial stroma of affected women. Its serum concentration is found to be increased exclusively in the proliferating stage [52, 53].

**Cell populations**

The patients with endometriosis present alterations in the normal lymphocyte count and in the monocyte-macrophage line. In particular the following is observed: increase in T suppressor lymphocytes (CD8+, CD11+) and in activated T lymphocytes (CD3+ ed HLA-DR+) [54,55], reduction in the circulating NK cells [56,57], and increase in the neutrophil/lymphocyte ratio (NLR) (consequence of the increase in circulating neutrophils) [58].

**Other immunological biomarkers**

Endometriosis is associated with an increase in the serum concentrations of the C5 and C4 complement fractions [59] and in the soluble forms of CD4 and CD23 [60-62]. A recent paper has shown the presence of high levels of peptides known as human neutrophil peptides 1, 2, 3 (HNP 1-3) in the peritoneal fluid of affected women [63].

**Adhesion molecules**

From the studies, the present authors have examined that it can be inferred that endometriosis is associated with an increase in the serum concentrations of the following adhesion molecules: ICAM-1 (particularly high in Stages I-II of the disease [64, 65], VCAM [66], E-cadherin (that does not present any particular correlation with the stage of the disease) [67], and finally, osteopontin [68].

**Growth factors**

A study has shown an increase in the serum levels of IGF-1 exclusively in Stages III-IV [69].

**Circulating cell-free DNA (ccf-DNA)**

Through real time PCR, it was possible to demonstrate a ccf-n DNA plasma concentration that was significantly greater in patients with endometriosis compared to controls; the test presents a sensitivity of 70% and a specificity of 87% [70].

**Hormones**

*Prolactin (PRL):* The association of hyperprolactinemia, galactorrhea with endometriosis, has been known for more than 30 years. Recent studies have shown the presence of hyperprolactinemia (PRL > 20 ng/ml) in 30% of women with endometriosis and infertility, whereas none of the fertile women with endometriosis and none of the controls presented raised levels of this hormone [71].

*Luteinizing hormone (LH), testosterone, cortisol:* Various studies have shown increased serum levels of this hormone in women with endometriosis; testosterone seems to be selectively associated with ovarian endometriosis and cortisol with advanced stage endometriosis (III-IV) [71, 72].

*Leptin and adiponectin:* Their serum levels are respectively increased and reduced in patients with endometriosis compared to controls [73-75].

**Angiogenetic factors**

Various studies have demonstrated the increase, in the advanced stages of this disorder, in serum concentrations of VEGF, and in one of its soluble receptors (sFlt-1) present in the serum and in the urine [18, 19, 76], Angiogenin [77], in FGF-2 [78] and finally in HGF [79].

**Proteomic markers**

The analysis of protein expression profiles in the serum and in the endometrium of women with the disorder is one of the most promising areas of research on potential biomarkers: the presence, absence, hypo- or hyper-expression of peculiar isoforms in the blood and/or endometrial tissue, could indicate new useful biomarkers. The protein peaks found, indeed, could be used to construct a diagnostic protein pattern in patients with endometriosis. The most important proteomic studies carried out so far are the following: Wang et al. [80] who have identified a pattern consisting of five protein peaks endowed with a sensitivity and specificity equal to 92% and 90%, respectively; the study by Kyama et al. have used two proteomic panels: the first, that examined endometriosis of Stages I-II, presented a sensitivity and specificity of 100%, and the second panel showed a sensitivity of 80% and a specificity of
70%. Furthermore, this latter study developed a protein panel suited to the diagnosis of endometriosis irrespective of the stage of the disorder that consists of five protein bands and presents a sensitivity of 89.5% and a specificity of 90% [81].

Other potential biomarkers

*Serum urocortin:* it presents considerably increased values in the ovaries of women with endometriosis; it is therefore useful in making a differential diagnosis of the ovarian mass. Sensitivity is 88%, and specificity is 90% [82]. In actual fact, a more recent study showed lower values: 72.6% sensitivity and 45.7% specificity [83].

*Protein PP14:* high especially in advanced stages [84];

*Tumor associated trypsin inhibitor (TATI):* sensitivity 34% and specificity 85% [85];

*Amyloid A:* increases in Stages III-IV;

*Paroxonase 1 (PON-1):* antioxidant glycoprotein. Its sensitivity is 98% and its specificity is 83% [86];

*Matrix metalloproteinase 9 and 2 (MMP-9, MMP-2) and phosphatase of regenerating liver 3 (PRL-3):* reach a sensitivity of 87.5% in Stages III-IV [87, 88].

*Urinary vitamin D-binding protein (VDBP):* sensitivity 58%, specificity 76% [89].

*Urinary cytokeratin-19 (CK-19):* initial studies have established a sensitivity and a specificity of 100% [90].

Panels

From the statistical analysis of the panels of biomarkers proposed in the literature of the last 28 years, those with greater diagnostic efficacy are:

- IL-6, IL-8, TNFα, hs-CRP, CA-125; CA19-9 (sensitivity = 92.2% specificity = 82%) [91];
- CA-125, NLR: (sensitivity > 86% specificity > 89%) [58];
- PGP9, VIP, substance P (sensitivity = 95% specificity = 100%) [92];
- CCR1 mRNA, MCP1, CA-125 (sensitivity = 92.2% specificity = 82%) [93].
- CA-125, CA19-9, survivin: (sensitivity = 87%) [94].

Discussion

The numerous difficulties encountered in pursuing the present objective are linked to various factors. First of all, a negative impact was due to the inherent characteristics of endometriosis such as: its multifactorial nature and the heterogeneity in terms of stage, site, and aspect of the lesions. Moreover, specific characteristics found in the various studies have proven to be important such as: inadequate patient sample (insufficient number, lack of confirmation of the diagnosis of endometriosis through laparoscopic exploration, lack of definition of recruitment criteria), and/or inadequacy of the group of healthy controls (limited number, not well-defined recruitment criteria, presence of co-morbidities); poor specificity of most of the biomarkers taken into account; the frequent disagreement among the data provided by various studies on the same biomarker (attributable to: method used, threshold value, timing of the sampling of the biological samples, adjustment of data to menstrual phase), and the lack of publication of studies with negative or irrelevant outcomes that could have provided useful insight [95].

With regards to the biomarkers, some of them, albeit presenting high sensitivity, do not have an adequate level of specificity, since they are implied also in physiological processes (cytokines) or in various pathologies. Some examples of biomarkers having low specificity are: CA-125 glycoproteins CA-19-9 [96], urinary IGF [97], VEGF and anti-cardiolipin antibodies [98], urocortin [99]. The diagnostic efficacy of biomarkers is considerably increased by the phases of the menstrual cycle, by the stage of the disease, and by the site of the lesions: elements that can cause conspicuous variations in terms of sensitivity and specificity of the values. The design of the studies the present authors selected is an important factor in evaluating the reliability of the results obtained. Indeed, even though rigid inclusion criteria were used, many works concerning the same biomarker often provided diverging results because of the wide variability in the threshold value taken into account, in the method used for the assay of the biomarkers, in the origin of the biological sample, in the method, timing of sampling and storage of the biological sample, in the selection criteria of the group of patients and controls and the breadth and scope of the results, and finally the statistical instrument used for processing the results.

The threshold selected significantly affects the diagnostic accuracy of the biomarkers; this is the case of CA-125 whose sensitivity ranges from 27% to 79% depending on the cut-off that was adopted [41-44], and of IL-6 with a sensitivity varying between 71% and 90% and a specificity ranging from 51% to 89% [4, 7, 8, 12]. Of considerable importance is also the biological sample, as regards the type of sample and the sampling and storage techniques. TNF-α [5] has a sensitivity and specificity of about 95% when serum assays are performed, and a sensitivity of 100% and specificity of about 89% when assays are performed on the peritoneal fluid. An adequate selection of the group of patients and controls is indispensable for the quality of the study. In many studies the control group was not adequately selected.

Indeed a fundamental factor is the heterogeneity of the control groups that should include healthy individuals as well as women with symptoms suggestive of endometriosis in whom however the disease has not been excluded with a laparoscopic test. At the same time, with reference to the studies that included among the controls women with benign gynaecological disorders, one cannot exclude that the pathologic condition of some women may have affected the outcome of the study. The handling difficulties instead are a limit to the application of the promising proteomic tests in clinical practice. Indeed it would be a good thing to be able to purify and identify protein molecules corresponding to the protein peaks, so as to introduce immunological tests that assay these proteins in the laboratory without necessarily having to use the SELDI-TOF-MS techniques.
Conclusion
At this point in time, endometriosis is a disorder with a high socio-economic impact whose diagnosis is made difficult by the poor knowledge of its etiopathogenesis, by the non-specificity of its symptoms, and by the lack of an effective non-invasive test. The aim of this study was to search for a biomarker or a panel of biomarkers with sensitivity, specificity, and ease of use suited to make a non-invasive diagnosis of endometriosis. Unfortunately, the present research data were not sufficient to identify a totally reliable non-invasive diagnostic protocol that could be immediately introduced into clinical practice, especially for the lack of very high quality studies, for the large discrepancy between the results of different studies carried out on the same biomarker, for the absence at the present time of a molecule or a panel of molecules that are exclusively correlated to the endometriotic disorder, and finally, for the difficult handling and/or costs of some tests.

References

New horizons in the non-invasive diagnosis of endometriosis
The role of serum adiponectin levels in women with polycystic ovarian syndrome

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Summary

Purpose of investigation: The aim of this study was to measure serum adiponectin concentrations in women with polycystic ovarian syndrome (PCOS) and to assess possible correlations between adiponectin and the hormonal or metabolic parameters of this syndrome. Materials and Methods: Serum adiponectin levels were evaluated in 20 women with PCOS and 22 women without PCOS whose age and body mass index (BMI) matched the patients. The levels of fasting blood glucose, fasting insulin, gonadotropin, and sex steroid hormones were evaluated in both groups. The homeostasis model assessment (HOMA) score was also calculated. The serum adiponectin levels were assayed by enzyme-linked immunoabsorbent assay (ELISA). Results: Serum adiponectin levels were significantly lower in obese women than in normal-weight women, and they were also significantly lower in PCOS patients with HOMA scores greater than 1.7 compared with those with HOMA scores lower than 1.7. When the subjects were divided in two groups based on serum adiponectin levels (> 40 µg/ml, < 40 µg/ml), 65% of patients with PCOS were included in the lower adiponectin group (p < 0.05). In addition, gonadotropin levels were increased, dependent on the adiponectin levels in women with PCOS. Conclusion: Adiponectin is regarded as a possible link between adiposity and insulin resistance (IR). From this data, the secretions of gonadotropin are implicated in the levels of adiponectin in women with PCOS. It is suggested that adiponectin may play an important role in the pathogenesis of PCOS.

Key words: PCOS; Adiponectin; Insulin resistance; Obesity.

Introduction

Polycystic ovarian syndrome (PCOS) has been shown to be identified by oligomenorrhea or amenorrhea as menstruation disorders, hyperandrogenism, and small multiple cystic follicles in the ovary on ultrasonography, and is usually found as a complex and heterogeneous endocrine disorder [1]. It occurs in about ten percent of women around reproductive age. In addition, it is associated with obesity in approximately 16% to 80% with PCOS. Recent work has identified that PCOS is often complicated with insulin resistance (IR) accompanied by compensatory hyperinsulinemia [2]. IR is suggested to be enhanced by the interaction between obesity and this syndrome [3]. These facts that both lean and obese PCOS patients show reduced insulin sensitivity and resultant hyperinsulinemia to some degree [4], suggest that hyperinsulinemia caused an increase in androgen biosynthesis [5] and a decrease in the levels of sex hormone-binding globulin (SHBG) [6]. These findings could possibly indicate the pathogenesis of hyperandrogenism. In addition to reproductive disorder, IR and hyperinsulinemia are recognized to increase the risk of long-term metabolic diseases, not only impaired glucose tolerance and type 2 diabetes [7], but also as cardiovascular disease [8].

Several studies have been reported to measure the circulating levels of adiponectin because of the importance of IR and obesity in PCOS [9, 10]. In recent years, it has been shown that adipocytes are secretory cells which produce various proteins with hormonal-type functions called adipocytokines. It is demonstrated that adiponectin is a 244-amino-acid protein, which is produced exclusively by adipose cells, and may have a role in preventing or counteracting the development of insulin resistance [11, 12]. In contrast to other adipocytokines, such as leptin, the production of adiponectin is decreased in obese subjects [12, 13].

The aim of this study was to clarify the determinants of adiponectin levels and to investigate the potential role of adiponectin in IR in women with PCOS. Furthermore, another objective of this study was also to clarify whether adiponectin is a marker of some degree in PCOS patients.

Materials and Methods

Twenty-seven consecutive reproductive-aged, amenorrheic women with PCOS were recruited at the Infertility and Endocrinology Clinic, Oita University Hospital, between January 2002 and December 2004. Exclusion criteria were excess alcohol consumption (n = 1), cigarette smoking (n = 2), previous or current oral contraceptive use (n = 3), and endurance physical training (n = 1).

Criteria for PCOS were chronic anovulation (fewer than six cycles in 12 months) or amenorrhea, elevated serum levels of luteinizing hormone (LH), with normal follicle-stimulating hormone (FSH), and LH/FSH of at least 1.5, and polycystic appearance of the ovaries on ultrasound, defined by ten or more follicles two to eight mm in diameter, with a tendency toward peripheral distribution and bright echodense stroma. Baseline characteristics included age, height, weight, body mass index (BMI), and hirsutism status. BMI was calculated as weight (kg) divided by height squared (m²). Subjects with Ferriman-Gallway scores exceeding ten were defined as hirsute [14]. None of the PCOS patients had evidence of an androgen-secreting neoplasm, pituitary adenoma, homozygous adrenal hyperplasia,
acromegaly, or Cushing syndrome in accordance with National Institutes of Health criteria. None of the subjects were taking any medication likely to affect muscle size, muscle strength, or body fat distribution. All women in the control group had normal ovulating cycles and no signs of hyperandrogenism.

In all women, the basal serum levels of serum gonadotropin (FSH, LH), estradiol 17β, testosterone, dehydroepiandrosterone sulfate (DHEAS), and androstenedione were measured using commercially available radioimmunoassays (RIAs). Serum levels of prolactin (PRL), glucose, and insulin were also measured.

Serum adiponectin was measured using a commercially available enzyme linked immunosorbent assay (ELISA). The intra-assay and inter-assay coefficients of variation for these RIAs and ELISA were 3%-5% and 8% to 10%, respectively.

IR in the fasting state was evaluated by using homeostasis model assessment (HOMA) and was calculated with the following formula: fasting plasma glucose (mg/dl)×fasting serum insulin (µU/ml) divided by 405. High HOMA scores denote IR [15]. The subjects were allocated to four groups on the basis of the adiponectin value and a diagnosis of PCOS. Hence, group 1 (n = 35) women had PCOS + adiponectin < 40 µg/ml; group 2 (n = 35) had PCOS + adiponectin > 40 µg/ml; group 3 (controls; n = 15) were ovulating without PCOS + adiponectin < 40 µg/ml; and group 4 (controls; n = 15) were ovulating without PCOS + adiponectin > 40 µg/ml.

Informed consent was obtained from each subject, and the study was approved by the Institutional Review Board, and was conducted in accordance with institutional guidelines and the Declaration of Helsinki.

Table 1. — Clinical and endocrine features of PCOS patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31.3 ± 4.7</td>
<td>30.3 ± 4.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.5 ± 5.5</td>
<td>157.6 ± 4.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.6 ± 11.0</td>
<td>55.8 ± 10.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 4.8</td>
<td>22.5 ± 4.3</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>9.3 ± 5.8*</td>
<td>4.7 ± 1.6</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.7 ± 1.5**</td>
<td>7.2 ± 1.5</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>1.7 ± 0.8*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>43.0 ± 24.0</td>
<td>36.6 ± 21.8</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>11.0 ± 6.1</td>
<td>12.4 ± 9.2</td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>34.2 ± 21.3</td>
<td>32.2 ± 13.7</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>93.0 ± 7.6</td>
<td>92.5 ± 7.2</td>
</tr>
<tr>
<td>IRI (pmol/l)</td>
<td>13.2 ± 12.1</td>
<td>7.9 ± 5.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.1 ± 3.0</td>
<td>1.9 ± 1.5</td>
</tr>
</tbody>
</table>

BMI = body mass index; LH = luteinizing hormone; FSH = follicle-stimulating hormone; E₂ = estradiol; PRL = prolactin; T = testosterone; FBS = fasting blood glucose; IRI = insulin resistance index; HOMA-IR = homeostasis model assessment-insulin resistance; *p < 0.01, **p < 0.05 for differences between PCOS and controls by the Mann-Whitney U test. Data represent mean ± SD.

Table 2. — The number of subjects on the basis of adiponectin levels in PCOS and controls.

<table>
<thead>
<tr>
<th>Adiponectin (µg/ml)</th>
<th>PCOS (n = 20)</th>
<th>Controls (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>13 (65%)</td>
<td>7 (35%)*</td>
</tr>
<tr>
<td>≥ 40</td>
<td>6 (27%)</td>
<td>16 (73%)*</td>
</tr>
</tbody>
</table>

*p < 0.05 for differences between PCOS with lower adiponectin levels and controls with higher adiponectin levels by the χ²-test.
The role of serum adiponectin levels in women with polycystic ovarian syndrome

**Results**

Patients and controls were equally distributed according to age, BMI, and degree of obesity (Table 1). LH and LH/FSH ratio were significantly higher in patients with PCOS compared with controls. However, no significant differences were observed between the BMI-matched groups.

The results of the univariate analysis of the effects of PCOS or of control status and of the degree of obesity are shown in Figure 1. Serum adiponectin levels were significantly lower in the ≥ 25 kg/m² BMI group than among normal-weight (BMI < 25 kg/m²) women among PCOS patients; however, these levels were not affected by obesity in controls.

These levels were also significantly lower in women with a HOMA score greater than 1.7, compared with those with an HOMA score less than 1.7 among PCOS patients. No difference was found in adiponectin levels among controls as shown in Figure 2.

Women with PCOS (subjects) were classified according to serum adiponectin levels as described in Methods. When PCOS patients and controls were divided into two groups by serum adiponectin level (< 40 µg/ml, ≥ 40 µg/ml), 65% of patients with PCOS were included in the lower adiponectin group (Table 2). LH and LH/FSH ratio were significantly increased in lower adiponectin group (group 1) compared with higher adiponectin group (group 2) among PCOS patients shown in Figure 3. By contrast, there were no significant differences between two groups in other hormone levels (Table 3).

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**Table 3.** Baseline characteristics and hormonal features in PCOS and controls.

<table>
<thead>
<tr>
<th></th>
<th>PCOS &lt; 40 µg/ml</th>
<th>PCOS ≥ 40 µg/ml</th>
<th>Controls &lt; 40 µg/ml</th>
<th>Controls ≥ 40 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29.4 ± 4.8</td>
<td>33.9 ± 3.3</td>
<td>30.0 ± 4.6</td>
<td>30.5 ± 5.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 5.5</td>
<td>20.3 ± 1.7</td>
<td>26.7 ± 3.5</td>
<td>21.0 ± 3.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0 ± 2.3</td>
<td>3.0 ± 4.2</td>
<td>3.4 ± 2.1</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>9.5 ± 6.5</td>
<td>12.3 ± 5.4</td>
<td>11.6 ± 8.0</td>
<td>12.7 ± 9.9</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>43.5 ± 20.9</td>
<td>47.0 ± 31.3</td>
<td>23.8 ± 5.8</td>
<td>41.4 ± 23.7</td>
</tr>
<tr>
<td>T (pg/ml)</td>
<td>40.4 ± 49.5</td>
<td>22.7 ± 19.2</td>
<td>36.2 ± 8.2</td>
<td>30.7 ± 15.3</td>
</tr>
</tbody>
</table>

BMI = body mass index; HOMA-IR = homeostasis model assessment; PRL = prolactin; E2 = estradiol; T = testosterone.

PCOS patients and controls were classified according to serum adiponectin levels as described in Methods. *p < 0.05 vs group 4 *p < 0.01 vs group 4 †p < 0.001 vs group 4 ††p < 0.05 vs group 2 †p < 0.01 vs group 3 for differences between four groups by Bonferroni-Dunn test. Data represent mean ± SD.
Discussion

In the present study, the authors investigated the relationship between endocrine parameters and adiponectin levels in PCOS patients. Adiponectin is thought to be almost exclusively produced in adipose tissue. It was demonstrated that obesity, IR, and type 2 diabetes were associated with low plasma adiponectin levels in previous study [13]. In this data, obese women (BMI ≥ 25 kg/m²) showed significantly decreased fasting serum concentrations of adiponectin as compared with those of matched lean women (BMI < 25 kg/m²) with PCOS.

It has been reported that serum adiponectin levels are decreased in PCOS patients [10, 16, 17]. Thus, this result may be particularly important in the context of the concurrence of obesity (9), IR [18, 19] and/or impaired glucose tolerance [20] in these women. It is well-recognized that IR is frequently observed and has been linked to the clinical and endocrine alterations, such as hyperandrogenism and reproductive disorders in PCOS patients [21, 22]. Likewise, hyperinsulinemia associated with IR might be physiological roles of not only impaired glucose tolerance and type 2 diabetes mellitus, but also atherosclerosis and cardiovascular disease observed in women with PCOS [7, 23].

Overall, these findings are based on the previous studies, in which significant lower adiponectin levels were evident, in obese women with PCOS [15]. On the other hand, lean women with PCOS did not show significant decreases in adiponectin levels as compared with the corresponding lean women in control group.

It is demonstrated that adiponectin is highly-expressed in white adipose tissue, and is by far the most abundant circulating specific protein derived from adipose tissues in humans [13]. The evidence that adiponectin has the potential to enhance insulin sensitivity and to improve glucose metabolism [11, 12, 24, 25] has been demonstrated in vitro and in vivo studies using rodents as a model. The mechanisms of improvement of IR and glucose metabolism by adiponectin are currently under investigation, although it is well-recognized that the effects of insulin-sensitizing agents have been implicated both in the liver and muscle [24].

Consistent with findings in a rodents’ model, the adiponectin levels were involved in obesity, type 2 diabetes mellitus, and cardiovascular disease [12, 26]. In this way, circulating low adiponectin levels in PCOS may not only determine the degree of IR, but could also provide a link to a higher risk of type 2 diabetes mellitus and cardiovascular disease [21].

The decreasing of adiponectin levels may contribute to IR in women with PCOS, because adiponectin is considered to reduce the triglyceride content of muscle, enhancing insulin signaling, and activates peroxisomal proliferator-activated receptor alpha (PPARα), resulting to increase energy combustion. Adiponectin also up-regulates fat oxidation and transport of muscle and inhibits the expression of enzymes with gluconeogenesis, reducing hepatic glucose production by phosphorylation of AMP-activated protein kinase [27].

Overall, one interesting point that arises from these results is that serum adiponectin levels are observed in hormonal differences (elevated LH and LH/FSH ratio) in PCOS, but not observed in controls. The fact that gonadotropin secretion is associated with adiponectin concentrations suggests that it may represent the role of adiponectin on the endocrine condition directly or indirectly in women with PCOS.

In conclusion, these data have shown that compared with controls of similar body weight, PCOS patients have altered adiponectin secretion. These differences may be caused by the result of altered adipose tissue function. Likewise, altered adiponectin secretion may still be involved in the characteristic IR of PCOS. Further studies will be needed to elucidate this issue.

Acknowledgement

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References

The role of serum adiponectin levels in women with polycystic ovarian syndrome


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Does tension-free vaginal tape and tension-free vaginal tape-obturator affect urodynamics? Comparison of the two techniques

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Summary

Aim: To evaluate the effects of tension-free vaginal tape (TVT) and tension-free vaginal tape-obturator (TVT-O) operations on urodynamics and subjective and objective outcomes. Materials and Methods: Thirty-six patients with stress or mixed urinary incontinence underwent TVT or TVT-O. Bristol Female Lower Urinary Tract Symptoms (BFLUTS) Questionnaire-Scored Form, one-hour pad test, Q-tip test, perineometer, and urodynamics were performed before and after the operations. Blaivas-nomogram was used for assessment of postoperative voiding difficulty. Results: Nineteen patients underwent TVT-O and 17 patients underwent TVT. Mean follow-up was 18.4 ± 6.8 months. There was no difference between two groups regarding demographic variables, degree of prolapse, type of incontinence, perineometer, Q-tip test, pad test, and urodynamics. There was a significant increase in the maximum urethral closure pressure (MUCP) and residual volume in TVT-O group. According to Blaivas-nomogram, five patients had mild, one had medium obstruction in the TVT-O group, whereas one had mild and three had medium obstruction in TVT group. Two bladder perforations occurred during TVT. One patient developed groin pain after TVT-O. Conclusions: TVT-O may lead to an increase in MUCP and residual urine volume. TVT-O is as efficient as TVT and leads to milder obstruction when compared to TVT.

Key words: Stress urinary incontinence; Urinary incontinence; Midurethral sling; Tension-free vaginal tape; Transobturator tape; Urodynamics.

Introduction

Stress urinary incontinence (SUI) is defined as incontinence secondary to increased abdominal pressure such as coughing, sneezing, and heavy lifting [1]. It affects approximately 30% of adult women. In the 20th century, more than 100 surgical techniques for the treatment of urinary incontinence were developed. The tension-free vaginal tape (TVT) procedure, initially described by Ulmsten et al. in 1996, was the first minimally invasive mid-urethral sling procedure with 84% cure and eight percent significant improvement rates at two years follow-up [2]. The complication rates are low for TVT and mainly include bladder injury, hematomas, and transient retention of urine with bladder injury being the most common and occurring in three to nine percent of cases [3-6]. There have been rare reports of bowel and vascular injury with TVT [7]. In order to overcome the bladder, bowel, and vascular injuries related to TVT, transobturator approach (TOT) was developed by Delorme, maintaining the efficacy of TVT and reducing or even eliminating the complications related to the penetration of the retropubic space [8]. In 2003, de Leval described the inside-out technique of transobturator approach (TVT-O) for better control of the vaginal passage [9].

Various studies have been conducted comparing the efficacy and complication rates of these two methods; however, literature lacks sufficient amount of reports concerning the effect of these methods on urodynamics and relationship with the success of these methods. In this study, the authors evaluated the effects of TVT and TVT-O operations on urodynamics and compared the two methods according to patient satisfaction and objective measures of success.

Materials and Methods

Thirty-six patients admitted to the present institution with the complaint of SUI or mixed urinary incontinence and operated were included in this prospective study. Informed consent was obtained from all patients. Ethics approval was obtained from the local ethics committee. The patients were randomly assigned and 19 patients underwent TVT-O and the remaining 17 underwent TVT. Preoperative and postoperative evaluations included urinalysis, urine culture, urogynecologic symptom assessment and gynecologic examination, one-hour pad test, four-day bladder diary, stress test, Q-tip test, and urodynamics were performed. Pelvic organ prolapse was evaluated using Baden-Walker Halfway System. The Bristol Female Lower Urinary Tract Symptoms Questionnaire-Scored form (BFLUTS) was used to evaluate the effect of SUI on the patient’s everyday life and for the quantification of the lower urinary tract symptoms [10]. Urodynamic studies (MMS UD-2000) included uroflowmetry, multichannel cystometry, and urethral pressure profile. In cases of grade 3 and more pelvic organ prolapse, normal anatomy was restored using a pessary or a vaginal tampon during the tests.

The same surgeon performed all of the surgical procedures. The operations were performed with spinal or general anesthesia according to patient preference in accordance with original techniques described by Ulmsten and De Leval. For TVT oper-
tions Gyneecare TVT, for TVT-O operations Gyneecare TVT Obturator System tension- free support for incontinence was used. Cystoscopy was routinely performed in all of the TVT procedures and in suspected cases during TVT-O operations.

Foley catheter was introduced during all of the operations and kept for 24 hours in cases of isolated midurethral sling operations, and kept for three days if anterior colporrhaphy was included. The residual urine volume was measured after the Foley catheter was removed and the patients were discharged when the residual urine volume was < 100 ml. In case of urinary retention, the catheter was inserted and kept in place for an additional 24 hours. Perioperative and postoperative complications were noted in all of the cases.

Patients were re-evaluated at three to 12 months after surgery. Groups were compared according to demographic variables, urinary leakage, pad usage, voiding problems, Q-tip test, stress test, pad test, uroflowmetry, cystometry, and urethral pressure profile. For the determination of postoperative bladder outlet obstruction, Blaivas nomogram was used [11].

Statistical analysis

Statistical analysis was performed with the computer program Statistical Package for the Social Sciences (SPSS) 11.0 for Windows by a professional statistician. Data are expressed as mean ± standard deviation. All univariate comparisons were performed using Student’s t-test in cases where the data were normally distributed. Normality assumption was performed and Mann Whitney U test, Wilcoxon signed rank test, Spearman correlation, chi-square test, and McNemar chi-square test were used for abnormally distributing data. All outcome comparisons were one-sided to compare the methods used in each group to assess the improved outcomes. Comparisons of patient characteristics were two-sided. A p value less than 0.05 was considered statistically significant.

Results

Thirty-six patients were included in the study. TVT-O was performed in 19 patients and 17 patients underwent TVT. The demographic variables of patients are summarized in Table 1. There was no statistically significant difference between the groups for age, body mass index, parity, menopause, history of previous prolapse surgery, previous anti-incontinence surgery, previous hysterectomy, total abdominal hysterectomy, vaginal hysterectomy, Manchester operation, vaginal hysterectomy + colporrhaphy posterior, vaginal hysterectomy + colporrhaphy anterior + posterior, Manchester operation, vaginal hysterectomy, total abdominal hysterectomy, vaginal hysterectomy + colporrhaphy anterior + posterior, and vaginal hysterectomy + colporrhaphy anterior + posterior.

Five patients (26%) in the TVT-O group and four patients (24%) in the TVT group had genuine SUI. Fourteen patients (74%) in the TVT-O group and 13 patients (76%) in the TVT group suffered from mixed urinary incontinence. Eighteen (96%) of the patients in the TVT-O group and all of the patients in the TVT group had a cystocele, two patients in the TVT group, and three patients in the TVT group had a rectocele, nine patients in the TVT-O group, and eight patients in the TVT group had uterine prolapse. The types of the operations performed are summarized in Table 2. Four patients in the TVT-O group and three patients in the TVT group did not have pelvic organ prolapse and underwent sling operation only.

No significant difference was observed between the TVT and TVT-O groups in terms of preoperative pad test, Q-tip test, perineometer results, and bladder diaries. All of the patients had urethral mobility before the operation. Only five patients in the TVT-O group and two patients in the TVT group had negative pad test results before the opera-

| Table 1. — Demographic variables of the two groups. |
|------------------|----------|----------|----------|
|                  | TVT-O (n = 19) | TVT (n = 17) | p        |
| Age              | 51.1 ± 9.3    | 50.6 ± 8.0 | > 0.05*  |
| Body mass index (kg/m²) | 30.9 ± 4.9   | 30.4 ± 4.3 | > 0.05*  |
| Parity           | 3.58 ± 1.54   | 3.06 ± 1.30 | > 0.05*  |
| Menopause        | 9 (47%)       | 9 (52%)    | > 0.05*  |
| Hormone replacement treatment | 3 (16%)      | 3 (17%)    | > 0.05*  |
| Previous anti-incontinence surgery | 1 (0.05%)    | 0          | > 0.05*  |
| Previous hysterectomy | 2 (10.5%)    | 3 (17.6%)  | > 0.05*  |
| Previous prolapse surgery | 2 (10.5%)    | 1 (5.8%)   | > 0.05*  |

*Student-t test; †Mann-Whitney U-test; °Fischer chi-square test.

| Table 2. — Operations performed in the two groups. |
|------------------|-----------|-----------|
|                  | TVT-O (n = 19) | TVT (n = 17) |
| Sling operation only | 4          | 3          |
| Prolapse operation included | 15         | 14         |
| Vaginal hysterectomy + colporrhaphy anterior + posterior | 6          | 5          |
| Colporrhaphy anterior | 1          | 0          |
| Colporrhaphy posterior | 3          | 0          |
| Colporrhaphy anterior + posterior | 1          | 3          |
| Manchester operation | 1          | 0          |
| Vaginal hysterectomy | 2          | 1          |
| Total abdominal hysterectomy | 1          | 3          |
| Vaginal hysterectomy + colporrhaphy anterior + posterior | 0          | 2          |

Chi square test, p > 0.05.

| Table 3. — One-hour pad test, Q-tip Test, and perineometer results of the two groups before and after surgery. |
|------------------|---------------|---------------|-----------|
|                  | TVT-O (n = 19) | TVT (n = 17) | p         |
| One-hour pad test (gr) | 16.1 ± 26.8   | 0             | > 0.05*   |
| (5.0)             | (0-100)       | (0-70)        |
| Q-tip Test (°)    | 62.5 ± 17.5   | 45.0 ± 20.4   | > 0.05*   |
| (35-90)           | (20-90)       | (35-85)       |
| Perineometer (cm H₂O) | 24.4 ± 13.4   | 25.4 ± 13.5   | > 0.05*   |
| (4-51)            | (3.0-45.0)    | (4-44)        |

*Mann-Whitney U test.

| Table 4. — Comparison of the preoperative and postoperative bladder diaries. |
|------------------|---------------|---------------|-----------|
|                  | TVT-O (n = 19) | TVT (n = 17) | p         |
| Mean amount of fluid intake/day (ml) | 1793 ± 494   | 1808 ± 594   | > 0.05*   |
| (600-2800)       | (600-2566)    | (837-3219)    |
| Mean daytime micturition number of micturition | 8.5 ± 2.6    | 6.5 ± 2.4    | 0.05       |
| (5-14)           | (4-12.5)      | (4-17)       |
| Mean number of urgency episodes/day | 4.6 ± 4.7    | 1.8 ± 1.0    | 0.05       |
| (0-15)           | (0-14)        | (0-16)       |
| Mean number of leakage/day | 2.2 ± 2.7    | 0.5 ± 0.9    | 0.05       |
| (0-8.5)          | (0-2.5)       | (0-14)       |

Statistical analysis

Statistical analysis was performed with the computer program Statistical Package for the Social Sciences (SPSS) 11.0 for Windows by a professional statistician. Data are expressed as mean ± standard deviation. All univariate comparisons were performed using Student’s t-test in cases where the data were normally distributed. Normality assumption was performed and Mann Whitney U test, Wilcoxon signed rank test, Spearman correlation, chi-square test, and McNemar chi-square test were used for abnormally distributing data. All outcome comparisons were one-sided to compare the methods used in each group to assess the improved outcomes. Comparisons of patient characteristics were two-sided. A p value less than 0.05 was considered statistically significant.

Results

Thirty-six patients were included in the study. TVT-O was performed in 19 patients and 17 patients underwent TVT. The demographic variables of patients are summarized in Table 1. There was no statistically significant difference between the groups for age, body mass index, menopausal state, hormone treatment, and surgical history. One patient in the TVT-O group had a history of periurethral injection, which was unsuccessful.

Five patients (26%) in the TVT-O group and four patients (24%) in the TVT group had genuine SUI. Fourteen patients (74%) in the TVT-O group and 13 patients (76%) in the TVT group suffered from mixed urinary incontinence. Eighteen (96%) of the patients in the TVT-O group and all of the patients in the TVT group had a cystocele, two patients in the TVT-O group, and three patients in the TVT group had a rectocele, nine patients in the TVT-O group, and eight patients in the TVT group had uterine prolapse. The types of the operations performed are summarized in Table 2. Four patients in the TVT-O group and three patients in the TVT group did not have pelvic organ prolapse and underwent sling operation only.

No significant difference was observed between the TVT and TVT-O groups in terms of preoperative pad test, Q-tip test, perineometer results, and bladder diaries. All of the patients had urethral mobility before the operation. Only five patients in the TVT-O group and two patients in the TVT group had negative pad test results before the opera-
Stress test was positive in five patients in the TVT-O group and four patients in the TVT group (Tables 3 and 4).

There was no significant difference in the preoperative urodynamic parameters between the two groups. Six patients (32%) with mixed urinary incontinence and two patients (10%) with SUI in the TVT-O group, and five patients (30%) with mixed urinary incontinence in the TVT group had detrusor overactivity during cystometry. SUI was observed in eight patients (42%) in the TVT-O and seven patients (37%) in the TVT group. There was no difference in the mean abdominal leak point pressure between the two groups. The results are summarized in Table 5.

The mean follow-up period was 18.4 ± 6.8 months. Cure was accomplished in 89.5% of the TVT-O group, 65% of the TVT group and 10.5% of the TVT-O and TVT group improved (p < 0.002). Stress test was negative in all of the patients. Only one patient in the TVT group and one patient in the TVT-O group did not have urethral mobility in the postoperative evaluation (Table 3). None of the patients who underwent TVT-O complained of SUI in the postoperative period, but two patients (10.5%) suffered from urge urinary incontinence. One had mixed urinary incontinence before the operation, and the other one developed de novo. One patient (5.8%) in the TVT group suffered from SUI, four patients (23.5%) had urge urinary incontinence, and one patient (5.8%) had mixed urinary incontinence. There was a significant difference between the two groups in total number of incontinent patients. When the postoperative pad test results were compared, none of the patients in the TVT-O group and only two patients in the TVT group had a positive pad test (p > 0.05).

BFLUTS results revealed no significant difference in the symptoms of obstructed voiding between the two groups, but in both groups, postoperative voiding dysfunction increased; 41% of the TVT-O group and 42% of the TVT group noted changing in the voiding pattern and hesitancy during voiding. Frequency of micturition and pad usage decreased (Table 6).

When the postoperative urodynamic parameters were compared, there was no significant difference in maximum flow rate, the time to reach maximum flow, micturition volume, residual urine volume, compliance, maximum detrusor pressure, abdominal leak point pressure,

<table>
<thead>
<tr>
<th>Table 5. — Comparison of uroflowmetry, cystometry, and urethral pressure profile before and after surgery.</th>
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</thead>
<tbody>
<tr>
<td><strong>Uroflowmetry</strong></td>
</tr>
<tr>
<td>Maximum flow rate (ml/s)</td>
</tr>
<tr>
<td>Preoperative: 28.3 ± 8.3 (13-43)</td>
</tr>
<tr>
<td>Postoperative: 17.8 ± 6.1 (11-42)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Maximum urethral pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 8.6 ± 0.8 (2.3-36)</td>
</tr>
<tr>
<td>Postoperative: 8.9 ± 3.9 (4-15)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Residual urine volume (ml)</td>
</tr>
<tr>
<td>Preoperative: 20.6 ± 21.4 (0-80)</td>
</tr>
<tr>
<td>Postoperative: 38.9 ± 52.4 (0-90)</td>
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<tr>
<td>p: = 0.047</td>
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<tr>
<td>Micturition time (sn)</td>
</tr>
<tr>
<td>Preoperative: 30.5 ± 11.6 (14-55)</td>
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<tr>
<td>Postoperative: 42.0 ± 24.5 (18-96)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td><strong>Cystometry</strong></td>
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<tr>
<td>First sensation of urine (ml)</td>
</tr>
<tr>
<td>Preoperative: 187 ± 57 (125-319)</td>
</tr>
<tr>
<td>Postoperative: 175 ± 45 (121-391)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Strong sensation of urine (ml)</td>
</tr>
<tr>
<td>Preoperative: 323 ± 256 (no-699)</td>
</tr>
<tr>
<td>Postoperative: 496 ± 131 (251-658)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
</tr>
<tr>
<td>Maximum bladder capacity (ml)</td>
</tr>
<tr>
<td>Preoperative: 595 ± 177 (184-720)</td>
</tr>
<tr>
<td>Postoperative: 609 ± 153 (317-717)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Compliance (ml/ml/cmH2O)</td>
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<tr>
<td>Preoperative: 75.9 ± 56.6 (13-210)</td>
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<tr>
<td>Postoperative: 124 ± 156 (13-450)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Maximum detrusor pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 10.3 ± 8.5 (3-36)</td>
</tr>
<tr>
<td>Postoperative: 14.5 ± 10.6 (3-85)</td>
</tr>
<tr>
<td>p: = 0.021</td>
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<tr>
<td>Abdominal leak point pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 78.8 ± 29.3 (40-131)</td>
</tr>
<tr>
<td>Postoperative: 85.2 ± 41.5 (22-141)</td>
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<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Detrusor leak pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 27.0 ± 11.9 (15-47)</td>
</tr>
<tr>
<td>Postoperative: 30.6 ± 16.8 (13-52)</td>
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<tr>
<td>p: &gt; 0.05</td>
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<tr>
<td>Detrusor pressure at micturition (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 35.7 ± 23.5 (5-85)</td>
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<tr>
<td>Postoperative: 25.0 ± 7.5 (12-32)</td>
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<tr>
<td>p: = 0.05</td>
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<tr>
<td>Maximum urethral pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 48.7 ± 25.8 (25-115)</td>
</tr>
<tr>
<td>Postoperative: 68.1 ± 34.0 (23-131)</td>
</tr>
<tr>
<td>p: = 0.05</td>
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<tr>
<td>Maximum urethral closure pressure (cm H2O)</td>
</tr>
<tr>
<td>Preoperative: 43.4 ± 30.8 (0-115)</td>
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<tr>
<td>Postoperative: 63.1 ± 25.8 (23-107)</td>
</tr>
<tr>
<td>p: = 0.031</td>
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<tr>
<td>Proximal urethral length (cm)</td>
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<tr>
<td>Preoperative: 3.1 ± 0.5 (2.2-3.9)</td>
</tr>
<tr>
<td>Postoperative: 2.9 ± 0.6 (1.5-3.7)</td>
</tr>
<tr>
<td>p: = 0.05</td>
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<table>
<thead>
<tr>
<th>Table 6. — Comparison of the symptoms of voiding difficulty of the two groups before and after surgery.</th>
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</thead>
<tbody>
<tr>
<td><strong>TVT-O</strong></td>
</tr>
<tr>
<td>Preoperative: 14 (58%)</td>
</tr>
<tr>
<td>Postoperative: 0 (0%)</td>
</tr>
<tr>
<td><strong>TVT</strong></td>
</tr>
<tr>
<td>Preoperative: 16 (78%)</td>
</tr>
<tr>
<td>Postoperative: 11 (55%)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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<thead>
<tr>
<th>Table 7. — Comparison of the complications encountered during or after TVT-O and TVT procedures.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complications</strong></td>
</tr>
<tr>
<td><strong>TOT</strong></td>
</tr>
<tr>
<td>Preoperative: 2 (12%)</td>
</tr>
<tr>
<td>Postoperative: 0 (0%)</td>
</tr>
<tr>
<td><strong>TVT</strong></td>
</tr>
<tr>
<td>Preoperative: 2 (12%)</td>
</tr>
<tr>
<td>Postoperative: 0 (0%)</td>
</tr>
<tr>
<td>p: &gt; 0.05</td>
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* Mann-Whitney U test; Wilcoxon rank test.
sure, detrusor pressure at micturition, maximum urethral pressure, and functional urethral length. The results are summarized in Table 5. The mean maximum urethral closure pressure was 63.1 ± 25.8 cm H₂O (23-107 cm H₂O) in the TVT-O group and 45.1 ± 18.3 cm H₂O (11-86 cm H₂O) in the TVT group. There was a significant increase in the maximum urethral closure pressure and residual urine volume in patients who underwent TVT-O operation (Table 4).

As shown in Figure 1, none of the patients in the TVT-O group had severe obstruction according to Blaivas nomogram. One patient (5.2%) was in the moderate and five patients (26%) were in the mild obstruction group. In the TVT group, three patients (17%) were in the moderate obstruction group and one patient (26%) was in the mild obstruction group.

Intraoperative and postoperative complications are summarized in Table 7. Postoperative urinary retention developed in two patients in both groups, but resolved completely shortly after. Groin pain developed in one patient in the TVT-O group. De novo urge incontinence was seen in one patient in the TVT-O group. Bladder perforation occurred in two patients in the TVT group.

Discussion

In this study, the effect on urodynamics and subjective and objective outcomes of TVT and TVT-O procedures have been evaluated. Subjective and objective criteria including urodynamics were used to compare the TVT and TVT-O operations. There was no difference between the two groups regarding age, parity, menopausal state, and the prevalence of mixed urinary incontinence; therefore the two groups were suitable for comparison in this study.

There was a significant difference in patient satisfaction and cure rates in the two groups with more patients cured in the TVT-O group. In this study, cure was defined as no leakage episodes after surgery. In another study regarding the success rate of TVT operation from the present institution with mean follow-up period of 11 months (1-24 months), the cure rate was 90% and 10% of the patients had improved [12]. The mean age of the population studied and the inclusion of other vaginal surgical procedures were similar to the present study group. Various other reports presented 90% cure-rate in the first year after surgery using TVT with reduction in the success rate when cases with intrinsic sphincter deficiency and pelvic organ prolapse were included [13-15]. In most of the studies evaluating the success rate of TVT operations, patients with pelvic organ prolapse and pelvic reconstruction surgery and previous anti-incontinence surgery have been excluded. Tsivian et al. [16] reported that when these cases are included, the cure rate declines to 78.9%. In a recent systematic review, retropubic procedures have shown greater objective success, but no difference in subjective outcomes [17]. The current authors did not accept the patients as cured when there were still symptoms (loss, urge, high residual urine volume), even if they had a negative pad test and stress test result and no leakage in urodynamics. However, this study demonstrates that TVT-O is as successful as TVT operation and the rate of complications is very low. In a study evaluating the patient perceptions of success after TOT and TVT, 65.5% of the patients in the TVT group and 63.4% of the TOT group reported no stress incontinence [18]. Similarly, in a recent multicenter randomized controlled trial using both objective and subjective outcomes, the success rate for TVT was 80.8% and for TOT was 77% [19] However, for subjective outcomes, success rates were 62.6% and 55.5% for TVT and TOT, respectively.

These two operations do not aim at correcting the urethral hypermobility. On the contrary, the persistence of urethral hypermobility after surgery is important for the dynamic movement of the urethra during increases in intra-abdominal pressure [20]. It was shown that urethral mobility was not affected after TVT [21, 22]. The continence mechanisms of TVT and TOT and TVT-O are similar. Fellipi showed the persistence of urethral mobility after TOT operation using cystography [23]. In another study using a Q-tip test, no effect of TOT on urethral mobility was found [24]. According to the present study, neither TVT nor TVT-O affected the urethral mobility in the postoperative period.

One of the major complications of both procedures is voiding difficulty, which may be observed after incontinence procedures. Significant portion of the patients began to suffer from hesitancy and voiding difficulty after the operations in both groups. Porena et al. reported voiding difficulty in 44% and 24% following TVT and TOT, respectively [24]. However, in other studies lower rates of voiding dysfunction were reported. Definitions of voiding difficulty vary between studies, so it is difficult to draw conclusions.
Persistence of urge incontinence or de novo urge incontinence may occur following the aforementioned procedures. In the present cohort, urge incontinence and de novo urge incontinence was not observed in the TVT group. One patient suffered from de novo urge incontinence in the TVT-O group. Thirteen patients in the TVT group had mixed urinary incontinence symptoms before surgery and this was reduced to five patients after surgery. Similarly, 14 patients had mixed urinary incontinence symptoms before the operation and only one patient after surgery had mixed symptoms in the TVT-O group. Segal et al. [25] studied the effect of TVT on urge urinary incontinence and detrusor overactivity. In this study, urge incontinence symptoms disappeared in 63.1% of the mixed urinary incontinence cases in the TVT group and 57.7% could stop their anticholinergic drugs. The present findings are in parallel. Similarly TVT-O leads to a reduction in urge incontinence symptoms in patients with mixed urinary incontinence.

Both TVT and TVT-O have high rates of success in the treatment of SUI and one would anticipate changes in the urodynamic parameters after these procedures. However, there was no significant difference in the urodynamics between TVT and TVT-O. There was a significant increase in the maximum urethral closure pressure in the postoperative evaluation in the TVT-O group. According to the present study, TVT-O might as well be effective in the treatment of intrinsic sphincter deficiency together with urethral hypermobility in patients. There was also a slight increase in the residual urine volume and micturition time. In the Blaivas nomogram, which shows voiding difficulty based on maximum detrusor pressure and maximum flow rate, there was no significant difference between the two groups. None of the patients in the TVT-O group had severe obstruction. Higher rates of voiding difficulty have been reported with TVT compared with TOT operation [16], possibly because it is more obstructive; but this was not seen in the study by Richter et al. [19].

The other complications following both techniques include bladder perforation, vascular injuries, hematomas, vaginal perforations, and groin pain [3-7]. In this study, there was a low rate of complications (Table 7). Two bladder perforations developed in the TVT group during the operations and the inserted needle was removed and re-inserted. No bladder perforations, vaginal sulcus injury or vascular injury developed in the TVT-O group, but one patient suffered from groin pain.

**Conclusion**

This study demonstrates that TVT and TVT-O procedures have high success rates with minimal effect on bladder storage and voiding functions. Both work well in patients with mixed urinary incontinence and pelvic organ prolapse. However, TVT-O procedure resulted in a higher cure rate with a significant increase in maximum urethral closure pressure than did the TVT procedure.


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Effect of short-term tibolone treatment on risk markers for cardiovascular disease in healthy postmenopausal women: a randomized controlled study

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Summary

Objective: The aim of this prospective randomized controlled cross sectional study was to evaluate the effect of a six month tibolone treatment in healthy postmenopausal women on biochemical CVD markers by calculating the changes of the blood serum levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (Tg), high-sensitivity C-reactive protein (hsCRP), homocysteine (Hcy), and endothelin-1 (ET-1) at the beginning of the treatment and after six months. Materials and Methods: Fifty-two healthy postmenopausal women were enrolled in a prospective, randomized, case-controlled outpatient trial. Group 1 (n = 26) received 2.5 mg/d tibolone for six months, while Group 2 (n = 26) received no treatment. Serum levels of TC, LDL, HDL, Tg, hsCRP, Hcy, and ET-1 were evaluated at baseline and after six months. Results: The two groups did not statistically differ at baseline characteristics. In Group 1 tibolone treatment decreased significantly TC (p = 0.01), HDL (p < 0.001), and Tg (p < 0.001) serum levels while a significant increase of hsCRP (p < 0.001) was observed. Finally no changes were noticed on LDL, Hcy, and ET-1 serum levels. Regarding Group 2, no changes were observed. Conclusion: Short-term tibolone treatment in healthy postmenopausal women exerts a mixed action, acting beneficially in some markers (TC, LDL, Tg, Hcy, and ET-1) where as detrimentally in others (HDL, hsCRP). Key words: Tibolone, cardiovascular disease; Risk markers; Postmenopausal women.

Introduction

Menopause is mainly connected to the gradual and massive reduction of the estrogen levels in women. This hormonal condition has different effect on various target organs such as the uterus, vaginal mucosa, skin, and endothelium. The protective role of estrogens on the endothelium has been proven by multiple studies and so menopause can induce endothelial dysfunction and lead to metabolic syndrome and cardiovascular disease (CVD), the first cause of death in women during the postmenopausal period [1, 2]. Several biochemical substances in the blood serum have been studied and used as present as valuable risk markers for CVD such as total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (Tg), high-sensitivity C-reactive protein (hsCRP), homocysteine (Hcy), endothelin-1 (ET-1), and many others both in men and women, mainly addressed to the endothelial function.

Hormone replacement therapy (HRT) in postmenopausal women had been welcomed with enthusiasm at the beginning, both by patients and clinicians due to the relief of the postmenopausal symptoms and the proven positive effect on the evolving osteoporosis during menopause and the positive effect on the prevention of CVD [3]. On the contrary the results of randomized-controlled studies showed that HRT has adverse effects on the cardiovascular system [4]. Further studies in the past decade has given more clarity in the safe length of HRT regimes and made patients less reluctant to the use of it [5].

Tibolone is a synthetic steroid with tissue-specific estrogenic, androgenic, and progestogenic properties. It mainly acts as an agonist at all Type I steroid hormone receptors [6]. It was primarily used against osteoporosis but nowadays is also used as an alternative to HRT for relief of menopausal symptoms. Though, acting as an estrogen, data suggest that tibolone may have cardio-protective role by acting positively on biochemical risk factors for CVD, when used in postmenopausal women [7]. The results among relevant studies on the topic are still conflicting.

Materials and Methods

Fifty-two Caucasian healthy postmenopausal women were enrolled in a prospective, randomized, case-controlled outpatient trial. All women presented at the Menopause Outpatient Clinic of the present university teaching hospital after referral for postmenopausal symptoms. After consultation the patients were randomized in two groups. Group 1 (n = 26) received 2.5 mg/d tibolone for six months, while Group 2 (n = 26) received no treatment. Randomization was carried out by using sealed envelopes containing computer-generated randomization numbers. Informed consent was obtained from all women and the study was approved by the regional ethical committee.
Inclusion criteria in the study were: the time interval since the last menstrual bleeding (MSM) more than 4 months; for surgical menopause time interval ≥ four months, and the body mass index (BMI) < 30 kg/m². In all patients follicular stimulating hormone (FSH) was > 40 IU/L. Moreover all patients were healthy without taking any medication. Exclusion criteria were: medical history of thrombophilia, arterial hypertension, CVD, hepatic or kidney disease, thyroid disease, diabetes mellitus, use of HRT more than six months prior to the study, and any type of neoplasia. All women were requested to avoid any diet and lifestyle modifications or commence any long-term medication during the trial.

During the first visit in the clinic, medical history was taken and also clinical examination, transvaginal ultrasound (TVUS) of the internal genital organs, and smear test collection were performed. Further bone densitometry (DEXA) of the hip was ordered and performed by the radiology department of this hospital. At the same day blood sampling was performed after 12 hours of fasting for the evaluation of serum levels of TC, LDL, HDL, Tg, hsCRP, Hcy, and ET-1. Six months later, another blood sampling was performed under the same conditions for the evaluation of the same markers.

**Assays**

ET-1 serum levels were measured using ET-1 ELISA kits. The sensitivity of the kit is 0.064 pg/ml. ET-1 concentrations were found to be in the range 0.401-2.83 pg/ml. HsCRP serum levels were measured using Cardiphase hsCRP ELISA kits. Expected values for healthy individuals are typically ≤ 3 mg/l. The sensitivity of the method is 0.175 mg/l. Serum Hcy levels were measured by fluorescence polarization immunoassay. Within-assy and between-assy CV were 1.4 – 2.2% and 2.9 – 4.8%, respectively. TC serum levels were measured by enzymatic method. Expected values for normal individuals were < 200 mg/dl. Within-assy and between-assy CV were 0.8% and 1.7%, respectively. LDL serum levels were measured by enzymatic method. Expected values for normal individuals were < 100 mg/dl. Within-assy and between-assy CV were 0.71-0.81% and 1.16-1.2%, respectively. HDL serum levels were measured by enzymatic method. Expected values for normal individuals were ≥ 55 mg/dl. Within-assy and between-assy CV were 0.58-0.9% and 1.3-1.85%, respectively. Tg serum levels were measured by enzymatic method. Expected values for normal individuals were < 130 mg/dl. Within-assy and between-assy CV were 1.5% and 1.8%, respectively.

**Statistics**

Statistical analysis was conducted with the use of SPSS 17.0 and STATISTICA 8.0. The Kolmogorov-Smirnov test was used to check normality assumptions. All data are expressed as mean ± standard error of mean (SEM). Differences between groups were evaluated with t-test or Mann-Whitney U-test, where appropriate. A repeated measures ANOVA was used for the assessment of group differences over time. Fisher’s post-hoc test was employed. All tests were performed at level α = 0.05. All values are expressed as mean ± SEM and statistical significance was set for confidence interval (CI) 95% (p < 0.05). In cases of p > 0.05, it was characterized as non-significant (NS).

**Results**

There was no statistical significant differences at the basic characteristics between the two groups (Group 1 vs Group 2) regarding the age (50.46 ± 0.52 vs 51.84 ± 0.54), BMI (25.44 ± 0.26 vs 24.84 ± 0.32), and MSM (16.8 ± 1.59 vs 18.81 ± 1.75) (Table 1).

At baseline, no statistical significant difference was found between the two groups (Group 1 vs Group 2) regarding TC (204.38 ± 4.33 vs 210.57 ± 6.2 mg/dl), LDL (130.80 ± 4.33 vs 134.46 ± 6.75 mg/dl), HDL (52.65 ± 2.39 vs 51.42 ± 2.38 mg/dl), Tg (106.08 ± 6.61 vs 113.04 ± 5.72 mg/dl), hsCRP (1.22 ± 0.15 vs 1.23 ± 0.13 mg/l), Hcy (10.26 ± 0.52 vs 9.98 ± 0.41 mmol/l), and ET-1 (1.29 ± 0.11 vs 1.03 ± 0.08 pg/ml) (Table 2).
pg/ml levels. In the group of women who did not receive tibolone serum levels of TC (206.34 ± 5.45 mg/dl), LDL (132.61 ± 5.8 mg/dl), HDL (50.53 ± 2.3 mg/dl), Tg (113.04 ± 5.72 mg/dl), hsCRP (1.08 ± 0.13 mg/dl), Hcy (10.16 ± 0.4 mmol/l), and ET-1 (0.98 ± 0.08 pg/ml) remained unchanged (Table 2).

Regarding the menopausal symptoms, all patients reported improvement during the six month use of tibolone without mentioning any side-effects.

Discussion

The systematic study of the endothelial function through biochemistry has established several markers in the serum of the blood that can diagnose dysfunction and possible tendency for evolving CVD in these patients. There are many studies including women as patients that examine the short-term effect of different regimes such as oral contraceptives, HRT, tibolone, and others on these markers. In these studies, though that use the same medication, there is an obvious difference in the number of patients participating, the number of markers included, and the length of the study.

In the present study the authors examined the effect of tibolone for a six months period on the most important CVD markers in postmenopausal women. While patients were asked not to change any dietary habits, the results show that tibolone significantly decreased TC, Tg, and HDL serum levels, which is a finding in the majority of the related studies [8-12]. Very few studies did not show any effect on these markers [11, 13], but definitely did not prove that tibolone can cause an increase in a similar period of time.

HsCRP is an acute-phase protein and also a valuable marker of inflammation, but in low levels and without any symptomatic pathology, can be a marker of low-grade chronic inflammation, endothelial dysfunction, and an established CVD marker. In most of the studies where tibolone was used, there was a significant increase of hsCRP serum levels [14-18] and very few studies showed that serum levels remained unchanged during similar time interval [19, 20]. From the present results, the authors agree that tibolone may increase hsCRP serum levels in postmenopausal women during a six-month period course, but not above the physiological range.

The non-significant impact of tibolone on LDL, Hcy, and ET-1 is at least favorable for the endothelial function of postmenopausal women and these results come into agreement with the existing literature regarding LDL [8, 12, 21] and Hcy [15, 22, 23]. Concerning ET-1, tibolone is known to lower the ET-1 levels from the limited existing literature [24, 25].

Limitations of the study can be considered the short-term interval of tibolone use (six months) and also the inclusion of both women with surgical and natural menopause, with different time-interval since the last menstrual period, taking though into consideration that it is still unclear if the CVD risk factors are age or estrogen-related [26]. The authors believe that similar studies only with patients shortly after surgical menopause will give stronger evidence on the subject.

Conclusion

The results of the present study suggest that the use of tibolone in postmenopausal women for six months may have a favorable effect on the endothelial function or at least not negatively affect other CVD markers, excluding HDL and hsCRP. After the safe length of tibolone is established, it would be valuable that more studies with further follow-up of these specific patients be announced in the future, with further biochemical and clinical follow-up and definitely, as in all clinical trials, a proper meta-analysis with adequate number of studies that will further clarify the effect of tibolone on CVD markers in postmenopausal women.

References


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Transvaginal removal of ectopic pregnancy tissue and repair of uterine defect for cesarean scar pregnancy

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Summary

Purpose: This work aimed to introduce a new surgical operation for cesarean scar pregnancy (CSP). Materials and Methods: Transvaginal removal of ectopic pregnancy tissue and repair of a uterine defect were performed in 17 CSP patients. Results: The new surgical operation was performed successfully in all cases. Conclusions: The new surgery operation is safe, effective, and minimally invasive in CSP patients.

Key words: Cesarean scar pregnancy; Transvaginal operation.

Introduction

Cesarean scar pregnancy (CSP) is rare type of ectopic pregnancy and belongs to long-term complications of low segment cesarean sectioning. With the increase in cesarean rates worldwide, the incidence of CSP gradually increased over the years. If not diagnosed and treated, CSP is potentially life-threatening and may lead to severe complications, such as uncontrolled hemorrhage and even hysterectomy [1-3].

Materials and Methods

From September 2011 to January 2012, transvaginal removal of ectopic pregnancy tissue and repair of a uterine defect were performed in 17 CSP patients which were diagnosed with transvaginal sonogram in this hospital. Regarding the surgical technique, the uterus, (Figure 1) low segment was exposed through the anterior vaginal wall and the peritoneum incision was folded back. The uterine defect was an obvious sag in the lower segment. Ectopic pregnancy tissue was removed after a low-segment incision was made to the uterus (Figure 2). The uterine defect and vaginal wall were then both sutured (Figure 3).

Figure 1. — Exposing of lower uterine segment.
Figure 2. — Transvaginal removal of ectopic pregnancy tissue.
Figure 3. — Repair of lower uterine segment.
Results

In all cases, transvaginal surgery was successfully performed. The average operation time was 40 minutes and average bleeding was 20 ml. Serum beta-human chorionic gonadotropin (β-hCG) levels declined to normal levels within a month after surgery.

Discussion

Transvaginal removal of ectopic pregnancy tissue and repair of a uterine defect is a novel surgical operation and it includes several advantages. Firstly, repair of the uterine defect can prevent secondary CSP, secondly, after transvaginal operation, patients have minimal trauma and a rapid recovery time. Lastly, it may save admission costs because the patients remain hospitalized for only three to four days. Overall, based on this observation of 17 treated cases, the transvaginal removal of ectopic pregnancy tissue and repair of the uterine defect is safe, effective, and minimally invasive in patients with CSP.

References


Threatened miscarriage in the first trimester and retrochorial hematomas: sonographic evaluation and significance

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Summary

Background: Vaginal bleeding during the first half of pregnancy occurs in approximately 25% of women and about half of these pregnancies terminate in abortion. In many instances a retrochorial hematoma (RCH) is sonographically found. Objective: The aim of the present study was to determine the frequency of a RCH in the group of threatened miscarriages and to examine the possible relationship of parity, previous miscarriages, hematoma size and localization, and duration of vaginal bleeding to pregnancy outcome. Materials and Methods: The study group consisted of 45 women of 852 (5.2 %) referred for ultrasound examination due to vaginal bleeding in the first trimester of pregnancy, who were found to have a RCH in the presence of a singleton live embryo. The control group consisted of 807 women with the same gestational age, with vaginal bleeding, and vital singleton pregnancy without sonographically proven RCH. All were followed with repeated sonograms at seven days intervals until bleeding ceased, the RCH disappeared or abortion occurred. The authors have examined the possible relationship of duration of vaginal bleeding, hematoma size and localization, parity, and previous miscarriages to pregnancy outcome (spontaneous abortion, term or preterm delivery). Results: The researches have shown that the previous miscarriages and deliveries do not affect the occurrence of RCH. In the group with a RCH on the back wall of uterus, as well as repeated bleedings affect higher frequency of spontaneous miscarriages. Hematoma size itself does not affect higher frequency of spontaneous miscarriage. Conclusion: Ultrasound is the method of choice for diagnosing the existence of a RCH. The frequency of RCH in the group of threatened spontaneous miscarriages is 5.2 %. A RCH on the back wall and repeated bleedings affect higher frequency of spontaneous miscarriages.

Therapy procedure is based on strict bed rest and administration of: pregnyl, gestagenic drugs, progesterone, antihistamines, and sedatives.

Key words: Retrochorial hematoma; Spontaneous miscarriage; Ultrasound; Therapy.

Introduction

Vaginal bleeding during the first trimester of pregnancy occurs in approximately 25% of women and about half of these pregnancies terminate in abortion [1]. The main reasons for vaginal bleeding are retrochorial hemorrhage and retrochorial hematoma (RCH) [2].

RCH may be detected sonographically in the first trimester by the presence of a crescent-shaped echo-free area outlining the intact gestational sac [3].

Its etiology is unknown [4]. The risk of abortion in early pregnancies complicated by RCH remains controversial. Bennett et al [5] concluded that fetal outcome depends on the size of the hematoma, whereas Pedersen and Mantoni [6] claimed that even large hematomas do not pose a serious threat. Tower and Regan and Mandruzzato et al. [7,8] concluded that miscarriage occurred in 17.7 % patients with a RCH.

Jouppila [3] in a broadly cited study concluded that there are no therapeutic options, and Ben-Haroush et al. [9] doubted the benefit of bed-rest. The aim of the present study was to determine the frequency of a RCH in the group of threatened miscarriages and to examine the possible relationship of parity, previous miscarriages, hematoma size and localization, and duration of vaginal bleeding to pregnancy outcome.

Materials and Methods

The present study included 852 women referred for ultrasound examination because of vaginal bleeding in the first trimester of pregnancy from 2010-2011 in the present Center.

The study group consisted of 45 women of 852 (5.2 %) who were found to have a RCH in the presence of a singleton live embryo. The control group consisted of 807 women with vaginal bleeding in the first trimester of pregnancy, which were not found to have a RCH in the presence of a singleton live embryo. The sonographic criterion for RCH in the first trimester was a crescent-shaped echo-free area outlining the intact gestational sac.

All patients were clinically followed at seven-day intervals, including bimanual and sonographic examination until the bleeding ceased, the RCH disappeared or abortion occurred.

All sonographic examinations were performed by experts. The women were followed prospectively from the time of the first bleeding episode and data were collected on gestational age at onset of vaginal bleeding, parity, previous miscarriage, duration and frequency of bleeding, size and localization of the RCH, and pregnancy outcome (spontaneous abortion, preterm or term delivery). The intensity and course of bleeding were monitored daily and the therapy was dosed accordingly, with the ultimate goal to stop bleeding. None of the patients suffered from: diabetes mellitus (laboratory analysis confirmed regular glucoregulation), hypertension or autoimmune diseases. The time of bleeding and the time of coagulation were within the limits of referential values in all the patients with a RCH. The number of thrombocytes was in the range of 150,000 - 400,000.

Categorical data were analyzed statistically with Chi-square, Fisher’s exact test, and Student t-test, as appropriate A p value less than 0.05 was considered statistically significant.
Results

In relation to parity the patients in the present study were almost equally proportioned: primiparas 53% and multiparas 47%. The percentage of patients bleeding in the period to eight weeks of gestation was 24.4%, and of the percentage of patients bleeding after eight weeks of gestation was 75.6%. Only five of the patients (11.1%) had one previous spontaneous miscarriage. The authors’ research has shown that previous deliveries and miscarriages do not affect the occurrence of a RCH, which is statistically confirmed ($p > 0.05$).

Eight pregnancies (17.7%) in the group with RCH ended in abortion, and 247 (30.6%) in the control group. In none of them did the weekly ultrasonographic or clinical follow-up reveal any signs of cervical incompetence. The present study has shown that a RCH does not increase the risk of spontaneous miscarriage. By observing the varying sizes of hematomas in the present study, miscarriage occurred with large but also with very small hematomas, which leads to the conclusion that the hematoma size in itself is not an initiating factor for the occurrence of miscarriage. The critical factor is the position of hematoma, as miscarriage was more frequent with localization on the back wall (62.5%), which might be explained by poorer circulation in the spiral arteries on the back wall of uterus (Table 1). The initiating factor for occurrence of miscarriage is bleeding. Miscarriages occurred in the patients with a RCH who bled twice heavily or several times mildly, although they were on an adequate therapy (Table 2). In the patients that bled once and in which bleeding stopped with the prescribed therapy experienced hematoma regression.

Discussion

In view of the presented results as well as the results of other authors’ studies [10,11], the present authors believe that an ultrasound examination is the method of choice for diagnosing the existence of a RCH in patients with signs of threatened miscarriage. In the present study, RCH was found in 5.2% of patients with clinical signs of threatened abortion, which is similar to the results of Stabile et al. [10]. Miscarriage occurred in 17.7% of the presented patients with RCH (mostly due to repeated bleeding), which complies with the results of other authors [7,8]. In the control group (without RCH), miscarriage occurred in 30.6% women. The present study has shown that RCH does not increase the risk of spontaneous miscarriage by itself. Abu-Yousef et al. [1] claim that the poor outcome of pregnancy with a RCH is in connection with the intensity of vaginal bleeding and increase of hematoma volume accompanied with pain. All the patients in this study that had miscarriage bled twice heavily or several times mildly, although they were on adequate therapy. In the patients that bled once and in which bleeding stopped with the prescribed therapy experienced hematoma regression. Other authors [3,5,12,13] reported similar results in their researches, whereas Ben-Haroush et al. [9] claimed that there was no association of pregnancy outcome with duration of vaginal bleeding. In the present study, miscarriage occurred in large but also in very small hematomas, which leads to the conclusion that the hematoma size itself is not an initiating factor for the occurrence of miscarriage. The initiating factor is bleeding, especially repeated bleeding. The study has shown that the critical factor for miscarriage is the position and not the size of the hematoma. Miscarriage occurred more frequently with hematomas localized on the back wall (62.5%), which might be explained by poorer circulation in the spiral arteries on the back wall and perhaps by more difficult discharge of hematoma due to its position. The important indication is the course of hematoma therapy is much bed rest throughout the duration of bleeding. Women who rested during vaginal bleeding had lower percentage of spontaneous miscarriages (9.9%) in relation to those that did not rest (23.3%), as claimed by Ben-Haroush et al. [9]. With the present study, the authors did not succeed in assessing the importance of bed rest in reducing the percentage of miscarriage, because severe cases were admitted to the clinic with signs of threatened miscarriage, so that the lowest percentage of miscarriages was in the group of two patients lying more than 20 days (0%) and less than ten days (6.6%) of bed rest, and the highest was in the group of the women with to to 20 days (46%) of bed rest. However, if the average of miscarriages is taken, regardless of the duration of bed rest, which is 17%, it complies with the results of Ben-Haroush et al. [9]. All the present patients with a RCH were monitored until the final outcome of pregnancy, which was: spontaneous miscarriage (17.7%), or delivery (82.3%) premature or on term. All patients had a vaginal delivery. Newborns had somewhat lighter weight, but without the need for a long term intensive care.

Table 1. — Localization of hematoma and miscarriage.

<table>
<thead>
<tr>
<th>Miscarriage</th>
<th>IZNAD</th>
<th>UN. USCA</th>
<th>Back wall</th>
<th>Front wall</th>
<th>Fundus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
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<td>5</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>16</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>19</td>
<td>18</td>
<td>6</td>
<td>2</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Hemorrhage and miscarriages in the examined group.

<table>
<thead>
<tr>
<th>Hemorrhage</th>
<th>Miscarriage</th>
<th>No miscarriage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once</td>
<td>0</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Twice</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>More than twice</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>37</td>
<td>45</td>
</tr>
</tbody>
</table>
Conclusion

Currently, an ultrasound is the method of choice for diagnosing the existence of a RCH in patients with signs of threatened miscarriage. The frequency of RCH in the group of patients with the signs of threatened miscarriage is 5.2%. RCH in this study group did not experience an increased risk of spontaneous miscarriages (17.7% vs. 30.6%). Parity and previous miscarriage did not cause a more frequent occurrence of a RCH. The size of a hematoma did not substantially affect the final outcome of pregnancy. A RCH localized on the back wall and repeated bleedings caused higher frequency of spontaneous miscarriages. Therapy procedure is based on strict bed rest and administration of: pregnyl, gestogenic drugs, progesterone, antihistamines, and sedatives.

References


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Introduction

For the induction of pneumoperitoneum, the pressures required to provide adequate intra-abdominal operational space (10-15 mmHg) during the laparoscopic surgery are usually higher than the normal physiological portal system circulation pressure (7-10 mmHg). This causes a decrease in micro- and macro-circulation of the abdominal organs and tissues, leading to hypoxia-anoxia especially in splanchnic organs, including the small intestine, liver, and kidneys [1]. In addition to this ischemic-hypoxic period, following deflation, which restores visceral perfusion of organs with oxygenated blood, the generation of reactive oxygen free radicals causes a second-hit to the cell, leading to cell death by both apoptosis and necrosis [1, 2]. As a consequence, laparoscopic surgery may cause ischemia-reperfusion (I/R) injury in the abdominal organs and tissues in a time- and pressure-dependent manner [3].

Hence, during the initial ischemic period, cells may die, which is known as necrosis; after that, following reperfusion of blood, apoptotic loss of cells will take place, requiring energy substituted from the blood stream [4]. Subsequently, cells undergo specific changes in enzyme activities, mitochondrial function, cytoskeletal structure, membrane transport, and antioxidant defenses in response to hypoxia, which then collectively predispose them to reoxygenation injury [5]. A number of mitochondrial enzymes decrease in activity, and expression of the multi-subunit cytochrome oxidase, and cytoskeletal changes could likely alter endothelial and epithelial permeability that can be observed as damaged ultrastructure [5]. All of these structural and morphological changes, owing to oxidative stress and inflammation, can only be correctly ascertained by a transmission electron microscope and not by a light microscope in the early stage, as in the present study. The light microscopic histologic findings are regarded as late stage [6].

To date, no study has investigated the effect of carbon dioxide (CO₂) pneumoperitoneum and different intraperitoneal pressures on the ovarian surface epithelium, ciliated fallopian tube epithelium, and ovarian endothelium. Moreover, studies investigating the effect of capnoperitoneum on the ultrastructure of parietal and visceral peritoneum were evaluated by scanning electron microscope (SEM) only and not by transmission electron microscope [7-11]. Intracellular organelles and DNA cannot be evaluated with SEM. Therefore, the ovarian surface epithelium (being a part of the peritoneum), ovarian endothelium as a surrogate of ovarian microcirculation, and ciliated epithelium of the fallopian tube were evaluated according to the structural configuration.

The aim of the experimental study was to analyze ultrastructural alterations to the integrity of the ovarian surface and fallopian tube epithelium generated by increased intra-abdominal pressure due to capnoperitoneum.
Materials and Methods

**Animals:** This study was performed at the Experimental Research Center of Baskent University. The Ethical Committee approval was obtained. Twenty-four mature (four months old) female, non-pregnant Wistar Albino rats weighing between 170 and 304 g were used as an experimental model. All rats were provided by Animal Laboratory of Baskent University. They were caged in a controlled environment of 22°C with 12 h light/dark cycles. Standard rat feed and reverse-osmosis purified water were provided ad libitum. All rats were allowed to have one week of acclimation to this environment before the experiment. Female Wistar rats were fasted overnight with free access to water containing 20% glucose.

The rats were randomized into three groups each one consisting of total of eight rats: Group 1 (control) had laparotomy and were left for 150 minutes after the incision. Groups 2 and 3 had laparoscopy and were left for 120 minutes under at 10 mmHg and at 15 mmHg of pressure, respectively. Thirty minutes after desufflation, laparotomy was also performed in Groups 2 and 3. In all groups, bilateral ovarioectomy and salpingectomy were performed. The ultrastructures of the ovarian surface epithelium, ovarian endothelium, and fallopian tube ciliated epithelium were evaluated by transmission electron microscope.

The Baskent University Committee on the Use and Care of Animals approved the experiments, and all investigations complied with the 1996 National Academy of Science’s Guide for Care and Use of Laboratory Animals.

**Surgical procedures:** All the rats were anesthetized with an intraperitoneal administration of 50 mg kg⁻¹ ketamine hydrochloric acid and five mg kg⁻¹ xylazine hydrochloric acid. They were immobilized on a standard rat surgery board. Before surgery, the abdominal skin was shaved and antisepsis was achieved with 10% povidone iodine solution. All the animals were kept on a controlled environment of 22°C with 12 h light and 10% humidity. They were caged in a controlled environment of 22°C with 12 h light and 10% humidity.

**Histologic examination:** The specimens were fixed in 2.5% glutaraldehyde in 0.11 of phosphate buffer, pH 7.3, for six hours. The fixative was washed out in buffer for two x 15 min, post-fixed in one percent osmium tetroxide (OsO₄) in the same buffer for 120 min, washed twice in buffer for two x 15 min, and dehydrated in a graded series of ethanol concentrations (25%, 50%, 75%, and 95% absolute alcohol) embedded with araldite 2-dodecyl succinic anhydride (CY 212, DDSA), benzylidimethyl amine (BDMA), and dibutyl phthalate. They were polymerized for 48 h at 56°C in an incubator. Uranyl acetate and lead-citrate dyed ultrathin sections were studied in a transmission electron microscope (LEO 906E EM).

**Analysis of transmission electron microscopy:** In accordance with literature, the normal findings of ultrastructural evaluation of the Ovarian Surface Epithelium (OSE) are described as follows: OSE is heterogeneous and shows deep invagination, and serous-villous like papillary projections. Usually OSE is composed of a single layer of cubic epithelium covered with short uniform villi and differentiated from each other by significant intercellular borders. Golgi apparatus, endoplasmic reticulum at apical cytoplasm, scattered polyomes in the perinuclear cytoplasm, and various numbers of mitochondria are located in the basal and apical zones of cells. Intercellular lateral connections are formed as interdigitation, and in some areas large, asymmetric, irregular gaps are observed. These gaps fill with a pale amorphous substance (intracellular liquid?).

The ultrastructural evaluation of the OSE was categorized into three main groups: apical surface specializations, lateral surface specializations, and organelle modifications. All results were recorded as positive or absent.

Stage 1: Deterioration of lateral face junctions, disordered microvilli distribution, no microvilli observed in the apical surface (M), deletion of mitochondria cristae (cristolysis) in 25% of the cells, swelling in the mitochondria, and vacuolization (V) formation inside the cell.

Stage 2: Cristolysis of mitochondria cristae, presence of residual bodies (R) in the cell, V formation in the cell, and observation of changes as presence of lipid droplets in more than 50% of the cells.

Stage 3: Cristolysis of mitochondria cristae, swollen mitochondria, presence of R bodies in the cell, V formation in the cell, and observation of changes like presence of lipid droplets in more than 50% of the cells.

Stage 4: No remnants of amorphous bodies between the cells, separation of large cytoplasmic bodies from the cell, formation of projections and blebs (B), and complete separation of the cells from the basement membrane.

**Statistical analysis**

The categorical data was evaluated by Chi-Square test. Because the case number for each cell was not sufficient, p value could not be given. Therefore the groups were compared in doubles. Each time point was evaluated separately, and p values less than 0.05/3 = 0.017 was considered significant. SPSS (Statistical Package for the Social Sciences, version 11.0) was used for all analysis.

**Results**

No apical or lateral surface changes or organelle modifications in ovarian surface epithelium were observed in the control group (Figure 1). Apical ovarian surface epithelium changes were statistically significant (p < 0.001) in Groups 2 and 3 in comparison to the control Group (Figures 2-4), but no significant difference was found between Groups 2 and 3 according to the apical
Does carbon dioxide pneumoperitoneum altering pressure levels lead to ultrastructural damage of fallopian tube and ovary?

In terms of lateral surface changes in ovarian surface epithelium, no statistically significant differences were observed among the groups. The organelle modification was only significant ($p < 0.001$) in Group 3 compared to the control group (Figure 5). The ultrastructure of the endothelium under the surface epithelium of the ovaries and the isthmus epithelium of the fallopian tube were not affected by pneumoperitoneum (Figure 6).

**Discussion**

In literature, studies show that CO$_2$ pneumoperitoneum and increasing the intra-abdominal pressure lead to ischemia and reperfusion damage and some dysfunctions of the organs. However ovarian surface epithelium, ovarian endothelium, and tubal sillier epithelium were not examined in such studies. In this study, the authors have shown that CO$_2$ pneumoperitoneum leads to alterations in...
ovarian surface epithelium’s ultrastructure, the degree of which is well-dependent on intra-abdominal pressure. In pneumoperitoneum models where insufflations pressures were compared to each other although the intra-abdominal pressure was above 7 mmHg, fairly “lower” (i.e. 10 mmHg) and “higher” (i.e. 15 mmHg) intra-abdominal pressures were used. The general finding of these studies is that when high intra-abdominal pressure is used, there is increased tissue-organ hypo-perfusion and damage, increased metabolic effects, and increased formation of free oxygen radicals.

The response of each tissue to ischemia and the entry into irreversible phase differs. Characteristically, it is noted that there are two phenomena which show that irreversible points are reached: mitochondria and plasma membrane damage. At this point, plasma membrane damage is central factor in pathogenesis. One of the important biochemical mechanisms having a role in membrane damage is a reactive oxygen particle, which causes ischemia and reperfusion damage. While reactive oxygen particles can be formed in the post-ischemic mitochondria by the insufficient reduction of oxygen or by the synthesis of superoxide ion by the ksatrin oxidase on the vascular endothelium, it is in fact secreted by polymorphonuclear leukocytes. As a result of all these, there is calcium charge into the cell and the cells move towards the irreversible point [28].

If ischemia continues, there will be irreversible damage in the cell. The transition from irreversible status to cell death is not biochemically clear. While the degeneration of the membranes in the cell may result, intracellular calcium flow into the mitochondria may be observed as well. This will result in the vascularization of the mitochondria and the formation of mitochondrial density residual items. The calcium charge to the cell will increase especially if the ischemic area is repurfused. There will be constant outflow of enzymes, proteins, metabolites, etc. from the cell. At this point, lysosomal enzymes will be secreted in the cell and cell death occurs [28].

In the present study, while both in the 10 mmHg and 15 mmHg groups apical surface changes and membrane damage in the ovarian surface epithelium were observed, in the entire 15 mmHg group, in addition to the above, mitochondrial degeneration was also observed. It is logical that while ischemia occurred during pneumoperitoneum, it initially damages the plasma membrane and apical modifications in the cell, when the intra-abdominal pressure increases organelles, from which mitochondria is initially damaged. Because, after ischemia, oxidative phosphorylation in the mitochondria and the energy carrier of the cell, ATP decreases, which stops the activities associated with aerobic circulation. The sodium pump does not work; intracellular ion and water balance become disrupted. Furthermore, there will be calcium charge into the cell and potassium discharge of the cell. As a result, the cell swells, microvilli and cell skeleton disrupts, protrusions on the cell membrane are formed, mitochondria swells and expands, myelin figures are formed within and outside the cell [28].

Although statistically non-significant, especially in Group 2 (15 mmHg), higher trend for changes in lateral surface modifications and widening of the intercellular junctions were found. However it is not clear whether these changes are either attributable to an inherent property of CO2 per se [12], leading to local acidosis or a direct pressure effect, leading to the temporary stretching and expansion of the peritoneal surface area by the pneumoperitoneum [13].
The other interesting finding was the mitochondrial degeneration that was found strikingly in high and partly low pressures of pneumoperitoneum. In contrast to the above-mentioned findings, the degenerative changes in mitochondria were most likely related to post-ischemic reperfusion damage-second hit effect (surrogate of irreversible cell damage) leading to influx of calcium and H$_2$O, and affecting the cell skeleton [14-16].

The final deleterious effect that resulted from either local acidosis or the direct compression is disturbed microcirculation and hypoxemia [8]. Hypoxic tolerance of various cell types differs, depending on the metabolic rate and intrinsic adaptive mechanisms of the tissue. The deterioration of blood flow during pneumoperitoneum was more prominent in solid organs, such as the liver, pancreas, spleen, and kidneys, compared to that in hollow viscous organs such as the intestine, while it was non-significant in the stomach [17]. This discrepancy suggests a potentially varying degree of sensitivity to ischemic insult among different tissues. Although in literature various splanchnic organs have been tested for pneumoperitoneum-associated ischemia and reperfusion injury, only one study evaluated the ovarian tissue [18-21].

Fallopian tube ciliated epithelial cells are extremely sensitive to hormones, in rat estrous cycle, such that their morphology can completely change in a 24-hour period. Constant change of morphology, especially ciliary movement after ovulation requires high energy and mitochondrial activity. Hence, in the initial stages of study, they were assumed to be effected by ischemia and reperfusion damage and were included in the study. However, the response of the cells to damage depends on the type, duration, and intensity of the damage. Furthermore, the types of cells and their general condition are also important in this response. Each cell has a different response to ischemia and a different period of entry into the irreversible period. While this period is one to two hours for liver cells, it is three to five minutes for neurons. This may be the reason for the difference observed in the fallopian tube ciliated epithelial cells received from the isthmus, which is relatively inactive compared to ampulla. Another reason may be the observation of the internal epithelia, which is protected from the direct mechanical effect of the increased intra-abdominal pressure, contrary to the external fallopian tube epithelia. Furthermore, in contrast to ovarian surface epithelium, as these cells were not in direct contact with CO$_2$, intercellular hypercapnia and acidosis may have occurred. If this experiment was conducted in the ampulla where ciliated cells are the most active, they may have less exposure to ischemic reperfusion damage (provided all subjects are in the estrus phase). The present authors revealed that in all groups ciliated tube epithelium was unaffected. Another explanations for these results may be avoiding exposure to direct CO$_2$ and stable intra-tubal pressure. Although SEM may be considered principally as an appropriate means for evaluating peritoneal surface changes, microvilli and organelles cannot easily be used for comparison because their number and appearance may vary greatly [23]. Hence transmission electron microscopy is more suitable for the evaluation of microvilli and intracellular organelles.

Although no standard CO$_2$ pneumoperitoneal pressures were identified in experimental studies, various studies used working pressure as low as four mmHg and as high as 20 mmHg [24-26]. In accordance with this finding, the present authors preferred to use high and low pressures in this study. In literature, nonetheless some studies proposed that pressures above eight to 10 mmHg in a rat model do not correlate well with working pressures in humans. Thus, the findings may not be applicable for humans. However, there were some methodological problems with the above mentioned recent study [26]. In this study, there was some variability in the end-tidal CO$_2$ baseline levels between the different pressure groups. This variability is the largest flaw of this study. The other criticism for this study is not measuring the central venous pressure, consequently lacking of close hemodynamic monitoring.

There are some limitations in the current study that must be acknowledged. There is a disadvantage in extrapolating data across species, as the immunologic properties of species are different. Additionally, rats were not mechanically ventilated due to technical constraints, as well as blood gas follow-up and close hemodynamic monitoring, especially in experiments in which a high intra-abdominal pressure model is used, in order to reduce evaluation errors that could result from differences in the insufflations system, and the intra-abdominal volume of the subject. Since the authors did not perform intubation and mechanical ventilation and did not follow up blood gases during the experiment, they cannot state whether hypercapnia or elevated intra-abdominal pressure influenced the results. Under full intubation, especially tissue perfusion being potentially different and effecting the results, comparison of low and high intra-abdominal pressure with regard to the present transmission electron findings are so significant that could not be disregarded even with such limitations.

A suggestion for a follow-up study and further analysis would be to examine the histological changes in ovaries under the same experimental conditions but one week later, to determine whether the changes are as significant and/or permanent.

The present authors found hazardous effects particularly ultrastructural damage on ovarian surface epithelium when the intra-abdominal pressure was set at 10 mmHg or 15 mmHg. They therefore planned a further study with lower intra-abdominal pressure (five mmHg) and different cytoprotective agents [27].

In literature, up until the period during which this study was conducted, no model on alternation of ovarian vascularization, the thin structure of the endothelium of the ovarian mucosa, due to increased pneumoperitoneum or intra-abdominal pressure, have been found; for this reason, it is not known how the ovarian microcirculation is affected from increased intra-abdominal pressure.

This experimental study demonstrated the depressed tissue blood flow and also prominent evidence of oxida-
tive stress injury in the ovaries during CO\textsubscript{2} pneumoperitoneum and proposed that the ovaries were also highly sensitive to ischemia. It was suggested that this hyperfusion period may cause significant detrimental effects on the ovaries especially in critical conditions related to the ovary, such as unexplained infertility, in which subtle changes in follicle development, ovulation, and the luteal phase may be important etiologic factors [22]. The post-operative fertility studies should be undertaken to determine any long-term fertility effects. The clinical significance of the findings regarding humans has yet to be established. For this purpose, similar studies on the human ovary are imperative [22].

References


Behaviour of lab parameters and neonatal weight loss in relation to neonatal breathing movements and cord clamping time

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³Complex Operative Unit of Gynecology and Obstetrics, G.B. Grassi Hospital of Ostia, Ostia (Italy)

Summary

Background: To date, delaying cord clamping two to three minutes after birth is considered effective for newborn well-being. This time does not consider the newborn’s breathing movements, which may also condition neonate well-being. Aim: To investigate the behaviour of neonatal weight loss and of some umbilical vein lab parameters, in relation to timing of newborn breathing and cord clamping. Materials and Methods: Time from birth to cord clamping and time from birth to first cry of the newborn were collected in 87 full-term healthy women. First cry is a sign of effective breathing. Birth weight loss at the first, second, and third day from birth and lab parameters were assessed in relation to: time from birth to cord clamping, time from birth to first cry, and cord clamping before or after the first cry. Results: Partial pressure of carbon dioxide (pCO2) decreased if cord clamping was performed after first cry and increased if first cry occurred after cord clamping, independently from the time elapsed from birth to first cry (p = 0.012). Calcium (Ca²⁺) concentration decreased if cord clamping was performed after the first cry and increased if first cry of the baby after birth was delayed (p = 0.021). Each second of delay from birth to cord clamping resulted in an increase in Cl⁻ concentration (p < 0.001). Each second of delay in cord clamping resulted in a reduction in the percentage of weight loss at the first day (p = 0.024), at the second day (p = 0.007), and at the third day (p = 0.028) after birth. Conclusions: Neonate breathing after birth should induce umbilical vein flow from placenta to lungs, conditioning the reduction of birth weight loss after birth and umbilical lab parameters modifications.

Key words: Delayed cord clamping; Neonatal breathing; Neonatal circulation.

Introduction

Delayed cord clamping has proven useful for newborn infants in the perinatal period and in the first year of life [1]. Delayed cord clamping provides neonates with an adequate blood volume and iron reserve [1]. Moreover, evidence from the literature suggests better adaptation for preterm babies and higher red blood cell flow to vital organs during the first few days of life for all babies [2-4]; additionally, behavioural benefits of delayed cord clamping may be helpful for fostering early breastfeeding [2].

Two pivotal concepts should be drawn from the aforementioned evidence. Firstly: one should wait a reasonable time to clamp cord to allow the transfusion of an adequate blood volume from cord and placenta to the neonate. Secondly: one should consider at least two minutes as the adequate time for clamping cord, as considered in randomized trials [5, 6]. The aforementioned concepts are however not congruent. Indeed, flow is a function of volume and time, and is a continuous variable. Therefore, the volume of blood transfused is in continuous relation with the time elapsed from birth to cord clamping. Mathematically, when the time from birth to cord clamping is infinite (if one does not clamp the cord), the flow from placenta and cord to neonate is infinite, since the blood volume in cord and placenta is not null.

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Some authors [1] consider it useful to delay cord clamping by two to three minutes because cord pulse stops within the same minutes, suggesting that placental flow has stopped. The authors do not agree with this concept: cord flow is directed from the left fetal heart to the placenta through the umbilical arteries, and from the placenta to right fetal heart through the umbilical vein. With breathing movements, the newborn infant induces a depression in the chest and shifts the direction of blood flow to the lungs from the placenta, through the umbilical vein, and right heart atrium and ventricle [7]. Moreover, intrauterine pressure after birth is higher than before delivery [8-10]. As explained by Laplace’s rule, the pressure within a sphere is inversely related to the sphere radius. Therefore, when the infant has been delivered, the uterine volume and, therefore, the uterine radius are reduced, leading to a rise in intrauterine pressure. The higher intrauterine pressure encounters blood pressure in the fetal umbilical arteries and favours flow through the uterine vein to the neonate lungs, for as long as the placenta is still within the uterine cavity. Therefore, there may be a time lapse in which umbilical arterial flow has stopped while umbilical vein flow is still present, with the effect of transferring the whole blood volume content in cord and placenta from the cord and placenta to the newborn infant.

As a logical consequence, one should consider the time from birth to cord clamping as a continuous variable that, along with time elapsed from birth to first breathing movements, may influence neonate well-being in a continuous way.
The following study will quantitatively investigate the behaviour of neonatal weight loss and of some umbilical vein lab parameters in relation to timing of newborn breathing and cord clamping.

**Materials and Methods**

A sample of 87 full-term healthy women who delivered vaginally was enrolled from March 2011 to November 2011 at the G.B. Grassi hospital of Ostia (Italy). Immediately after delivery, time from birth and cord clamping was collected by stopwatch and expressed in seconds. The time from birth to the first cry was collected in the same way. Crying is considered an objective sign of at least an appropriate breathing movement. The midwife was free to decide the time of cord clamping after birth and did not know the aim of the study. Therefore the study is observational and does not modify the current practice of the facility. Immediately after cord clamping, a blood sample from the umbilical vein was collected to instantly assess the following lab parameters: partial pressure of oxygen (pO2), pH, bases excess (BE), sodium (Na+), potassium (K+), partial pressure of carbon dioxide (pCO2), calcium (Ca2+), chlorine (Cl_), hematocrit. An analyser was used for specific assessment of such parameters. Capillary bilirubin and glucose levels were assessed on the first day after delivery, as routine screening tests of newborn infants. Birth weight was collected at birth and on days one, two, and three after birth. Weight loss on the same days was expressed using a percentage scale. Each of those variables was considered as dependent variables in regression models. The independent variables considered in each multivariable regression model were: time from birth to cord clamping (seconds), time from birth to first cry (seconds), cry after cord clamping (yes/no).

Moreover, to check the interdependence among dependent variables, a three-component, rotated, factor analysis was built, in order to aggregate the associated variables. By checking the interdependence among the dependent variables, it is possible to determine which dependent variables are linked and, therefore, which ones vary together, according to trends found in regression models.

SPSS 16.0 package was used for statistical calculations and p < 0.05 was set as minimum significance.

**Results**

Mean time from birth to cord clamping was 95.6 seconds (± 66.6). Mean time from birth to first cry was 38 seconds (± 29.4). Fourteen (16.1%) patients underwent cord clamping before the baby’s first cry, and 73 (83.9%) patients underwent cord clamping after first cry. Table 1 reports the mean values with standard deviations of lab parameters assessed in umbilical vein samples and the mean values with standard deviations of the first, second, and third day of weight loss.

Regression models found significant relationships for pCO2, Ca2+, Cl−, and for weight loss at first, second, and third day after birth (Table 2). pCO2 decreased if cord clamping was done after first cry and increased if first cry occurred after cord clamping, independently from time elapsed from birth to first cry (partial regression coefficients (B) = -5.951, 95% confidence intervals (CI) -10.580 - -1.323) (p = 0.012). Moreover, each second of delay from birth to cord clamping increased pCO2 (B = 0.039, CI 95%

![Table 1. — Descriptive statistics including mean values with standard deviations of continuous variables.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Means</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.31</td>
<td>± 0.08</td>
</tr>
<tr>
<td>pO2</td>
<td>34.4 mmHg</td>
<td>± 13.6 mmHg</td>
</tr>
<tr>
<td>pCO2</td>
<td>39.7 mmHg</td>
<td>± 7.6 mmHg</td>
</tr>
<tr>
<td>Bases excess</td>
<td>-5 mmol/l</td>
<td>± 1.9 mmol/l</td>
</tr>
<tr>
<td>Na+</td>
<td>134 mmol/l</td>
<td>± 3.6 mmol/l</td>
</tr>
<tr>
<td>K+</td>
<td>5 mmol/l</td>
<td>± 0.9 mmol/l</td>
</tr>
<tr>
<td>Ca2+</td>
<td>5.08 mg/dl</td>
<td>± 1.54 mg/dl</td>
</tr>
<tr>
<td>Cl_</td>
<td>107.5 mmol/ml</td>
<td>± 3.72 mmol/ml</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.87 mg/dl</td>
<td>± 0.49 mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>90.1 mg/dl</td>
<td>± 20.5 mg/dl</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>53%</td>
<td>± 11%</td>
</tr>
<tr>
<td>1st day percentage of weight loss</td>
<td>3.5%</td>
<td>± 1.68%</td>
</tr>
<tr>
<td>2nd day percentage of weight loss</td>
<td>5.7%</td>
<td>± 2.03%</td>
</tr>
<tr>
<td>3rd day percentage of weight loss</td>
<td>5%</td>
<td>± 3.48%</td>
</tr>
</tbody>
</table>

![Table 2. — Regression analyses and factor analysis. Time in seconds between birth and cord clamping between birth and first cry of the baby and the effects on lab tests and neonatal weight loss.](image)

<table>
<thead>
<tr>
<th>Cry after cord clamping</th>
<th>Time from birth to first cry</th>
<th>Time from birth to cord clamping</th>
<th>Variables interdependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ph</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>pO2</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>pCO2</td>
<td>-5.951</td>
<td>0.039</td>
<td>p = 0.012</td>
</tr>
<tr>
<td>Bases excess</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Na+</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p = 0.024</td>
</tr>
<tr>
<td>K+</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Ca2+</td>
<td>-1.081</td>
<td>0.01</td>
<td>p = 0.021</td>
</tr>
<tr>
<td>Cl_</td>
<td>N.S.</td>
<td>0.022</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>1st day percentage of weight loss</td>
<td>N.S.</td>
<td>-0.216</td>
<td>p = 0.024</td>
</tr>
<tr>
<td>2nd day percentage of weight loss</td>
<td>N.S.</td>
<td>-0.328</td>
<td>p = 0.007</td>
</tr>
<tr>
<td>3rd day percentage of weight loss</td>
<td>N.S.</td>
<td>-0.441</td>
<td>p = 0.028</td>
</tr>
</tbody>
</table>

0.013 - 0.065) (p = 0.003). Ca2+ concentration decreased if cord clamping was performed after the first cry and increased if first cry of the baby after birth was delayed (B = -1.081, 95% CI -1.996 - -0.165) (p = 0.021). Each second of delay from birth to cord clamping resulted in an increase in Ca2+ concentration (B = -0.01, 95% CI -0.005, - -0.015) (p < 0.001) and an increase in Cl− concentration (B = -0.022, 95% CI 0.011 - 0.033) (p < 0.001).
Each second of delay in cord clamping resulted in a reduction in the percentage of weight loss at the first day (B = 0.216, 95% confidence interval (CI) -0.403 - 0.029; p = 0.024), at the second day (B = -0.328, 95% CI -0.564 - -0.092; p = 0.007), and at the third day (B = -0.441, 95% CI -0.832 - -0.050; p = 0.028) after birth.

The three-component rotated factor analysis highlights interdependence among variables (Bartlett’s test of sphericity: p < 0.001). The interdependence is strong with weight loss variables (marked with 1 on Table 2, right-hand column). Interdependence is less strong for pCO2, BE, Na+, K+, Ca2+, Cl− (marked with two in Table 2, right-hand column). Additionally, pO2, pH, pCO2, BE, Na+, bilirubin, and hematocrit depict a scanty interdependence (marked with three in Table 2, right-hand column).

Discussion

This study aimed to assess if time lapse from birth to cord clamping can independently influence neonate well-being, as measured by neonatal weight loss, and if it is related to breathing.

Interestingly, pCO2 rises if the sample is taken before a breathing movement and when cord clamping is delayed. A pCO2 behaviour similar to the one reported has been reported by Wiberg et al. [11]. These authors found an increase in pCO2 levels both in the artery and vein most markedly at 45 minutes after birth. Interestingly, De Paco et al. [12] did not find an increase in pCO2 at two minutes from birth, but pO2 increased after more than two minutes from birth. Taken together, those data suggest that pCO2 in the umbilical vein is strongly related to breathing movements, which usually occur some seconds after birth. Therefore, when the umbilical vein cord clamp is performed two minutes after birth, a healthy neonate will have already taken a breath in at least the majority of the cases. A logical conclusion drawn from this pCO2 behaviour, is that lung function is needed for the ventilation of CO2 in newborn infants after birth, and that the placenta is not needed to ensure respiratory function during the few minutes after birth, because pO2 increases in umbilical blood vein if cord clamping is delayed [12].

Ca2+ behaviour would suggest that pCO2 modifications in the umbilical cord vein are linked with umbilical vein flow. Ca2+ concentration in peripheral venous blood increases due to blood stasis [13]. Ca2+ increases in the umbilical vein may be linked to blood stasis as well: if the neonate cries (denoting breathing), the Ca2+ concentrations decrease. Therefore, breathing movements induce blood flow through the umbilical vein, and are able to induce CO2 ventilation through the lungs.

Cl− concentration changes follow the Ca2+ and the pCO2 modifications. This is demonstrated by the second cluster of interdependence found by factor analysis (the one marked with two in the right-hand column of Table 2). Such interdependence could be explained by anionic gap behaviour in the very special condition of the umbilical vein of the newborn infant some seconds after birth. It was reported by Wiberg et al. [11] that lactate increases 45 and 90 seconds after birth in the umbilical vein. Even if the increase of lactic acid was not assessed in the present study, it does indeed occur. Usually, the rise in lactates does alter the anionic gap in an adult, reducing the Cl− concentration, and is buffered by bicarbonates [14], producing CO2 and H2O.

In the umbilical vein, however, due to blood stasis, the authors found that Ca2+ increases, thereby explaining the rise in Cl− in order to maintain electrical neutrality. Therefore the excess of anions could be neutralized by the rise in Ca2+ concentrations.

Another weak interdependence (marked as 3 in the right-hand column of Table 2) was found among pO2, pH, pCO2, BE, Na+, bilirubin, and hematocrit value. This behaviour may influence some metabolic and respiratory parameters in a pathophysiological relationship, as depicted by the interdependence found in the present study. Interestingly, the blood volume of neonates is higher in the case of delayed cord clamping, rising by about 32% when cord clamp is delayed by at least three minutes [15]. The interdependence relationships found by the present authors and results from Nelle et al. [15, 16] lead to consider that delaying cord clamping supplies both blood cells and water to the newborn infant. This idea impacts neonatal well-being, since neonatal weight loss at first day after birth is reduced. Consequently, this supply of water impacts weight loss at second and third days after birth too, as proven by the strongest interdependence (one on the right-hand column of Table 2).

Caution should be used in interpreting the percentage of reduction of weight loss from partial regression coefficients (B). As suggested by large intervals of confidence for each coefficient of regression, the percentage of weight loss varies very much for each newborn, and it may be explained by other variables not considered in the multivariable regression models (such as, breastfeeding or milk formula supplements). Therefore the authors judged that the reduction of the weight loss percentage predicted by timing of cord clamping may be overestimated.

In summary, the authors depict the following evolution of cord flow after birth. Umbilical arteries restore the placental bed until cord pulsation stops. Then, placental and cord blood volume halts until the first breathing movements occur, accumulating CO2, lactate, O2, and Ca2+. This could be due to oxygenation in the placenta and to the anaerobic metabolism of red blood cells in the cord, producing lactic acid, buffered by bicarbonates. Vein stasis leads to increased Ca2+ ions that neutralize anions. With breathing, a quantity of blood volume stored in the placental vascular bed and umbilical vein is shifted to lungs, supplying blood cells, iron, and water to the newborn infant. Each second of delaying cord clamping supplies the neonate with blood volume for perfusing lungs and removing CO2. The supply of water prevents newborn weight loss in the days after birth.

An intriguing speculation suggests that blood flow through the umbilical vein may exist until blood volume in the placenta is detectable (five days after birth, according to Nelle et al. results [16]). This blood volume could be helpful for avoiding neonatal weight loss, substantiating...
the lotus birth practice [17]. The topic will require appropriate investigations that quantify the blood volume transfusion through the umbilical vein in relation to neonatal breathing movements and time elapsed from birth.

**Conclusion**

Delaying cord clamping reduces newborn weight loss during the first days after birth. The hypothesis that umbilical vein flow after birth would not stop with artery pulse seems to be supported by data variations of lab parameters in relationship with breathing. Therefore, it is useful to clamp cord after the initial newborn breathing movements, and breathing movements are needed for perfusing lung vascular bed.

**References**


Role of psychological intervention in fetoscopic laser surgery of twin-to-twin transfusion syndrome

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Summary

Objective: This study aims to investigate the influence of application of psychological intervention in fetoscopic laser surgery of twin-to-twin transfusion syndrome (TTTS) on perinatal outcome. Materials and Methods: A total of ten cases of pregnant women diagnosed with TTTS from January 2007 to December 2009 in the present hospital were selected. Their gestational weeks ranged from 16 to 29 weeks. Under the location of B ultrasound, the method of intra-amniotic fetoscopic laser occlusion of chorioangiopagous vessels (FLOC) plus amnioreduction was conducted for treatment. Contemporarily, psychological intervention was also carried out. Results: Preoperative, intraoperative, and postoperative behavior controls of all pregnant women were good, and all operations were successfully completed to achieve the desired purpose of rehabilitation discharge. Conclusion: Fetoscopic laser surgery is an effective treatment for TTTS and competent psychological intervention is one of important measures for successful operation and pregnant woman rehabilitation discharge.

Key words: Twin-to-twin transfusion syndrome; Laser; Psychological Intervention.

Introduction

Twin-to-twin transfusion syndrome (TTTS) refers to obvious hemodynamics differences between twins and a series of pathological and physiological changes caused, due to placental vascular anastomoses during twin pregnancy [1]. Perinatal mortality rate is extremely high. If treatment is not conducted, its mortality rate can reach 80% to 90% [2]. At present, fetoscopic laser occlusion of chorioangiopagous vessels (FLOC) is the internationally-preferred method for TTTS treatment. According to domestic reports, TTTS incidence rate is low, and such operation is conducted in only a few hospitals. However, fetoscopic laser surgery also causes many psychological reactions to pregnant women. It is reported that the surgically treated pregnant women with TTTS have obvious psychological stress reactions or mental disorders [3], and surgery itself can induce some reactions such as agrypnia, anxiety, and depression that inevitably influence the life quality of patients [4]. As pregnant women cannot understand and accept its damages to fetuses, they are bound to not accept surgery as an option. The present summarizes the treatment results of ten cases of patients with TTTS receiving FLOC and the psychological intervention from 2007 to 2009 to assist pregnant women to actively respond to the intervention, relieve anxiety extent of pregnant women intraoperatively, and enhance compliance in order to provide a reference for smooth implementation of treatment and postoperative rehabilitation of pregnant women with TTTS.

Materials and Methods

General data

Ten cases of pregnant women diagnosed TTTS from January 2007 to December 2009 receiving surgical treatment in the present hospital were selected. Their average age was 28.3 years, and gestational weeks ranged from 16 to 29 weeks. They had no history of disease of vital organs and no medication and radiation exposure history during pregnancy. Among them, one case was in stage I pregnancy, one case was in stage II pregnancy, four cases were in stage III pregnancy, and four cases were in stage IV pregnancy.

This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the Affiliated Hospital of Hangzhou Normal University. Written informed consent was also obtained from all participants.

Surgical methods

FLOC: after various routine examinations of pregnant women were completed, local anesthesia was conducted at uterine fundus or anterior uterine wall rather than placental attachment skins under the location of B ultrasound. Trocar punctured the skin to enter the amniotic cavity. After amniotic fluid outflowed, a fetoscope was positioned to seek the transportation vascular branch near amnion at placenta bottom. In the handle hole, 365.0 µm laser transmission optical fiber was inserted to aim at the vessels. Subsequently, energy and frequency were set (1.0 - 2.0 J/10Hz). Laser was used to cauterize and occlude vessels. Intraoperatively, several vessels were respectively cauterized. After surgery was completed, partial amniotic fluids were slowly released until the deepest amniotic fluid area was five to six cm. The surgical process was strictly monitored by B ultrasound and fetal heart and fetal movement of two fetuses were normal. The surgical process was strictly monitored by B ultrasound and fetal heart and fetal movement of two fetuses were normal. Finally, amniotic fluid index and umbilical artery’s pulsatility index (PI), resistance index (RI), and systolic/diastolic (S/D) values were measured.

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Psychological intervention method

Psychological problems and requirements of pregnant women were understood by the specialized intervention team, which was composed of a primary nurse, head nurse, and obstetrician that evaluated the psychological changes of the pregnant women, and appropriate measures were promptly taken preoperatively, intraoperatively, and postoperatively [5].

Preoperative psychological intervention: 1) Provide information. As fetoscopic laser surgery used for TTTS was a newer treatment technique, information acquisition routes of pregnant women and families were fewer and there were more worries. Therefore, it was necessary for doctors to provide the actual information of FLOC used for TTTS to pregnant women and their families before surgery, including both subjective and objective information. The subjective information provided intraoperative impressions, intraoperative potential problems, and coordinating measures regarding FLOC to pregnant women. The objective information was to introduce details of FLOC, existent achievements of FLOC, possible complications and precautions to patients and their families before surgery. 2) General supportive psychotherapy. It was very important to understand whether emotions of pregnant women were stable to conduct psychological intervention. In the preoperative discussion with pregnant women, doctors avoided using medical nomenclature and allowed pregnant women and their families to set forth as many problems as possible and explain the fetoscopic laser surgery process by use of a graphic method to eliminate some wrong concepts and unrealistic ideas. Among the ten cases, one case overcame psychological concerns and finally signed the surgical cognitive consent at after six hours of repeated psychological counseling. 3) Strengthening the doctor-patient communication. The operating nurse strengthened ward communications of medical care staffs with pregnant women and their families, visited pregnant women and their families before surgery to introduce anesthesia method, surgical process, surgical room environment, etc. and provided timely feedback of the problems and needs of the patients and their families to medical care staff within the ward. The medical care staff timely resolved the problems of pregnant women and their families. Therefore, it greatly relieved the tension of both the patients and their families and reduced various interferences of families towards surgery. 4) Teaching the physical and mental relaxation methods to allow patients [6] to learn self-adjustment. The physical and mental relaxation method attempted to eliminate patient distractions and calm mind and body through self-training. Specific method: the nurse guided pregnant women to naturally sit up, with eye closure and two palms placed on both knees. Also, their attentions focused on their two foot arches. They uniformly and slowly breathed for three to four minutes to relax each group of muscles for extending to systemic relaxation. Subsequently, they slowly opened their eyes. In this fashion, relaxation was carried out once daily for less than 30 minutes.

Intraoperative psychological intervention: fetoscopic laser surgery of TTTS was conducted under B ultrasound location and local anesthesia, and surgery continued for about 60 minutes. The pregnant women were always in the waking state of consciousness. The authors observed that the patients often intraoperatively guessed and imagined the surgical process. In order to avoid undue psychological distress of the patients, unrelated conversations and communications were minimized as much as possible, and professional terms were used in the communications [7]. While doctors conducted surgery, they offered comfort and explained the surgical progress to the patients, while nurses offered encouraging words at head side of the patients, such as “you are good!”, “you are fantastic!”. In the interim, they closely observed vital signs and psychic reactions and dispersed attentions of pregnant women. For some particularly-nervous patients, the nurses constantly communicated with them (talking about some families or friends, work, and other unrelated matters) to disperse their attentions and timely updated the surgical progress to offer psychological support to them. As a result, ten cases were completed without any complications.

Postoperative psychological intervention: if the patients presented anxiety, dysphoria, and other symptoms due to wound pain or discomfort and other reasons within 24 hours after FLOC, the medical care staffs strengthened tour inspections to carefully observe systemic symptoms, monitor vital signs, monitor fetus situations, abdominal incision to confirm whether there were threatened premature delivery symptoms, and timely treated uncomfortable situations of pregnant women, foreseeing resolved requirements of pregnant women and enhanced the trust of the patients towards medical staff. Twenty-four hours postoperatively, the conditions of pregnant women were relatively stable. At this time, a majority of pregnant women expressed concern regarding fetal state and its survival. Therefore, medical care staffs actively communicated with pregnant women to highlight rest importance. At the postoperative third day, the patients had absolute bed rest. The nurses timely explained fetal monitoring situations, illustrated the monitoring of the fates to the patients, and attempted to obtain comprehensive care for meeting the patients’ requirements while winning their active cooperation. Individual patients became irritable, and the nurses guided these to use the attention dispersing method. According to habits, hobbies, and cultural literacy of pregnant women, the excessive concern of pregnant women to fetal prognosis was transferred. Relaxing and soothing music or video materials were selected to transfer pregnant women’ mood and disperse their attention [8, 9], and better results were obtained.

Results

All patients with TTTS had different extents of psychological problems in the perioperative period. Among them, 70% had an operable contradictory psychology before surgery and worried about threatening fetal life and unsuccessful surgery. In the perioperative period, the number of pregnant women confident to successful surgery greatly increased after implementation of psychological intervention, and ten cases underwent surgery in a healthy mood. As a result, preoperative, intraoperative, and postoperative moods of the cases were well controlled.

Ten cases were compliant with medical care staffs to complete surgery. Postoperative vital signs of pregnant women were stable, and postoperative B ultrasound re-examination showed that except for biparietal diameter, femur length, scalp edema, and pyeptonitenum of two fetuses, umbilical artery blood flow indices PI value, RI value, S/D value of the remaining were normal [10]. No complications occurred and the desired treatment purpose was achieved. On average, the patients were discharged after seven days of hospitalization.
Discussion
In China, fetoscopic laser surgery used for TTTS is a novel technique. The Affiliated Hospital of Hangzhou Normal University is one of the first hospitals to conduct such treatment and better results have been obtained. The success of this treatment is not only related to doctors’ experience, but also closely related to the psychological intervention of medical staff.

For patients with TTTS and gestational weeks less than 26 weeks, FLOC is the preferred treatment method. Rossi [11] summarized 611 cases of TTTS cases and drew a conclusion that fetuses receiving laser therapy more easily survived than fetuses receiving amnioreduction. Especially in stages III and IV, advantages of laser therapy are more obvious [12]. Compared with continuous amnioreduction, FLOC can increase the survival rate of perinatal period and reduce the incidence rate of nervous system [13].

Abroad, FLOC treatment is more consolidated. It is reported at home that TTTS incidence rate is low, and such an operation is carried out only in a few hospitals. Fetoscopic laser surgery used for TTTS is a new technique. As there are a fewer reports on disease conditions and treatment information of TTTS, pregnant women and their families obtain with difficulty the relevant knowledge. Some studies [14] suggested that if patients did not know the disease condition in detail, they easily generated doubt, fear, and random guess psychologies. These are unfavourable for psychological health and disease treatment, while effective communications and common investigations on disease-related knowledge and treatment schemes are useful for the treatment and rehabilitation of patients. According to this phenomenon, the authors provide the information in the form of images and words for pregnant women and their families, allowing them to understand the disease conditions and surgical process, allowing them to better home internal supports during hospitalization. Therefore, confidence and courage of pregnant women to surgical treatment are enhanced and treatment compliance is increased [15, 16].

Due to the initial experience to this traumatic operation, a majority of pregnant women will exaggerate the fetal surgical risk, which causes them to generate larger psychological changes and generate anxiety and fear. Many studies confirm that in case of high anxiety level, muscle tension increases, while pain threshold decreases. Therefore, it increases the pain experience of patients during surgery and renders it more difficultly for them to cooperate, whereas cooperation extent influences diagnosis and treatment efficiency. Therefore further psychological support is provided [17] and guidance and encouragement of both patients and their families to express their feelings by use of one-to-one support expression method. According to the psychological requirements and existent problems of pregnant women, explanation, encouragement, and comfort are timely given. In addition, pregnant women generate fear reaction towards surgery and generate anxiety due to excessive concerns regarding fetal safety. Studies suggest that people only focus on a matter at a time. If the attention or accompanied bad mood is transferred to the interest task or work attracting the attention, the link between the conditioned stimulus and response can be prevented. Therefore, dispersing attention through communication can act as a way of relieving psychological stress reaction towards surgery [18, 19]. The attention dispersing method used by medical staff is simple and convenient and it can independently provide auxiliary measures of relieving psychological stress reaction.

A number of practices [20] prove that relaxation training can offset negative influences of physiological and psychological stresses to restore the balance and coordination of human body, psychology, and spirit. It not only can apparently relieve general mental tension and nerve disorder, but also can treat stress-induced psychosomatic reactions. The physical and mental relaxation method adopted by the authors is a more utilized behavior method for the relaxation before obstetric operation [21]. As a result of operability, safety, and convenience of relaxation training, a majority of pregnant women are willing to accept such a method and obtain better effectiveness of relieving psychological perplexity from it.

The results of this study show that fetoscopic laser surgery used for treatment of women with TTTS has different extents of negative psychological problems and the psychological intervention used during the perioperative period can improve the psychological status resulting in the smooth operative implementation and satisfactory postoperative rehabilitation. As limited researched samples, it is necessary to carry out verifications and researches on a larger range. At the same time, after the post-discharge psychological intervention of pregnant women is combined, it will improve post-discharge quality of life of the patients.

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Role of environmental organochlorinated pollutants in the development of endometriosis

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Summary

Endometriosis is a gynecological disease, which involves the growth of endometrial tissue outside the uterine cavity, commonly in the pelvic region. The etiology of the disease is unclear, but multiple factors may contribute to its pathogenesis. Environmental organochlorinated pollutants, particularly dioxins and polychlorinated biphenyls (PCBs), are thought to play a role in the development of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis. Their effects on cytokines, immune system, hormones, and growth factors are thought to increase the risk of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis. Their effects on cytokines, immune system, hormones, and growth factors are thought to increase the risk of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis. Their effects on cytokines, immune system, hormones, and growth factors are thought to increase the risk of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis. Their effects on cytokines, immune system, hormones, and growth factors are thought to increase the risk of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis. Their effects on cytokines, immune system, hormones, and growth factors are thought to increase the risk of this disease; however, the results of clinical trials are discordant, and it is not clear how the effect of exposure to these compounds is linked to endometriosis.

Key words: Endometriosis, TCDD; PCB; Persistent organic pollutants; Organochlorinated pesticides; Dioxin-like compounds.

Introduction

Endometriosis, affecting about 10% of women of reproductive age, is often associated with pelvic pain and/or infertility. Pain symptoms can be severe particularly in the presence of deep invasive endometriosis and can affect the quality of life of these patients [1]. According to the literature, the number of cases and the severity of the disease are increasing and the actual incidence of the disease may be higher, owing to the requirement of surgical visualization for diagnosis [2, 3]. Moreover, the disease tends to recur even if the recurrence risk factors are not well clarify. Nevertheless, adhesions and previous surgery seem to have a role [4]. The etiology of endometriosis is unclear, but it is probably multifactorial involving hormonal, genetic, immunologic, and environmental factors [5]. The possibility that exposure to environmental chemicals is a contributing factor to the development of endometriosis has been a matter of scientific debate for 20 years.

Review

A compound, which has been of great concern, is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an undesired byproduct of many combustion processes. It is the prototype of a group of substances which have similar chemical characteristics and spectrum of effects and are both persistent and bioaccumulative. Chemicals belonging to this group are polyhalogenated aromatic hydrocarbons (PHAHs) and they may contain multiple chlorine and/or bromine atoms at three or more lateral positions on the multiaromatic ring structure [6]. They include polyhalogenated dibenzo-p-dioxins (PCDDs and PBDDs), dibenzofurans (PCDFs and PBDFs), biphenyls (PCBs and PBBS), and naphthalenes (PCNs and PBNs). Polychlorobiphenyls (PCBs) include 209 different congeners which are divided into ‘dioxin-like’ (DL-PCBs) and ‘non dioxin-like’ according to their structure. Dioxin-like congeners have no or only one chlorine in the ortho position while non-dioxin-like PCBs are characterized by two or more chlorines in the ortho position. PCBs, polychlorodibenzo- furans (PCDFs and PCDDs, commonly referred to as ‘dioxins’) are resistant to degradation and they bioaccumulate at higher levels in the food chain due to their lipophilicity. Food is thus the most important source of exposure to these pollutants [7]. Humans and animals are exposed to complex combinations of such chemicals; however, most studies focus only on single toxicants.

Some dioxin-like and non-dioxin-like PCBs and organochlorinated pesticides (such as p,p‘-DDE, a metabolite of DDT) seem to interfere with the endocrine (as an endocrine-disruptor) and the immune systems, causing reproductive disorders such as endometriosis. Endocrine disruptors (EDCs) are compounds that may interfere with the endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. They can mimic, reduce, and in some cases, completely block the effects of endogenous hormones.

The hypothesis that exposure to environmental pollutants could play a role in disease etiology was first suggested by Rier et al. [8]. The study conducted in monkeys, which were chronically exposed to TCDD, found a dose-dependent increase in the incidence and severity of spontaneous endometriosis. Although strongly criticized by some scientists [9, 10], this paper opened new ways for further research investigating the relationship between endometriosis and environmental pollutants.

The effects of TCDD, dioxins, and PCBs have been studied by numerous investigators and yielded contrasting results [3, 5, 11-14]. In 2002, Eskenazi et al. [15] evaluated the role of TCDD in the development of endometriosis in...
women exposed to a great amount of this toxicant. The study subjects were those who lived in Seveso, Italy, in July 1976, when a chemical explosion dispersed large quantities of TCDD into the atmosphere. The researchers did not find a significant association between endometriosis and TCDD concentrations in serum, but only a trend.

Many studies have investigated the relation between endometriosis and exposure to dioxins and DL-PCBs. All these compounds bind to the aryl hydrocarbon receptor (AhR), expressed in both the endometrium and immune cells, eliciting the same spectrum of toxicological activities. The binding affinity and the toxic potency of each congener is expressed in relation to the most toxic compound of the group (the TCDD), termed as the toxicity equivalency factor (TEF). The concentration of a mixture of congeners is therefore expressed in toxicity equivalents (TEQs), multiplying the analytical concentration of each congener by its TEF. TEQs for each single congener, are then summed to obtain the total TEQ, which characterizes the overall toxicity of the mixture [12].

Heilier et al. [13] provided epidemiological evidence linking endometriosis with increased concentrations of dioxin and dioxin-like compounds. This study conducted in women with peritoneal and/or deep infiltrating endometriosis found that they had higher serum TEQ levels than controls. Tsukino et al. [14] did not confirm this association, finding lower TEQ levels in patients with endometriosis than controls. However, Tsukino et al. included in the control group patients with Stage I endometriosis and infertile women, whereas in the study of Heilier et al., the control group was constituted only by healthy women with no infertility or endometriosis [13]. These differences in the selection of control groups probably contributed to the differing results.

The mechanisms involved in the deleterious effects of such compounds on reproduction are still under evaluation. AhR mediates most of the toxic effects of “dioxins” on cell functions, and activates several genes including cytochrome P450. Exposure to AhR agonists may influence the immunological functions of endometrial-like pattern of cell-cell interaction in the human endometrium, which interferes with progesterone’s ability to suppress matrix metalloproteinases (MMPs) expression in both epithelial and stromal cells. Progesterone exposure during the secretory phase of the menstrual cycle serves to down-regulate the endometrial MMP system, so that endometrial breakdown does not occur before menstruation. Under normal circumstances endometrial tissue, which has reached the peritoneum due to retrograde flow of menstruation, is eliminated by the innate immune system. Several studies show that inflammatory-like processes caused by dioxin-like toxicants can interfere with the normal physiology of the endometrium and the innate immune system. This condition may permit the persistence and the development of endometrial tissue within the peritoneal cavity [12, 17].

Exposure to PCBs may be linked to an altered endocrine status in humans, which may cause development of reproductive tract dysfunctions and diseases. Some studies suggest that endometriosis is linked to exposure to certain PCBs [3, 5, 13], while other studies do not confirm such a link [14, 18, 19].

In our studies we found a significant association between increased levels of some PCBs and endometriosis, but did not find any difference in blood concentrations of dioxin-like chemicals (PCDDs, PCDFs, and the 12 dioxin-like PCBs) in women with different stages of the disease [3, 5]. We also examined the immunological functions of patients with endometriosis serum level of PCBs and p,p'-DDE to verify the impact of these environmental contaminants on the dysregulation of immune functions and they observed that increased concentrations of these compounds were associated with altered natural killer (NK) immune responses [20].

The different results obtained in the published studies may be influenced by differences in control groups, methods used for compound analysis, type of congeners investigated, and the statistical tests employed.

Selection of the control group is a possible source of error in an epidemiological study investigating the association between PCBs and endometriosis. Women living in the same area as the test subjects should be recruited as controls, so that both the groups are likely to have been similarly exposed to organochlorines. The development of endometriosis as a co-morbidity factor in infertile women may confound the interpretation of studies enrolling infertile subjects without the disease as controls [14].

Another potential bias is the method used to exclude the presence of endometriosis in controls, as laparoscopic examination remains the only reliable diagnostic tool to assess the presence or the absence of the disease.

In two studies that confirmed a link between exposure to PCBs and endometriosis, laparoscopy was performed in both cases and controls to confirm or exclude the presence of the disease [5, 21].

Lactation is an important PCB excretory route, which leads to a significant decrease in the body burden of organochlorine compounds. To avoid the confounding factor of breast-feeding, only nulliparous or non-nulliparous women, who have never breastfed should be enrolled [5, 22].

Furthermore, the type of endometriosis may also influence the results. Heilier et al. [13] found that concentrations of PCBs and dioxin-like compounds in the serum were associated with a significantly increased risk of developing deep endometriotic nodules of the recto-vaginal septum, although the risk of developing peritoneal endometriosis was not statistically significant. The authors also suggested that organochlorines might mainly cause development of deep endometriosis. Future studies should consider peritoneal endometriosis and deep endometriotic nodules as distinct entities, in order to assess the possible etiological contribution of organochlorines.

Genetic predisposition and environmental factors have been suggested to concur to the onset and progression of endometriosis. Genetic susceptibility was explored by studying mutations in genes responsible for detoxifica-
tion, such as glutathione transferase (GST), as a possible risk factor to endometriosis per se and in association with exposure to PCBs. Vichi S et al. [23] showed that the GSTs polymorphisms per se do not increase per se the risk of developing endometriosis. However, a gene-environment interaction was observed for GSTP1 and GSTM1 null genotypes, modulating the effect of total PCBs on disease risk.

Research should also focus on the risk of developing endometriosis by exposure to environmental chemicals in the womb, during early childhood, puberty, and adulthood.

In conclusion, accumulated evidence supports the hypothesis that exposure to organochlorine pollutants may induce endometriosis. The mechanisms involved are still unclear. They may act as immune toxicants and/or endocrine disruptors, enhancing estrogen synthesis and disruption of progesterone-dependent remodeling responses, which under normal circumstances prevent development of endometriosis. Additional standardizing studies in humans and animals are needed to better investigate the link between exposure to these toxicants and development of endometriosis and to identify the mechanisms involved.

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Corticotropin-releasing hormone and progesterone plasma levels association with the onset and progression of labor

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Summary

Purpose of Investigation: To examine the relationship between maternal plasma progesterone along with corticotropin-releasing hormone (CRH) plasma levels and the progression of labor. Materials and Methods: Maternal serum CRH and progesterone were measured during the latent phase of labor, active labor, and 24 hours postpartum in women who went into spontaneous labor and delivered vaginally at term. Progesterone (P) levels in women delivered by an elective cesarean section at term were also measured as baseline. Results: Mean maternal plasma P was 18% higher in the active phase than in the latent phase of labor (p < 0.001), and declined significantly by 24 hours postpartum (p < 0.001). Mean level of serum CRH was 24% higher in the active phase than in the latent phase of labor (p < 0.01), and subsequently declined significantly by 24 hours postpartum (p < 0.001). Conclusions: As labor progresses, P and CRH increase and subsequently decrease precipitously in the immediate postpartal period. P levels tend to drop in women who are in early labor compared with non-laboring full-term women.

Key words: CRH; Progesterone; Phases of labor; Term labor; Latent phase of labor; Active labor; Postpartum.

Introduction

Human pregnancy is maintained by a complex endocrine balance involving autocrine and paracrine signaling [1]. Although the precise mechanisms that control the onset of labor have not as yet been fully explained, accumulating data suggest that progesterone and corticotropin-releasing hormone (CRH) play substantial roles.

Progesterone (P) maintains pregnancy by promoting myometrial relaxation and quiescence [2]. It is thought to actively block myometrial contractility and its withdrawal converts the myometrium to the laboring state. Meanwhile, maternal plasma CRH is closely linked to the timing of parturition in human pregnancies [3]. Placental CRH is synthesized by human trophoblast, amnion, chorion, and decidual cells [4] and is secreted in maternal and fetal plasma [5]. It plays a key role in the initiation of parturition and in regulating the cascade of events involved in the birthing process [4, 6]. In addition, CRH may interact with the declining P levels which leads to the onset of labor [7], although this has not as yet been studied in detail.

The authors aimed to examine the relationship between maternal plasma P and CRH levels and the onset and progression of labor. Hypothesizing that the onset of labor is associated with a rise in CRH accompanied by a drop in P levels, P and CRH maternal serum levels were compared in the latent phase, active labor, and postpartal period spontaneously laboring women at term. Additionally, serum P from third-trimester non-laboring women was measured as baseline. Studies undertaking further examination of the fluctuation occurring in the plasma levels of CRH and P during labor and postpartum will shed additional light on the mechanisms of normal labor, while the conclusions of this study could be applied in the ongoing research of preterm labor.

Materials and Methods

Fourteen women at term were included in the study: nine of them presented in spontaneous early labor and delivered vaginally and the remaining five were admitted for an elective cesarean section by maternal request. None of the subjects was on any medications or had any documented medical or antenatal problems. None of the women who delivered vaginally received epidural anesthesia. Blood samples were taken from all subjects in the latent phase of labor (n = 9), in the active phase of labor (n = 9), and prior to the elective cesarean section (n = 5), and postpartum. Gestational age was confirmed by a first-trimester dating ultrasound. All subjects gave informed consent for participation in the study. The study was approved by the Ethics Review Board of the hospital.

Collection of blood samples

Ten milliliters of venous blood was collected from each participant by venipuncture of the antecubital vein. Blood samples were centrifuged at 1,600 rpm for 15 min at 0°C. Plasma was collected in duplicate aliquots. Plasma was frozen at -80°C and each aliquot was thawed on the day of the assay quantification.

Radioimmunoassay assessment of hormone levels

Plasma was extracted and processed for radioimmunoassay (RIA) by using a conventional RIA Kit according to the manufacturer’s instructions. CRH was extracted from three ml of plasma with Sep-Pak C-18 cartridges and eluted with Buffer B (60% acetonitrile, 1% TFA, and 39% distilled water). The extracts

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were evaporated, reconstituted in assay buffer, and assayed for CRH immunoreactivity. The RIA kit had a detection rate ranging from 0.1 to 67 pg/tube. A CRH-specific rabbit antiserum was used as the probe. CRH iodinated with I125 served as the tracer. Serum P was similarly assayed by a conventional RIA kit.

**Statistical analysis**

Data were distributed normally and are presented as means ± standard deviation. Mean maternal plasma P and CRH concentrations in the latent phase of labor (cervical dilation < 4 cm), in the active phase of labor (cervical dilation ≥ 4 cm), and 24 hours postpartum were compared in the women (n = 9) who delivered vaginally at term (over 37 weeks of gestation) by two-way analysis of variance (ANOVA). The sources of difference underlying effects revealed by ANOVA were detected by Fisher’s post hoc analysis. Mean plasma concentrations of P during the latent phase of labor in the above women were compared with the levels of P in the women (n = 5) who were delivered by an elective cesarean section at term by student t-test. A p < 0.05 was considered as level of statistical significance.

**Results**

**P and CRH level changes during progression of labor**

Table 1 and Figure 1A demonstrate a significant effect of labor phase (latent, active, postpartum) on P levels (p < 0.001). Specifically, the mean maternal plasma concentration during active labor was 18% higher than the mean level during the latent phase of labor (p < 0.01). A steep decline in P levels was observed following delivery: mean maternal plasma P concentration at 24 hours postpartum was significantly lower than active labor mean level (p < 0.001).

Likewise, there was a significant effect of labor phase (latent, active, postpartum) on CRH level (Table 1 and Figure 1B). Mean maternal plasma CRH concentration during active labor was 24% higher than that during the latent phase of labor (p < 0.01). Similarly to the pattern observed in P levels, there was a precipitous decline in CRH concentrations following delivery. Mean maternal plasma CRH concentration 24 hours postpartum was roughly 1/34th of active labor mean level (p < 0.001).

**P levels elevated with spontaneous occurrence of labor**

Table 2 illustrates that mean P concentrations in women who were at term and in early labor differed from those who were full term but not in labor (p < 0.10). Although this does not reach statistically significant levels, there is a trend showing that the mean maternal plasma concentration of P in the non-laboring group was higher than in the latent phase laboring group, a determination likely to be further confirmed with a larger number of participants.

**Discussion**

The fluctuation of maternal CRH and P levels during different stages of labor was examined in this study. P levels were lower in full-term pregnant women who labored spontaneously compared to gestation-matched women who did not labor, suggesting that a drop in P levels is linked to the initiation of labor. In addition, there was a parallel increase in P and CRH levels as women progressed from the latent to the active phase of labor. Both hormones subsequently dropped rapidly to non-pregnant levels as compared to standard laboratory values of non-pregnant women by day one after delivery (Figure 1).

The observed pattern in P levels complements the findings of Winkler et al. [8] who assessed P receptor (PR) concentrations in the human lower uterine segment at different stages of cervical dilatation during parturition at term. They found that PR concentration diminished significantly as women progressed from two to four cm cervical dilatation to four to six cm cervical dilatation and then increased to > six cm cervical dilatation.

The finding of the increase in CRH levels as women progressed from latent to active labor followed by a precipitous postpartal decline accords with data from other studies [9, 10]. Beyond the characteristic rise of CRH in the third trimester [11], CRH rises dramatically during the active phase of labor [9] and declines rapidly towards the non-pregnant levels by the first day postpartum [10]. It is interesting to note that the rapid drop in CRH and subsequently in CRH-induced cortisol in the immediate postnatal period is likely to be responsible for the ‘baby-blues’ commonly observed at postpartum.

CRH, the primary regulator of stress via its management of the hypothalamic-pituitary-adrenal axis (HPA), acts on the fetal pituitary-adrenal axis as well as on the uterus. This multi-sited action possibly maintains a positive feed-back loop between the fetal pituitary-adrenal axis and the placenta, which leads to an up-regulation of fetal secretion of cortisol [12] and dehydroepiandrosterone-sulfate (DHEA-S) [13]. Fetal cortisol, which is essential for the maturation of the fetal lungs [14], sequentially stimulates CRH release from the placenta [8, 11]. Meanwhile, DHEA-S stimulates placental estrogen production, which is also hypothesized to play a major role in the initiation of parturition [15]. CRH receptors exist in the myometrium [16, 17], fetal membranes [18], and placenta [19], indicating that CRH has multiple targets. In addition, placental and fetal membrane secretion of

| Table 1. — Progesterone (ng/ml) and CRH (pg/ml) levels (mean ± SD) in full term mothers during latent labor, active labor and post delivery. |
| Latent phase | Active phase | Post delivery | F-test | Effect of time |
| Progesterone | 103.2 ± 17.6 | 121.8 ± 11.3 | 12.2 ± 8.2 | 221.2 p < 0.001 |
| CRH | 778.9 ± 226.6 | 968.9 ± 240.3 | 28.5 ± 16.3 | 118.1 p < 0.001 |
| < 0.01 vs latent phase, p < 0.001 vs active phase. |

| Table 2. — Progesterone (ng/ml) levels (mean ± SD) in full term women in latent phase of labor (n = 9) compared with full term women not in labor (n = 5). |
| Latent labor | Not in labor | t-test | Statistical significance |
| Progesterone | 103.2 ± 17.6 | 123.2 ± 19.4 | 1.965t | p < 0.1 |
| (Prob = 0.073) |
Corticotropin-releasing hormone and progesterone plasma levels association with the onset and progression of labor

Prostaglandins E2 and F2a is up-regulated in response to CRH [20, 21]. The ability of CRH to potentiate the action of oxytocin may also contribute to the onset of labor both at term and prematurely [22, 23]. Similarly, the CRH binding protein, which is thought to delay CRH-controlled pituitary-adrenal stimulation by binding and eliminating the free potent CRH, falls rapidly around 20 days prior to spontaneous labor, while placental CRH secretion continues to rise as labor approaches [24].

It becomes apparent that initiation of labor involves complex mechanisms that initiate autonomic and central functions which coordinate myometrial contractility and cervical dilatation. In addition, CRH and its related peptide uroctin 1 increase local metalloproteinase-9 (MMP-9) activity in placenta and fetal membranes, which may trigger the initiation of labor [25]. Studies in second-trimester amniotic fluid from pregnancies that went on to preterm labor revealed raised levels of ADAM-8, a metalloproteinase, and cortisol [26]. This finding further supports the theory of the existence of a ‘CRH placental clock’ which determines the length of the pregnancy from an early stage [24]. Furthermore, there are accumulating data strongly indicating that CRH and P initiate a cascade of immune responses in the myometrium also contributing to synchronization of the onset of labor [27].

P has an inhibitory effect [7] on the secretion of CRH from the placenta [28], presumably by prohibiting the initiation of a positive feedback loop between CRH, adrenocorticotropic hormone and cortisol [29]. It has been suggested that the inhibitory effect of P is exerted by its binding to glucocorticoid receptors (GRs) on trophoblast cells [30]. At term, CRH-induced high levels of cortisol displace GR-bound P [31], whereby the action of cortisol is initiated. Based on the above, the parallel drop and increase in CRH and P levels and in particular the rise of P levels while labor advances (Figure 1), which was shown in this study, seems a paradoxical finding. A possible explanation for this is that a sequential effect of prostaglandins may take place during labor. Mesiano [32] concluded that functional P withdrawal is mediated by an increase in the myometrial PR-A/PR-B expression ratio. The PR-A isoform opposes P actions mediated by its counterpart, the PR-B isoform. Hence, women with a higher PR-A/PR-B ratio are more likely to deliver earlier than those with lower values. Prostaglandin E2 (PGE2) increases both PR-A and PR-B isoforms without changing the PR-A/PR-B ratio; on the other hand, prostaglandin F2α (PGF2α) selectively induces the expression of PR-A, thereby increasing the PR-A/PR-B ratio [32]. In the present study, the initial diminishing levels of P in women experiencing spontaneous early labor may be a result of a primary PGF2α-mediated increase in the PR-A/PR-B ratio, followed by an increase in PGE2, which does not affect the PR-A/PR-B ratio and may enable the subsequent rise of P levels while labor progresses. Further studies are needed to elucidate the sequential effect of P on the expression and action of various prostaglandins.

The authors conclude that the onset of spontaneous labor is associated with a drop in P levels, which is followed by a parallel rise in the levels of CRH and P while labor progresses. Both hormones decrease rapidly, almost to the pre-pregnancy levels, in the immediate postnatal period. By enhancing an understanding of the mechanisms related to the onset and progression of labor at term, the same principles in preterm labor, one of the main causes of perinatal mortality, and in which area little improvement has been achieved over the last few decades, can be assessed.
References


Operative hysteroscopy preserving virginity: a new technique

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Summary
Objective: To present a new technique of virginity-preserving operative hysteroscopy in the treatment of intrauterine pathologies. Materials and Methods: The details of operative hysteroscopy in which the hymenal orifice was left intact to preserve virginity are presented. The technique briefly involved the following steps: holding the cervix with a tenaculum and its traction to the immediate posterior hymenal opening with use of office hysteroscopy, which was then followed by operative conventional hysteroscopy. Results: The technique was performed successfully in all patients with an annular hymenal morphology. The technique enabled complete resection of intrauterine pathologies in all cases. There was no case of inadvertent hymenal injury during the procedure. Conclusion: The presented technique makes it possible to easily treat intrauterine pathologies while preserving the hymen. It can be preferred in groups of patients in whom it is necessary to preserve virginity.

Key words: Operative hysteroscopy; Vaginoscopy; Virginity.

Introduction
Hysteroscopy is a significant method commonly used in the evaluation of the vagina, cervix, and endometrium [1]. Currently, it has become the gold standard as it is minimally invasive and can be performed on an outpatient basis. Although it is widely used for quite a large group of indications, some restrictive factors may occasionally limit its use. One of these factors is virginity.

As virginity refers to the intactness of the hymen and sexual integrity in many cultures, it is directly related to female social life. It is of great importance in China and Mediterranean cultures, as well as in Muslim communities [2, 3]. Therefore, interventions through the vaginal route are found unacceptable in these cultures. It is obvious that there is a need to develop virginity-preserving methods.

The present study aims to specify the details of a new technique developed to preserve virginity.

Materials and Methods

In the present study, retrospective records of five cases were examined in whom hymen-preserving hysteroscopic technique through the vaginal route was performed. All of the patients and their parents were informed about the technique in detail, and written consents were obtained. The procedure was conducted under intravenous sedo-analgesia. The technique is performed in patients with annular hymenal morphology. Briefly, in a lithotomy position, an office hysteroscope was inserted through the hymenal opening and vaginoscopy was conducted without using a vaginal speculum. A panoramic image of the cervix was obtained and external cervical orifice was rendered more visible. Then, with the visual guidance of office hysteroscopy, a tenaculum was inserted through hymenal orifice and the upper cervical lip was grasped. The cervix was then pulled down through the vagina as close as possible to the proximity of the hymenal orifice (Figure 1). After adequate traction, the cervix was dilated by Hegar dilators through hymenal orifice up to nine mm and an operative hysteroscope was introduced into the endometrial cavity. The cervix was firmly held in traction throughout the procedure. The rest of the procedure was conducted in line with routine operative hysteroscopy.

Results
In this study, the aforementioned technique was performed in five cases. All the cases were virgins and had annular hymens. All the cases had a complaint of abnormal uterine bleeding which did not respond to medical treatment. Two of the patients had submucous leiomyomas and three had endometrial polyps. Mean operation time was 16 ± 3 minutes. Fluid deficit was 340 ± 80 ml. None of the patients had intraoperative or postoperative complications. Hymenal integrity was preserved in all patients.

Discussion
The findings of the present study have shown that operative hysteroscopy can be safely performed when hymenal integrity is a concern. There are only few studies about virginity-preserving gynecological interventions. Most of them are small case series that have reported the use of office hysteroscopy mainly in cervical pathologies [4, 5]. All the cases in the present study had intrauterine pathologies, and in this respect it is the first of its kind in the literature. The described method has some restrictions. Social, cultural, and religious values of the patients are the main obstacle to the vaginal approach [2]. This situation seems to be the most common limitation which
can be overcome by adequate information. Variations in hymenal morphology, size, and shape of hymenal orifice are also significant intrinsic factors that can limit the use of this operative hysteroscopy. This present method cannot be used in septate or cribiform hymenal structures. However, this type of hymenal morphology is found in about three percent of patients, therefore this is a limited concern [6]. Another potentially limiting factor is the inability to provide adequate cervical traction. As stated above, traction of the cervix is the essential step of the method. Conditions like endometriosis, pelvic infections, and nulliparity may compromise the amount of descensus provided.

The approach proved that operative hysteroscopy is a viable option in virgin patients whose main concern is preservation of hymenal integrity. The method was successfully applied in all five cases. However applicability of this technique in all virgin patients still remains to be answered due to the aforementioned limitations.

In conclusion, the technique the authors have described may enable the treatment of intrauterine pathologies requiring operative hysteroscopy while preserving hymenal integrity.

References


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Figure 1. — Grasping of cervix during vaginoscopy.
Introduction

Pain due to delivery is a normal physiological phenomenon, but severe persistence causes primiparas to experience fear along with pain, but it is also a neuroendocrine reaction induced by this stress that causes adverse effects in puerperant delivery process and fetuses [1]. Due to fear of delivery pain, partial primiparas will select cesarean section to avoid it. As a result, cesarean section rate of China with dominant primiparas constantly increases, and short- and long-term complications are increasingly apparent, which has become a serious public social problem [2]. With the progress of society, development of medicine, and change of obstetrics service, a safe, effective, and pain-relieving delivery has become an urgent need for gravidas, and it is an important issue in clinical researches. In recent years, although the delivery analgesia technology is increasingly effective, its popularity rate is still low in China: less than ten percent. So far, there is still no satisfactory, safe, simple, economical, and popular delivery analgesic method and drug suitable for the national conditions of China. In addition, it is always contestable whether it will delay labor and increase cesarean section, postpartum hemorrhage, and neonatal asphyxia rates [3].

Therefore, the authors use the delivery analgesia method with spinal epidural anesthesia plus a psychological Doula support in a prospective study in order to investigate its analgesic effect and its influences on mother and baby in providing a reference for promoting natural delivery and reducing cesarean section rate.

Materials and Methods

Clinical data

The primiparas laboring in the present hospital from May 2010 to May 2012 were selected, and their ages ranged from 20 to 34 years. For all primiparas, pregnancy months were adequate. Also, each primipara only delivered one fetus through cephalic presentation. In addition, there was no cephalopelvic disproportion, obstetric or internal medicine complications, and epidural anesthesia contraindications. During labor, 200 primiparas voluntarily selected to deliver with analgesia (observation group). At the same time, 200 primiparas delivered without analgesia (control group). For age, gestational week, and fetal size, there was no significant difference between the two groups. This study was conducted in accordance with the Declaration of Helsinki, and with the approval of the Ethics Committee of Beijing Tongren Hospital of Capital Medical University. Written informed consent was also obtained from all participants.

Doula and anesthesia analgesia

In the observation group, from initial labouring to two hours postpartum, each primipara was accompanied with one Doula midwife. During the delivery accompanying process, Doula midwife conducted psychological, physiological, and physical care, and explained delivery-related concepts to primiparas and their families and provided mental and spiritual support. When uterine orifice of the primiparas was dilated by about two to
three cm, a catheter was positioned for spinal analgesia. A first dose of anesthetic solution (ropivacaine 2.5 mg plus sufentanil 2.5 µg) was infused in the subarachnoid space. Subsequently, a solution of 0.1% ropivacaine plus one µg/ml sufentanil was infused into epidural cavity via the epidural catheter using a micro self-controlled pump at a rate of five to six ml/h for maintaining analgesia until uterine orifice was completely opened and during episiotomy oxytocin intension and amniotomy were performed to maintain satisfactory uterine contraction frequency and intensity. If the labor was complicated with fetal distress, abnormal fetal position, and protracted labor without resolution, cesarean section was performed.

Analgesic effect

Pain indicator: the visual analogy scoring method (VAS, 0-10 scores) was used [4]. 0: no pain; below 3 scores: slight pain, tolerable; 4 to 6 scores: pain affected sleep, tolerable; 7 to 9 scores: intolerable; 10 scores: sharp pain. According to the scores, pain situations of two groups of primiparas in the latent period, the active phase, and the second and third stages of labor were evaluated.

Blood gas analysis

As it was confirmed that primiparas were in the second stage of labor, one-ml radial artery blood specimen was acquired, sealed, and immediately sent for testing. After a fetus was delivered and before crying, one-ml of umbilical artery blood specimen was immediately acquired, sealed, and sent for testing.

Recording clinical data

Vital signs, labor times, visual analogy scores, amniotomies, oxytocin applications, delivery modes, neonatal asphyxia, and postpartum hemorrhage were recorded.

Statistical analysis

SPSS10.0 software was used for t-test and chi-square (χ²) test. If p < 0.05, a significant difference could be observed.

Results

Comparison of pain situations between two groups of primiparas

During labor, there were respectively, 20 and 38 cases receiving cesarean section due to fetus, delivery force, and other factors in the observation and control groups, and pain scoring was not conducted in them. For VAS score of pain before analgesia, there was no significant difference between two groups (p > 0.05). After analgesia, the pain of the observation group was significantly relieved. Between two groups, there was a significant difference for VAS score of pain (p < 0.05) (Table 1).

Comparisons of labor time and medical intervention measures between two groups

For the active phase time, the time of the second and third stages of labor, amniotomy intervention rate, and oxytocin application rate, there was no significant difference between two groups (p > 0.05) (Table 2). In addition, two groups of primiparas receiving cesarean section were excluded from the statistics.

Table 1. — Comparison of pain VAS scores at different times between the two groups (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Active phase</th>
<th>Second stage</th>
<th>Third stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>180</td>
<td>8.1 ± 1.3</td>
<td>3.6 ± 1.3</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Control</td>
<td>162</td>
<td>8.3 ± 1.7</td>
<td>8.8 ± 1.0</td>
<td>9.1 ± 0.6</td>
</tr>
</tbody>
</table>

For comparison between two groups, *p < 0.05.

Table 2. — Comparison of labor time and medical intervention measures between the two group (x ± s) n (%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Natural delivery</th>
<th>Assisted vaginal delivery</th>
<th>Cesarean section</th>
<th>Neonatal asphyxia</th>
<th>Postpartum hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>200</td>
<td>145 (72.5)</td>
<td>55 (27.5)</td>
<td>35 (17.5)</td>
<td>20 (10.0)</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Control</td>
<td>162</td>
<td>141 (72.5)</td>
<td>51 (31.2)</td>
<td>31 (19.2)</td>
<td>19 (11.7)</td>
<td>7 (4.3)</td>
</tr>
</tbody>
</table>

For comparison between two groups, *p < 0.05.

Comparisons of delivery mode and delivery outcome between two groups

Although the assisted vaginal delivery rate of the observation group was higher than that of the control group, the cesarean section rate was low and there was a significant difference between two groups (p < 0.05). However, there was no significant difference for neonatal asphyxia and postpartum hemorrhage rates between two groups (p > 0.05) (Table 3). In addition, two groups of primiparas receiving cesarean section were excluded from the statistics.

Comparisons of blood gas analysis results of primiparas and their neonates between two groups

For comparison of blood gas analyses, results of primiparas and their neonates, there was no significant difference between two groups (p > 0.05) (Table 4). In addition, two groups of primiparas receiving cesarean section were excluded from the statistics.

Discussion

The ideal delivery analgesia should cause minor psychological impact to mother and baby and it should be easily administered. Furthermore, it should satisfy all the operative requirements of delivery analgesia and avoid complications [5]. According to this analysis, the delivery analgesia method of self-controlled combined spinal epidural anesthesia of low-concentration ropivacaine and low-dose sufentanil analgesia plus Doula, is a method with satisfactory effectiveness.

In the delivery process, drastic uterine contraction pain cause primiparas to feel anxious, frightened, and nervous. Primiparas hope to receive treatment from healthcare providers to relieve psychological tension. Doula delivery
[6] provides a one-to-one new delivery care service mode for primiparas. It not only relieves emotional tension of primiparas and provides a spiritual pillar, but personalizes the assistance throughout the delivery process. This methodology was applied to the observation group. Therefore, the whole labor process was conducted under the active management and close cooperation of anesthetist, obstetrician, and midwife. In addition, the drug effect apparently mitigated or entirely relieved pain sensation of primiparas. This kind of delivery also appears to mitigate tension and anxiety of primiparas that can better cooperate with the obstetrical team enabling the delivery progression.

Application time, type, and dose of analgesic drugs, extension of analgesia, and blocking range determine the results of delivery anesthesia [7]. Local anesthetic is a most widely-used painless delivery of epidural anesthesia. Gor-mar et al. [8] reported that the action of this method was slow, not always satisfactory, and it could block motor nerves. In the two groups, during labor, a pain VAS scoring was conducted. Pain VAS scores of the observation group in various stages were respectively, 8.1 ± 1.3, 3.6 ± 1.1, 3.2 ± 1.1, and 2.6 ± 1.4, and pain VAS scores of the control group were respectively, 8.3 ± 1.7, 8.8 ± 1.0, 9.1 ± 0.6, and 5.4 ± 1.6. Based on the pain VAS score, no significant difference before analgesia was found between two groups (p > 0.05) (Table 1). After analgesia, the pain of in the observation group was mitigated, with a sustainable self-control activity, feeding, and micturition. In the second stage of labor, they can freely use abdominal pressure to actively participate in the delivery process. Therefore, uterine inertia, postpartum hemorrhage, and other complications can be avoided.

Some authors [12] believe that painless delivery would not delay labor, while others [13] believe that painless delivery would not delay the second stage of labor and that it could be useful for natural delivery. The key reason for controversy is possibly related to the type and dose of analgesic drugs, level of anesthesia, control of blocking range, and other factors. Some studies [14] suggest that if anesthetic dose was higher, anesthetic could block pelvic floor muscle and rectal sensory nerves, reducing motility and could inhibit uterine contraction and thus weaken delivery force, delay labor, and increase the possibility of cesarean section and assisted vaginal delivery. The solution is to mainly add opioid drugs such as sufentanil, to reduce the dosage of local anesthetic. In this study, the delivery analgesic method of self-controlled combined spinal epidural anesthesia of ropivacaine and low-dose sufentanil was used to observe the time of the active phase, and the second and third stages of labor. The time of various stages of the observation group was respectively, 6.1 ± 2.1 h, 86.6 ± 20.1 min and 11.2 ± 3.1 min, and the time of various stages of the control group was respectively, 5.8 ± 1.7 h, 82.6 ± 29.2 min, and 10.1 ± 1.6 min. Between the two groups, there was no significant difference for the time of various stages of labor. In addition, some studies [15] showed that painless delivery increased the artificial amniotomy and oxytocin intervention rates during labor. This study reports that the artificial amniotomy and oxytocin intervention rates of the observation group were respectively, 33.9% and 21.1%, and those of the control group were respectively, 33.3% and 21.6%. For the oxytocin intervention rate during labor, there was no significant difference between the two groups. According to the aforementioned results, it can be suggested that painless delivery does not affect labor progression and does not increase artificial amniotomy and oxytocin intervention rates. For the influence of painless delivery on delivery mode, one study [16] reports that painless delivery obviously increased the rates of assisted vaginal delivery and cesarean section. This group of data show that assisted vaginal delivery rate and ce-
sarean section rate of the observation group are respectively, 17.5% and 10.0%, and those of the control group are respectively, 10.5% and 19.0%. Although painless delivery of the observation group increases the assisted vaginal delivery rate to a certain extent, its cesarean section rate is significantly lower than that of the control group; there is a significant difference between the two groups. It is indented that this delivery analgesic method cannot obviously influence labor progression, but can reduce cesarean section rate and promote vaginal delivery. The present analysis results are possibly related to the applications of novel local anesthetic ropivacaine and low-dose opioid sufentanil and the control of implementation and closing time of analgesia. Under the premise of good analgesic effect, the combination of sufentanil and low-concentration ropivacaine reduces ropivacaine concentration and thus mitigates blocking of motor nerves. Delivery analgesia begins in the latent period in cases of uterine orifice of two to three cm, and primiparas justly feel obvious pain; at this time, it is best to conduct analgesia. Primiparas keep quiet and can actively cooperate. As uterine orifice is nearly fully open, analgesia pump is timely closed. At the second stage of labor, the relaxation effect of analgesia on vagina and perineum fades away incompletely. Therefore, it reduces the resistance to birth canal and mitigates the inhibition of analgesic to abdominal muscle and levator ani muscle. At this time, primiparas have accumulated their energy, which helps them to hold force in case of uterine contraction and is useful for smooth progress of labor. In addition, the authors believe that even if painless delivery increases a certain cesarean section rate, its influence is minor when compared with cesarean section rate caused by other factors, such as the ratio of primiparas fearing pain that require cesarean section (16.69%), as reported by the literature [17]. High cesarean section rate caused by this kind of social factor has an important significance especially in China. Primiparas in China are numerous, and fear and anxiety towards delivery increase cesarean section rate to a larger extent. Painless delivery updates the concept that delivery is certainly pained and it reduces unnecessary cesarean section. In this sense, it undeniably reduces cesarean section rate.

Neonatal asphyxia and postpartum hemorrhage incidence rates are the objective indicators of directly evaluating influences of delivery analgesia on mother and baby. For the influence of spinal analgesia on fetal heart rhythm, it is always contestable. Lee et al. [18] thought that the infusion of opioid drugs into subarachnoid space used for delivery analgesia could increase the risks of slow fetal heartbeat and postpartum hemorrhage, but it could not increase cesarean section rate. The study of Grondin et al. [19] showed that epidural low-concentration sufentanil infusion had no inhibition to neonate breathing. Ropivacaine used in this study is a novel long-acting local anesthetic. After the delivery analgesic method of self-controlled combined spinal epidural analgesia with low-concentration ropivacaine and low-dose sufentanil analgesia plus Doula, the results showed that neonatal asphyxia and postpartum hemorrhage rates of the observation group were respectively, 4.5% and 6.6%, and those of the control group were respectively, 5.5% and 5.5%; between two groups, there was no significant difference. It is suggested that this delivery analgesic method is effective and exact and it has no influence on postpartum hemorrhage and neonatal asphyxia incidence rates and its safety is also high. Sufentanil belongs to opioid drugs, and its application in small amounts can reduce ropivacaine dosage in order to achieve the purpose of minimal motor block and no influence on uterine contraction and labor progress. During delivery, mother and baby are treated as a single entity. Fetal oxygenation status is not only influenced by the fetus' own metabolism, but is also related to maternal acid-base status and uteroplacental blood flow. Therefore, blood gas results of mother and baby can accurately reflect maternal acid-base status and fetal intrauterine anoxia inhibition extent. In this study, blood gas analysis result showed that PO2 of primiparas and neonates in the observation group were respectively, 105.32 ± 13.45 mmHg and 25.48 ± 3.51 mmHg, and PO2 of primiparas and neonates in the control group were respectively, 102.38 ± 12.51 mmHg and 24.31 ± 4.53 mmHg; blood gas analysis result showed that PCO2 of primiparas and neonates in the observation group were respectively, 30.45 ± 3.51 mmHg and 43.32 ± 2.51 mmHg, and PCO2 of primiparas and neonates in the control group were respectively, 36.37 ± 3.35 mmHg and 46.21 ± 4.82 mmHg. According to the aforementioned results, partial pressures of oxygen of primiparas and neonates of the observation group are higher than those of the control group, while partial pressures of carbon dioxide primiparas and neonates are lower than those of the control group. Between the two groups, there are significant differences. It is suggested that delivery analgesia cannot only relieve pain, but can also increase vital capacity, improve lung function, and facilitate fetal oxygen supply. Simultaneously, it can mitigate stress reaction, avoid neonatal hypoxemia, and acidosis caused by apnea of gravidas in case of uterine contraction, improve uterine blood flow, and increase PO2 of umbilical arterial blood, which is useful for both mother and baby. Bolukbasi et al. [20] also found that self-controlled epidural delivery analgesia could decrease the stress reaction and oxygen consumption of primiparas and reduce fetal acidosis incidence rate, by detecting plasma adrenaline and noradrenaline, blood sugar, and blood gas of umbilical arterial blood of primiparas; it is in line with the viewpoint of this study.

In conclusion, the delivery analgesia method of self-controlled combined spinal epidural anesthesia applying the mixture solution of low-concentration and low-dose ropivacaine and trace sufentanil plus Doula has a rapid action and an exact analgesic effect, and it is also easily administered. It can meet the analgesic requirements of the entire labor process, and greatly mitigate related delivery pain. In addition, its influence on mother and baby is small, and its safety is high, therefore, it is easy for primiparas to accept it. As a result, it reduces the cesarean section rate caused due to the “social factor” fear to pain, saves medical costs, and avoids medical risks.
References


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Balloon tamponade for prevention and treatment of vaginal hemorrhages in gynecology

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Summary
The preliminary experience of balloon tamponade in planned vaginal surgery and in emergency vaginal bleeding using a new device (Vagistop) is reported. The results show the advantages of the system in comparison with vaginal gauze packing.

Key words: Vaginal surgery; Vaginal balloon tamponade; Vagistop.

Introduction
Vaginal packing with gauze is commonly used in vaginal surgery for different conditions. This type of tamponade is performed with the aim to stop venous bleeding of the dissected vaginal tissue and to avoid subsequent bleeding and haematomas formation. In most cases, the tamponade is removed after 24 hours [1-3].

However, the long gauze used for this procedure may absorb blood and conceal considerable blood loss. Moreover, retaining and removal of vaginal gauze is not comfortable for the patient.

The authors describe their experience with balloon tamponade after vaginal surgery in 85 consecutive cases using a new tamponade system, Vagistop, a balloon specifically created for vaginal distension and inspection (Figure 1). This preliminary experience has encouraged the authors to use Vagistop for severe vaginal obstetric haemorrhages [4].

Materials and Methods
Vaginal balloon tamponade with Vagistop was used at the end of vaginal surgery in 80 consecutive cases of planned vaginal surgery and in five cases of emergency vaginal surgery (Table 1), from January 2011 to June 2012. The 80 planned cases underwent vaginal surgery for prolapse, in most cases with associated hysterectomy.

At the end of surgical procedure, Vagistop was applied. The device is made of a flexible tube with a balloon tip, connected to a syringe through a valve that allows inflating the balloon continuously, without pulling out the syringe. The balloon is inserted until the posterior vaginal fornix or cuff. After insertion, the balloon is inflated with air using a 20-ml syringe connected to the inflation tube through the valve (Figure 1) until the whole vaginal space is occupied (Figure 2); at this point, the balloon internal pressure is about 50 mmHg, greater than the venous pressure in the pelvis. The material of the surface of the balloon is a polymer that allows the device to adhere to the vaginal walls. The total capacity of the balloon is 250 ml. The air volume used ranged from 180 to 40 ml, with an average of 90 ml.

Table 1. — Cases with emergency vaginal balloon tamponade.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Type of problem</th>
<th>Volume used</th>
<th>Time in place (hours)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laparotomy for retroperitoneal mass</td>
<td>180</td>
<td>24</td>
<td>good</td>
</tr>
<tr>
<td>2</td>
<td>Bleeding after Bartholin’s cyst removal</td>
<td>140</td>
<td>24</td>
<td>good</td>
</tr>
<tr>
<td>3</td>
<td>Bleeding after vaginal cyst removal</td>
<td>140</td>
<td>24</td>
<td>good</td>
</tr>
<tr>
<td>4</td>
<td>Hemorrhage 3 days after vaginal cyst removal</td>
<td>140</td>
<td>24</td>
<td>good</td>
</tr>
<tr>
<td>5</td>
<td>Hemorrhage 2 days after conization</td>
<td>140</td>
<td>24</td>
<td>good</td>
</tr>
</tbody>
</table>

Results
The balloon was deflated and removed 24 hours later. In ten cases Vagistop was expelled spontaneously between two and six hours after surgery. Expulsion occurred in all cases with severe postoperative vomiting. In all cases, the nurse easily removed Vagistop. None of the patients complained of discomfort (VAS score 0/10). No bleeding occurred in any of the cases.

Discussion
Vaginal tamponade with gauze is a commonly used procedure at the end of different vaginal surgical procedures [1-3]. The aim is to avoid bleeding and haematomas caused by venous bleeding of the dissected vaginal tissue. Gauze packing is however a matter of discomfort for the woman and may absorb a lot of blood before evidence of persisting bleeding is recognized.

As far as the authors know, only one case of balloon tamponade for vaginal hemorrhage in gynecology has been published [5]. The authors’ previous positive experience in obstetrics with vaginal balloon tamponade with Vagistop [4] lead them to apply Vagistop in substitution of gauze packing in vaginal surgery. Simple and rapid application and removal, both in planned and in emergency surgeries (without use of anaesthesia), compliance of the patients, as well as optimal adhesion to vaginal walls, are the main advantages of Vagistop in comparison with gauze packing.

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Conclusions

The results obtained in this preliminary case justify the use of vaginal balloon tamponade to prevent and treat vaginal bleeding and haematomas in vaginal surgery, as well as in an emergency setting.

References


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Prevalence of women’s worries, anxiety, and depression during pregnancy in a public hospital setting in Greece

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Summary

Many studies have examined the prevalence and risk factors of postnatal depression. However, only a few studies have explored the prevalence of anxiety and depressive symptoms in pregnancy. The aim of this study was to investigate the prevalence of worries, antenatal anxiety (AA), and antenatal depression (AD). The sample of this study consisted of 163 pregnant women with gestational age from 11 to 26 weeks. Worries were measured with Cambridge Worry Scale (CWS), anxiety was measured with State-Trait Anxiety Inventory (STAI), and depression was measured with Center for Epidemiologic Studies-Depression scale (CES-D). Depressive symptoms were found in 32.7% of the participants and 44.4% had STAI scores indicating anxiety symptoms of clinical significance. The mean score for total CWS was 26 (SD = 12.3). It is noteworthy that the most important worries in the study sample were “the possibility of something going wrong with the baby”, “giving birth”, and “financial problems”. The prevalence of antenatal anxiety and depression identified in this study is of concern. Screening for antenatal anxiety and depressive symptoms with validated instruments is crucial.

Key words: Anxiety; Depression; Worries; Pregnancy; Prevalence.

Introduction

Pregnancy and the transition to parenthood involve major biological and psychosocial changes [1]. These changes have been linked to an increase in anxiety symptoms (AS), depression symptoms (DS), worry, and stress [2]. World Health Organization (WHO) estimates that depressive disorders will be the second leading cause of global disease burden by 2020 [3]. Postnatal depression shares similar prevalence ratings to those of depression in the general population, ranging from 12%-20%, with a commonly-reported estimate of 13% [4]. Although many studies have examined the prevalence and risk factors of postnatal depression, only a few studies have explored the prevalence of DS, and even fewer studies have addressed the prevalence of AS in pregnancy. A meta-analysis of 21 studies on depression during pregnancy indicated that the prevalence of antenatal depression (AD) was approximately 10.7%, ranging from 7.4% in the first trimester to 12.8% in the second trimester [5]. However, the rate of AD in individual studies ranges from 4.8% up to 40% [4, 6-12]. Moreover, the incidence rate of anxiety during pregnancy has been reported to range between 6.8% and 59.5% [4, 6, 9, 12, 13]. It is noteworthy that the estimation of the incidence of stress and worries during pregnancy has been a relatively neglected area of research.

Anxiety, depression, and other stressful feelings during prenatal period can easily lead to more severe diseases, which may be harmful to the mother, fetus, and the expectant newborn’s health [6]. Anxiety and depression during pregnancy have been associated with prematurity, low birth weight, and fetal growth retardation [14-16], obstetric complications, increased nausea and vomiting, planned cesarean delivery [17], postpartum depression [4], and may have a negative impact on child development [18]. Therefore, it is essential to investigate the prevalence of anxiety, stress, and depression of the pregnant women in order to implement interventions to reduce adverse pregnancy outcomes. The current literature suggests that low income and unemployment are major risk factors of antenatal anxiety and depression [4, 9, 10]. Therefore, the prevalence of antenatal anxiety, stress, and depression in a country with a major financial crisis and high unemployment rates as in Greece would be worthy of attention.

Materials and Methods

Sample and data collection

The study was conducted in one of the largest hospitals in Athens, Greece to achieve a representative database. The questionnaire was administered to a sample of 163 pregnant women with a gestational age of between 11 and 26 weeks, who were booked for antenatal screening in the antenatal clinic of a public hospital of Athens. Following ultrasound scanning, a midwife of this research team contacted the eligible women. The pregnant women were informed of the study aim and protocol, and once they voluntarily agreed to participate, they were given an envelope containing the questionnaires and an informed consent form. The completed questionnaires and the signed consent form were returned directly or by mail to the researcher (within two to three weeks).

Study instruments

Worries during pregnancy were measured with the Cambridge Worry Scale (CWS) developed by Green et al., in 2003 [19]. The CWS contains items concerning worries during pregnancy, such as the baby’s health, financial issues, and giving birth. Each item is scored on a six-point Likert-type scale ranging from not a worry (0) to major worry (5). The CWS scale can be used throughout pregnancy. Depending on the pregnancy week, additional context-specific items can be added or removed as appro-
prite. The CWS used in this study comprised of 16 items, which allowed a total sum score that ranged from 0 to 80 to be calculated. According to the instrument developers and the Greek validation outcome, the CWS has a four-factor structure: (1) socio-economic aspects of having a baby: giving birth, going to hospital, internal examinations, and coping with the new baby, (2) socio-economic issues: money, employment problems, housing, and the law, (3) health of mother and baby: miscarriage, something going wrong with the baby, and own health, and (4) relationships with partner, family, and friends. A higher score reflects higher worries. The CWS was adapted to the Greek language and has been found to have satisfactory psychometric properties (e.g., construct validity).

State and trait anxiety was measured with the State Trait Anxiety Inventory (STAI) [20]. State anxiety is defined as an unpleasant emotional condition that emerges in case of threatening demands or dangers. Therefore, it should be low in non-threatening situations and high if circumstances are perceived to be threatening or dangerous. The state scale consists of 20 items that ask people to describe how they feel at a particular moment in time, rated on a four-point scale ranging from not at all (1) to very much so (4). The trait scale consists of 20 items and asks people to describe how they generally feel (e.g., confident), rated on a four-point frequency scale ranging from (1) almost never to (4) almost always. Total scores for state and trait anxiety range from 20 to 80. The STAI was adapted to the Greek language and has been found to have satisfactory psychometric properties [21]. A cut-off score of 43 or was used in this study as a point indicating high-state anxiety [22]. Cronbach’s alpha of 0.84 (state) and 0.87 (trait) were obtained in the present study.

The Center for Epidemiologic Studies-Depression scale (CES-D) was used to assess depression symptoms of the study population [23]. CES-D is a self-reporting 20-item scale that covers affective, behavioural, and somatic symptoms experienced during the past week. Responses to item statements are graded from 0 (rarely or none of the time) to 3 (most or all of the time). Four items are reverse-scored items. Scores for each item in the CES-D scale are summed to obtain an overall score. The overall score ranges from 0 to 60, where the higher the score, the more frequent the depressive symptoms. A cut-off score of 16 or higher was used in this study as that point indicative of significant or mild depressive symptomatology in many studies addressing depression during pregnancy [24, 25]. The CES-D was adapted to the Greek language and has been found to have satisfactory psychometric properties [26]. A Cronbach’s alpha of 0.86 was obtained in this study.

Basic demographic and medical information included: age, gestational age, parity, previous miscarriages, previous deliveries, complications during previous pregnancy and labour, previous infertility problems, marital status, educational level, economic level, and employment status. The educational level was categorized as low (up through elementary school), medium (high school certificate) or high (university degree). The annual income level was categorized as low (€ 9,600-17,999 or USD 13,300-25,000), medium (€ 18,000-35,999 or USD 25,001-50,170) or high (> € 36,000 or > USD 50,171) [27].

Statistical analysis

Statistical analysis was performed using SPSS version 17.0. Descriptive statistics, such as means, standard deviations, and frequencies, were used to represent the demographic characteristics of the participants. Mean values and standard deviations of the total sum scores of the CWS, STAI, and CES-D, were also calculated; p values less than 0.05 were considered significant.

Results

Characteristics of participants

The mean age of participants was 31.2 years (SD 4.2 and range 22-44). Sixty-two percent had education beyond high school and 37% had high school, and one percent had less than a high school education. Eighty percent of women participated in the work-force and 96% were married. For 46% of the sample, this was their first pregnancy, 36% had already a child, 22% of the women had experienced previous miscarriages, and 12% of the participants had experienced a complication during previous pregnancy or previous labour.

Prevalence of antepartum anxiety and depressive symptoms

The means for STAI-state and trait scores were 41.5 (SD = 8.4) and 39.7 (SD = 8.3), respectively. The mean score for CES-D was 13.4 (SD = 9.2). Of the 163 participants assessed at the first and second trimesters of the pregnancy, 32.7% had CES-D scores ≥ 16, indicating depressive symptoms, and 44.4% had state anxiety scores ≥ 43, indicating anxiety symptoms. Specifically, 34.4% of participants with gestational age between 11 and 14 weeks had a CES-D score ≥ 16 and 46.9% of participants with gestational age between 11 and 14 weeks had STAI-state score ≥ 43. Moreover, 32.3% of participants with gestational age between 15 and 26 weeks had a CES-D score ≥ 16 and 43.8% of participants with gestational age between 15 and 26 weeks had STAI-state score ≥ 43.

Prevalence of antepartum worries

The mean score for total CWS was 26 (SD = 12.3). It is noteworthy that the most important worries in the sample were the “possibility of something going wrong with the baby”, “giving birth”, and “financial problems”.

Discussion

According to the authors’ knowledge, this is the first study that reports on the incidence of anxiety, depression, and worries in a sample of pregnant women admitted to a Greek public hospital. The main findings of this study suggest that AD occurs in one-third of pregnant women and AA in almost half of pregnant women. The rate of anxiety is in agreement with previous reports from both high-income [4, 12] and low-income countries [13]. Nevertheless, the rate of depression in this study was higher than those reported in countries such as USA [28], Sweden [27], Australia [4], Hungary [9], and China [6]. The high prevalence of depressive symptomatology in this study could be attributed to special socio-economic circum-
Prevalence of women’s worries, anxiety, and depression during pregnancy in a public hospital setting in Greece

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stances, such as financial crisis and high rates of unemployment. In addition to that, the direct association between poverty and depression is well-documented in high-income countries [4, 9]. Moreover, the worries related to the financial problems, ranked third in this study, whereas in previous relevant studies it did not rank top [29-31]. Therefore, financial issues may have caused significant worries among Greek pregnant women.

Conclusion

According to the findings of this study, about 50% of pregnant women experience anxiety symptoms and 30% experience antenatal depression, that not only had deleterious effects on the woman but also on her baby. The prevalence of antenatal anxiety and depression identified in this study is of concern. Midwives and healthcare professionals, who recognize the signs and symptoms of antenatal depression and anxiety, and the risk factors associated with these disorders, can help to identify and prevent them. The signs and symptoms of depression in pregnancy do not differ from depression at any other time. However, antenatal depression may go undiagnosed because the depressive symptoms could be considered complaints of pregnancy and could be attributed to the physical and hormonal changes associated with pregnancy [32]. Therefore, screening for antenatal anxiety and depressive symptoms with validated instruments is crucial.

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Preventive nursing of neonatal clavicular fracture in midwifery: a report of six cases and review of the literature

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Summary

Purpose: To summarize and analyze the obstetric factors and medical care for neonatal clavicle fracture during delivery. Materials and Methods: In 4,456 vaginal deliveries, only six newborns were found with a clavicle fracture in our hospital from October 2002 to October 2011. Results: Clinical findings showed that dystocia and improper midwifery manoeuvres are the two major reasons which lead to newborn clavicular fractures. Conclusion: More attention should be paid to non-violent traction and proper treatment of shoulder dystocia.

Key words: Midwifery; Newborn; Clavicle fracture; Nursing.

Introduction

Newborn clavicle fracture is a typical kind of birth injury in obstetrics [1]. The injuries cause severe psychological pressure on the parents and midwives of the newborn although it includes simple therapy and good prognosis. It is critical to reduce the incidence and carry out early diagnosis and treatment [2]. Six patients in 4,456 infants born through vaginal delivery were found clavicle-fractured in our hospital from October 2002 to October 2011.

Materials and Methods

A total of 4,456 fetuses weighing from 1,900 g to 4,350 g underwent vaginal delivery from October 2002 to October 2011 in our department, including 422 cases of multipara, 4,034 cases of primipara, 367 cases by forceps delivery, 248 cases with shoulder dystocia, and 61 cases with nuchal cord around neck. Six fetuses suffered from neonatal clavicular fractures at gestational age of 38-40 weeks, weighing from 3,450 g to 3,850 g, total laboring time four to 19 hours, and second stage of labor time 30 min to 1.5 hours. Out of these six, three had shoulder dystocia and three cases had forceps application together with Kristeller maneuver, while in five cases the fracture was in the distal third of the clavicle.

The injured babies cried, especially when the affected upper arm was moved. The injured upper arm was limited in movement, had local swelling, extravasated blood, bony crepitus, and reduced or disappeared embrace reflex in the ipsilateral clavicle. The earliest fracture time in one case was at delivery (fractural sound heard at shoulder delivery during labor). Other five cases were found at routine clavicle palpation within 24 hours and confirmed by X-ray.

The confirmed injured babies were set in supine position with chest expanded to mitigate the affected upper limb movements. In one case, an eight-style bandage was used for fracture dislocation. The fractured site was X-rayed and was well-reduced after the bandage was removed after two weeks. The remaining patients were not specially fixed. All patients were discharged together with their mothers.

Results

Callus growth was found in the fractured ends through X-ray examination at three weeks postpartum and all had healed at six weeks postpartum as confirmed during the normally scheduled follow-up visits. All patients were discharged with their mothers at the same time. Before leaving the hospital, individualized breastfeeding, bathing, and nursing education were performed. The follow-up contact cards were established to contact patients and encourage them for re-examination in the hospital. The follow-up visits were scheduled in four to six weeks in order to assess the healing conditions of the fractured limbs.

Discussion

Neonatal clavicular fracture is associated to the laboring manner, vaginal dystocia, fetal weight, and midwifery technique [3]. The fracture rate over vaginal dystocia is significantly higher than vaginal delivery and cesarean operation [4]. Five cases of this group occurred over vaginal dystocia. Therefore, dystocia is a fundamental factor in birth trauma that is elicited by mechanical factors. Midwifery manoeuvres are thus one of the vital causes for the injuries [5]. Providing that the posterior shoulder is raised prematurely when the anterior shoulder is not adequately delivered, the clavicle of the anterior shoulder is bound to press below the pubic arch causing the clavicle to fracture due to excessive forces [6]. Persistent occipitotransverse position or occipitoposterior position, fetal excessive weight (> 3,500 g), oversized fetal shoulder circumference, and premature uplift of posterior shoulder when the anterior shoulder is not adequately delivered, will lead to fracture through excessive pressure of anterior shoulder on the clavicle [7].

It is critical to avoid and prevent neonatal clavicular fracture by controlling the delivery for cephalic presentation and abnormal fetal position, properly treating shoulder dystocia against violent traction, and constantly improving the
childbirth technique [8]. In the event of shoulder dystocia, the McRobert method is immediately adopted. Three cases of neonatal clavicular fracture associated to improper midwifery way occurred in 74 cases of shoulder dystocia. Obstetricians should keep vigilant over this [9].

It is important to timely identify the neonatal clavicular fracture through careful examination. In the event of neonatal clavicular fracture, psychological care and health education should be enhanced for parents in order to establish a good nurse-patient relationship, to reduce or prevent complications, as well as to avoid medical disputes [10]. Five cases in the group with neonatal clavicular fractures were found by the nurses through conventional clavicle palpation within 24 hours after childbirth, and they underwent X-rays, orthopedic consultation, and immediate care. The affected limb was immobilized to ensure healing during breastfeeding and bathing. A good social supportive system is created to allow the parents to care for the newborns with scientific approaches, to benefit the affected limb recovery, shorten the disease course, and reduce or protect the complications. Detailed discharge guidance and regular follow-up visits are conducive to the healing of the fracture and improve the doctor-patient relationship to reduce medical disputes.

Conclusion

Generally bone remodelling will complete within six to 12 months in good condition and even recover its normal aspect, along with the stress change in limb in severe shortened angular deformed callus without any future sequelae or repercussions.

References


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Introduction

Uterine prolapse is one of the most common types in pelvic organ prolapse (POP), and its exact etiology is still unknown. The researches showed that the abnormal collagen metabolism in pelvic floor fascia connective tissue was the key of uterine prolapse; matrix metalloproteinases (MMPs) was involved in the occurrence and development of POP through regulating the collagen catabolism [1-6]. Recent researches showed that transforming growth factor-β1 (TGF-β1)-connective tissue growth factor (CTGF) pathway regulated the collagen metabolism; however, the function in uterine prolapse is still unknown [7-10].

Multiple studies have shown that superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) constitute the antioxidation defense system of organisms, the decrease of the antioxidation enzymes activity up-regulate the oxidative stress in cells, then affect the activity of collagen metabolism enzymes, such as MMPs and tissue inhibitor of metalloproteinases (TIMPs). One study found that the concentration of selenium and GPx was lower in camel with uterine prolapse than that in the normal camel.

So, the authors hypothesized that the decrease of GPx activity in pelvic floor fascia tissue would reduce the antioxidation ability. It may break the balance of oxidation and antioxidation in pelvic support structure, and may induce an increase of ROS level and the down-regulation of TGF-β1-CTGF pathway. It could inhibit the anabolism of collagen and injury the pelvic support structure, thus promoting the occurrence and development of POP.

Materials and Methods

Materials included: rabbit anti-human GPx1 polyclonal antibody, rabbit anti-human TGF-β1, CTGF polyclonal antibody, horseradish peroxidase labelled goat anti-rabbit polyclonal antibody, DBA, and an SP Kit.

Samples: approximately 100 mg of tissue sample was obtained with a sample intraoperatively from the pubocervical fascia tissue from each patient.

Methods: samples of the cervical fascia tissue were collected from 50 women undergoing vaginal hysterectomy at the present hospital from September 2010 to June 2011. Thirty of the patients with POP studied were placed into Group 1 (n = 10), Group 2 (n = 10), and Group 3 (n = 10), according to Pelvic Organ Prolapse Quantification (POP-Q). POP-Q II is group 1, POP-Q III is group 2, and POP-Q IV is group 3. Twenty cases with other benign gynecological disease were selected as the control group.

Control and prolapse subjects who were smokers or had concomitant malignant pelvic diseases or had been receiving local or systemic hormone replacement therapy, under anti-inflamm-
tory or steroid medications, were excluded from the study. All patients were matched to exclude possible influencing factors such as age, parity, and body mass index. Informed consents were obtained from all participating subjects and the Ethics Committee approval was obtained.

Immunohistochemical staining for GPx1 was performed to determine the presence and distribution of this protein in the pubocervical fascia tissue of POP patients. Semi-quantitative score was used to analyze the staining result. Two investigators who had no idea of the patients’ clinical information independently assessed the staining intensity. Preimmune sera was used as a negative control.

The data were analyzed by Chi-square test and Spearman rank correlation analysis. Significance was accepted at $p < 0.05$.

## Results

### Expression of TGF-$\beta_1$, CTGF and GPx1

The positive granules of TGF-$\beta_1$ and CTGF appeared dark brown or filemot, which presented a diffuse or focal distribution throughout the cytoplasm (Figures 1A-1D). TGF-$\beta_1$ expression showed significant decrease, $\chi^2 = 27.242$, $p < 0.05$ (Table 1). CTGF expression also showed significant decrease, $\chi^2 = 23.958$, $p < 0.05$ (Table 2).

The positive granules of GPx1 appeared dark brown or filemot, which presented a focal or diffuse distribution throughout the cytoplasm (Figures 2A-2B). GPx1 expression also showed significant decrease, $\chi^2 = 9.545$, $p < 0.05$ (Table 3).

### Correlation between the expression of TGF-$\beta_1$ and the POP-Q, CTGF and the POP-Q or GPx1 and the POP-Q

As the ordered category variables, there was a negative correlation between the POP-Q and expressions of TGF-$\beta_1$. With the degree of POP-Q increasing, the expression of TGF-$\beta_1$ decreased correspondingly (Table 1). It also could be seen between POP-Q and expression of CTGF (Table 2), and between POP-Q and expression of GPx1 (Table 3).

The correlation analysis between the expression of TGF-$\beta_1$ and CTGF, CTGF and GPx1 or TGF-$\beta_1$ and GPx1

As the ordered category variables, there was a positive correlation between TGF-$\beta_1$ and CTGF. The synergistic change trend was found between TGF-$\beta_1$ and CTGF (Table 4) It could also be seen in CTGF and GPx1 (Table 5) and between TGF-$\beta_1$ and GPx1 (Table 6).

## Discussion

It is generally considered that pregnancy and vaginal childbirth are associated with POP, but the exact etiology is still unknown. Female pelvic tissues were in a complex biomechanical environment with pregnancy, childbirth, high abdominal pressure (chronic cough, constipation, and obesity) etc. In the pathogenesis of POP, some researchers focused on the changes of extracellular matrix components, such as collagen-I, collagen-III, MMP, TIMP, and elastin in connective tissues. So, the decrease of mechanical prop-

### Table 1. — The expression of TGF$\beta_1$ in pubocervical fascia of four groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of TGF$\beta_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>POP-QII</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>POP-QIV</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>Total POP</td>
<td>50.00 (15/30) 50.00 (15/30) 0 0 50.00 (15/30)*</td>
</tr>
</tbody>
</table>

*The comparison between total POP and control, the $\chi^2 = 27.242$, $p < 0.05$. The correlation coefficient between TGF$\beta_1$ and POP-Q was $-0.572$, $p < 0.05$.

### Table 2. — Expression of CTGF in pubocervical fascia of four groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of CTGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>POP-QII</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>POP-QIV</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>Total POP</td>
<td>50.00 (15/30) 50.00 (15/30) 0 0 50.00 (15/30)*</td>
</tr>
</tbody>
</table>

*The comparison between total POP and control, the $\chi^2 = 23.958$, $p < 0.05$. The correlation coefficient between CTGF and POP-Q was $-0.409$, $p < 0.05$.

### Table 3. — Comparison of expression of GPx1 protein in pubocervical fascia among four groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of GPx1 [% (n/n)]</th>
<th>Total positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>0.00 (0/20) 20.00 (4/20) 40.00 (8/20) 30.00 (6/20) 10.00 (2/20) 80.00 (16/20)</td>
<td></td>
</tr>
<tr>
<td>POP-QII</td>
<td>10.00 (10/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10)</td>
<td></td>
</tr>
<tr>
<td>POP-QIV</td>
<td>20.00 (20/100) 20.00 (20/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100)</td>
<td></td>
</tr>
<tr>
<td>Total POP</td>
<td>53.33 (15/20) 46.67 (14/30) 0 0 46.67 (14/30)*</td>
<td></td>
</tr>
</tbody>
</table>

*The comparison between total POP and control, the $\chi^2 = 9.545$, $p < 0.05$. The correlation coefficient between GPx1 and POP-Q was $-0.606$, $p < 0.05$.

### Table 4. — Correlation of expression of TGF-$\beta_1$ and CTGF in the POP patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of TGF$\beta_1$</th>
<th>CTGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>0.00 (0/20) 20.00 (4/20) 40.00 (8/20) 30.00 (6/20) 10.00 (2/20) 80.00 (16/20)</td>
<td></td>
</tr>
<tr>
<td>POP-QII</td>
<td>10.00 (10/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10)</td>
<td></td>
</tr>
<tr>
<td>POP-QIV</td>
<td>20.00 (20/100) 20.00 (20/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100)</td>
<td></td>
</tr>
<tr>
<td>Total POP</td>
<td>53.33 (15/20) 46.67 (14/30) 0 0 46.67 (14/30)*</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. — Correlation of expression of GPx1 and TGF-$\beta_1$ in the POP patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of GPx1 [% (n/n)]</th>
<th>Total positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>0.00 (0/20) 20.00 (4/20) 40.00 (8/20) 30.00 (6/20) 10.00 (2/20) 80.00 (16/20)</td>
<td></td>
</tr>
<tr>
<td>POP-QII</td>
<td>10.00 (10/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10)</td>
<td></td>
</tr>
<tr>
<td>POP-QIV</td>
<td>20.00 (20/100) 20.00 (20/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100)</td>
<td></td>
</tr>
<tr>
<td>Total POP</td>
<td>53.33 (15/20) 46.67 (14/30) 0 0 46.67 (14/30)*</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6. — Correlation of expression of GPx1 and TGF-$\beta_1$ in the POP patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expression of GPx1 [% (n/n)]</th>
<th>Total positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP-QI</td>
<td>0.00 (0/20) 20.00 (4/20) 40.00 (8/20) 30.00 (6/20) 10.00 (2/20) 80.00 (16/20)</td>
<td></td>
</tr>
<tr>
<td>POP-QII</td>
<td>10.00 (10/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10) 0.00 (0/10)</td>
<td></td>
</tr>
<tr>
<td>POP-QIV</td>
<td>20.00 (20/100) 20.00 (20/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100) 0.00 (0/100)</td>
<td></td>
</tr>
<tr>
<td>Total POP</td>
<td>53.33 (15/20) 46.67 (14/30) 0 0 46.67 (14/30)*</td>
<td></td>
</tr>
</tbody>
</table>

The correlation analysis between the expression of TGF-$\beta_1$ and CTGF, CTGF and GPx1 or TGF-$\beta_1$ and GPx1

As the ordered category variables, there was a positive correlation between TGF-$\beta_1$ and CTGF. The synergistic change trend was found between TGF-$\beta_1$ and CTGF (Table 4) It could also be seen in CTGF and GPx1 (Table 5) and between TGF-$\beta_1$ and GPx1 (Table 6).
The expression of glutathione peroxidase-1 and the anabolism of collagen regulation pathway transforming growth factor-β1, etc.

The property induced by matrix remodeling was the key to the occurrence of POP [13].

Collagen is the main component in ligament and fascia, which determines the toughness of connective tissue. The connective tissue in pelvic floor mainly contained collagen-I and collagen-III [14-17]. The metabolic balance between collagen synthesis and collagen catabolism was broken, which led to pelvic floor tissue becoming weak and lax. It would ultimately result in the occurrence of POP [18, 19].

Under normal physiological conditions, the oxidation-antioxidation system maintains dynamic balance, which not only guarantees the physiological function of the normal oxidative stress reaction, but also prevents the injury of ROS. Only with the ROS overload or insufficient expression of antioxidation enzymes, the dysequilibrium of oxidation-antioxidation system would injure cells and tissues. The oxidation-antioxidation system is the basic of the health.

GPx is an important selenium protein in organism. Selenium is the active center of the enzyme, and its activity can reflect the level of selenium. GPx1 is one of the isozymes, and is widely distributed in the cytoplasms and mitochondria of every tissue cells. The expression of GPx1 reflects the level of selenium in tissue, and also is closely related to the ability of antioxidant. Some studies found that oxidative stress interferes with collagen metabolism in fibroblast cells [20-21].

Other factors such as pregnancy, childbirth, chronic constipation, and chronic cough, which increase intra-abdomi-
inal pressure, also are the important causes of POP. Dan found that mechanical strain changes the fibroblast cell morphology in uterosacral ligament and regulates the expression of collagen-I, collagen-III, and MMP-1 [22]. Excessive mechanical strain increases the level of ROS in cells, and then up-regulates the activity of MMPs to fasten the degradation of collagen [23-25]. Therefore, pregnancy, childbirth, chronic constipation, and chronic cough may result in the occurrence and development of POP by inducing the oxidative stress.

In this study, it was first found that the expression of GPx1, an antioxidase, decreased significantly in the pelvic floor fascia tissue of patients with POP, which negatively correlated with the degree of POP-Q. This study suggests that the increasing of mechanical strain or the decreasing expression of GPx1 could break the oxidation-antioxidation system balance of fibroblast cells in pelvic floor supporting tissue, and up-regulate ROS to disturb the metabolic balance of collagen synthesis, which was the key to the occurrence of POP.

In addition, the authors also found that the TGF-β₁-CTGF regulating pathway was decreased in the pelvic floor fascia tissue of patients with POP, and negatively correlated with the degree of POP-Q and positively correlated with GPx1. It could confirm that the expression of GPx1 decreased, which would make the antioxidation weak, increase ROS level in cells, down-regulate TGF-β₁-CTGF pathway, and inhibit the collagen synthesis. The increase of ROS in cells would up-regulate the activity of enzymes such as MMPs and fasten the collagen decomposition.

**Conclusion**

The expression of the antioxidase GPx1 in pelvic support structure of POP women decreased, which resulted in the antioxidation reduced. It could break the balance of oxidation and antioxidation in pelvic support structure, and may induce the increase of ROS level and the down-regulation of TGF-β₁-CTGF pathway. It could inhibit the anabolism of collagen and injure the pelvic support structure, thus promoting the occurrence and development of POP. In conclusion, the authors provide the hypothesis that the mechanism of POP may be the oxidation-antioxidation system disequilibrium. So, how to regulate the balance is the key to prevent and cure POP.

**Acknowledgment**

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**References**


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Female pseudohermaphroditism associated with maternal steroid cell tumor, not otherwise specified of the ovary: a case report and literature review

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1Department of Obstetrics and Gynecology, and 2Department of Pathology, Fujita Health University School of Medicine, Toyoake, Aichi (Japan)

Summary

Maternal virilization in pregnancy with or without fetal female pseudohermaphroditism has several etiologies. Of these, pregnancy luteoma is the most common cause of maternal virilization during pregnancy, and approximately 20 cases have been reported in recent years. Moreover, four cases of pregnancy luteomas with female pseudohermaphroditism have been reported. However, the extremely rare steroid cell tumor, not otherwise specified (NOS), has been reported only once as a cause for maternal virilization. Herein, the authors report the first case of maternal virilization with female pseudohermaphroditism associated with steroid cell tumor-NOS along with the clinical course, pathological features, and a review of the literature.

Key words: Female pseudohermaphroditism; Maternal virilization; Steroid cell tumor; Not otherwise specified; testosterone; Pathological diagnosis.

Introduction

Steroid cell tumors of the ovary are rare and account for approximately 0.1% of all ovarian tumors. These are classified into three subtypes: stromal luteomas, Leydig cell tumors, and steroid cell tumors, not otherwise specified (NOS), which account for approximately 60% of all steroid cell tumors [1]. Steroid cell tumors-NOS produce virilization in 56%-77%, hyperestrogenism in 6%-23%, and Cushing’s syndrome in 6%-10% of cases [2, 3]. Steroid cell tumors-NOS may produce the full range of steroid hormones seen in the other types. As menstrual abnormalities are common, pregnancies in the setting of this tumor are very rare. Only one case of maternal virilization by a steroid tumor NOS in pregnancy with a male fetus has been reported in the literature [4], and this case was not associated with fetal male pseudohermaphroditism. Herein, the authors report a case of maternal virilization and female pseudohermaphroditism caused by steroid cell tumor-NOS, along with the clinical course, histopathological features, and the literature review.

Case Report

A 36-year-old primigravida woman was admitted to this hospital with preterm rupture of membranes and the onset of labor at 22 weeks of gestation. She had a history of an exploratory laparotomy five years prior for bilateral solid ovarian tumors, which were initially suspected to be malignant. A left salpingo-oophorectomy was performed, and the tumor was thought to be benign on intraoperative gross inspection. The right ovarian tumor was not removed so as to not compromise fertility. The left ovarian tumor was initially diagnosed as a leiomyoma. The original tissue blocks were not available at the time this case report was drafted. The patient had irregular menstrual cycles since menarche at age 11. She was treated for infertility for four years and eventually became pregnant following ICSI (intracytoplasmic sperm injection). Magnetic resonance imaging (MRI) performed at 15 weeks of gestation, prior to referral showed a 75 x 80 mm solid tumor in the right pelvis, with heterogeneous low to intermediate signal intensity on T2-weighted imaging without contrast enhancement (Figure 1a).

On admission, her height was 160 cm, weight 51 kg, and her blood pressure was 118/70 mmHg. She presented with virilization manifested by increased facial, abdominal, and lower extremity hair, worsening acne, and a slightly enlarged clitoris. Virilization of the patient was not noticed in previous hospital during treatment for infertility. On ultrasonography, a 76 x 71 x 80 mm solid tumor was detected in the pouch of Douglas; Doppler evaluation of the tumor demonstrated hypervascularity. There was no morphological abnormality in the maternal adrenal gland. The fetus measured appropriate for gestational age and had normal anatomy and appeared to have male genitalia; however, the structure of the scrotum was obscure. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and free-T3 or T4 were all within the normal range. The serum testosterone level was markedly elevated (32 ng/ml, normal range; 0.1–0.7 ng/ml). The levels of serum tumor markers, carcinoembryonic antigen (CEA), CA19-9 were normal; however, the CA125 was slightly elevated (73 U/ml). A right ovarian sex-cord stromal tumor that produced testosterone was suspected, based on the physical, laboratory, and radiological findings. The patient was treated for preterm labor after admission to this hospital; however, she eventually developed chorioamnionitis and entered into active labor at 29 weeks of gestation. She delivered by cesarean section and underwent a right ovarian cystectomy at the same time. There was no evidence of extra-ovarian tumor or metastatic disease. A small amount of ascites was seen in the cul de sac.

The tumor measured seven by eight cm in diameter and was a well-circumscribed, grayish-yellow mass without apparent area of necrosis or degeneration (Figure 1b). The tumor was easily separated from the grossly normal-appearing ovarian tissue. Cytological examination of ascites was negative for malignancy. Histological examination of the tumor demonstrated an encapsulated, non-infiltrative pattern. The tumor included areas in which cuboidal or polygonal cells with oval to polygonal nuclei, small dis-
distinct nuclei, and abundant eosinophilic cytoplasm were arranged in a diffuse pattern of columns or nests. These columns were surrounded by spindle cells with central, small, round-to-oval nuclei with small nucleoli. These cells lacked typical Reinke’s crystals commonly seen in Leydig cell tumors. Only a few microscopic areas of necrosis were identified. The cellular atypia was scant and mitotic figures were found in less than two per ten high-power fields (Figure 2a, 2b). Both cell types were focally positive for fat stains by oil red and Sudan III.

Immunohistochemical staining was performed for AE1/AE3 (anion exchange protein) (1: 100 dilution), CAM 5.2 (1: 40 dilution), alpha-smooth muscle actin (SMA) (clone 1A4, 1: 200 dilution), vimentin (clone V9, 1: 400 dilution), desmin (clone DE-R-11, 1: 200 dilution), inhibin-alpha (clone R1, 1: 50 dilution), estrogen receptor (clone SP1, 1: 2 dilution), progesterone receptor (clone 1E2, 1: 2 dilution), testosterone (1: 50 dilution), and Ki-67 (clone 1E6, 1: 200 dilution) using the streptavidin-biotin-peroxidase complex method. Consequently, immunohistochemical staining of the two-component cell types was negative for cytokeratin (CAM5.2 and AE1/AE3), estrogen receptor, and progesterone receptor, and positive for inhibin-alpha and vimentin. Only the spindle cells were positive for smooth muscle actin SMA and desmin. Importantly, testosterone staining was positive in both components (Figure 2c–2d). The Ki-67 labelling index was 2.5% throughout the specimen. The final pathological diagnosis was a steroid cell tumor-NOS of the ovary.

The maternal serum testosterone level immediately normalized following tumor resection and her hirsutism slowly decreased. She has had no evidence of recurrence for five years, and her serum testosterone level has remained normal.

The neonate weighed 1,280 g at birth, and had Apgar scores of 9 at one minute and 9 at five minutes. The neonate was admitted to the neonatal intensive care unit. The neonate had ambiguous genitalia with a small penis without an obvious scrotum or palpable testis in the inguinal or genital region. Cyto genetic investigation on blood lymphocytes of the baby revealed a normal female karyotype 46, XX and was negative for the SRY gene. The neonate exhibited complete masculinization of the external genitalia with the external urethral meatus opening at the apex of the penis and complete labial fusion (Prader type V) [5] (Figure 3a).

MRI findings revealed a small uterus; the uterine corpus and the cervix were not distinguishable, and the vagina was closed just beneath the labia (Figure 3b). While the neonate initially grew normally, she developed hydrocephalus secondary to a cerebellar tumor. The tumor was resected and was consistent with a medulloblastoma with extensive nodularity and advanced neuronal differentiation. The tumor recurred and the infant died of disease progression at one year of age.

Discussion

The differential diagnosis of maternal virilization in pregnancy is divided into adrenal, ovarian, and iatrogenic causes. Ovarian tumors or tumor-like lesions which produce androgens include pregnancy luteoma, hyperreactio luteinalis, granulosa cell tumor, thecoma, Sertoli-Leydig cell tumor, steroid cell tumors including pure Leydig cell tumor, stromal luteoma and steroid cell tumor-NOS, stromal hyperthecosis, and ovarian tumors with functioning stroma including cystadenoma, cystadenocarcinoma, Brenner tumor, dermoid cyst, and Krukenberg (metastatic) tumor [6].

Pregnancy luteoma is the most common cause of maternal virilization during pregnancy, and approximately 20 cases have been reported in recent years [7]. Moreover, four cases of pregnancy luteomas with female pseudohermaphroditism have been reported in the English literature [6, 8-10]. This lesion is characterized by spontaneous disappearance of the tumors and normalization of the androgen levels after delivery. Imprudent surgical intervention should be withheld except for ovarian torsion or obstructed labor. However, three of four cases with female pseudohermaphroditism underwent surgery to obtain the accurate pathological findings at the same time of cesarean section or puerperium. Wang et al. reported a case of a nulligravida woman suffering from bilateral hydronephrosis and recurrent pyelonephritis caused by

Table 1. — Cases of female pseudohermaphroditism.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mat. age and para</th>
<th>Tumor size at diagnosis (mm)</th>
<th>Radiological findings</th>
<th>Histological diagnosis</th>
<th>Maternal testosterone level</th>
<th>Prader class</th>
<th>Maternal therapy</th>
<th>Maternal outcome</th>
<th>Fetal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massa V. [8]</td>
<td>34 nulligravida</td>
<td>13 R: 50</td>
<td>US; small hypo-echoic and hyperchoic area</td>
<td>bilateral pregnancy luteoma</td>
<td>T: 2.000 ng/ml (normal range: 50-300 ng/ml)</td>
<td>V</td>
<td>cesarean section with bilateral tumor resection at 39 weeks gestation</td>
<td>improvement of hirsutism</td>
<td>feminizing genitoplasty</td>
</tr>
<tr>
<td>Wang Y.C. [9]</td>
<td>27 nulligravida</td>
<td>35 R: 70x60x50 L: 90x64x50</td>
<td>solid mass and multiple nodules</td>
<td>bilateral pregnancy luteoma</td>
<td>T: 11.539 ng/ml (normal range: 20-86 ng/ml)</td>
<td>NS (17)</td>
<td>vaginal delivery at 36 weeks</td>
<td>bilateral ovaries normalized and improvement of hirsutism</td>
<td>NS</td>
</tr>
<tr>
<td>Spitzer R.F. [6]</td>
<td>36 nulligravida</td>
<td>post-partum</td>
<td>MRI; heterogeneously; predominantly solid</td>
<td>r-pregnancy luteoma</td>
<td>T: 10.6 nmol/l (normal range: &lt; 2.9 ng/ml)</td>
<td>II-III</td>
<td>vaginal delivery at 36 weeks and RSO and OMT on the 18th postpartum cesarean section with LSO at 35 weeks gestation</td>
<td>improvement of hirsutism</td>
<td>considering urogenital sinus repair</td>
</tr>
<tr>
<td>Ugaki H. [10]</td>
<td>33 nulligravida</td>
<td>35 L: 60</td>
<td>l-pregnancy luteoma</td>
<td>l-pregnancy luteoma</td>
<td>T: 6.11 ng/ml (normal range: 0.85 ± 0.28 ng/ml)</td>
<td>NS (111)</td>
<td>vaginal delivery at 36 weeks</td>
<td>improvement of hirsutism</td>
<td>feminizing genitoplasty</td>
</tr>
<tr>
<td>Current case</td>
<td>36 nulligravida</td>
<td>15 R: 75x30</td>
<td>heterogeneous low to intermediate signal intensity on T2-weighted imaging</td>
<td>r-SCT-NOS</td>
<td>T: 3.2 ng/ml (normal range: 0.1-0.7 ng/ml)</td>
<td>V</td>
<td>cesarean section with right-ovarian tumor resection at 29 weeks gestation</td>
<td>improvement of hirsutism</td>
<td>died of cerebellar tumor</td>
</tr>
</tbody>
</table>

R: right; L: left; T: Testosterone; ADS: Androstenedione; NS: not specified; RSO: right salpingo-oophorectomy, OMT: omentectomy, LSO: left salpingo-oophorectomy.
Female pseudohermaphroditism associated with maternal steroid cell tumor, not otherwise specified of the ovary: a case report etc.

Figure 1. — a) MRI showing a 75 x 80 mm solid tumor in the right pelvis, with heterogeneous low to intermediate signal intensity on T2-weighted imaging without contrast enhancement (arrow indicates the tumor). b) Macroscopic findings. The tumor measured 7 x 8 cm in diameter and was a well-circumscribed, grayish-yellow mass without apparent area of necrosis or degeneration.

Figure 2. — a), b) Pathological examination. The tumor includes areas in which cuboidal or polygonal cells with oval to polygonal nuclei, small distinct nucleoli, and abundant eosinophilic cytoplasm are arranged in a diffuse pattern of columns or nests. These columns are surrounded by spindle cells with central, small, round-to-oval nuclei with small nucleoli. These cells lack typical Reinke’s crystals. Only a few microscopic areas of necrosis are identified. The cellular atypia are scant and mitotic figures are found in less than two per ten high-power fields. [a] hematoxylin and eosin (H&E), original magnification x100, [b] H&E, x400] c), d), e), and f) Immunohistochemical study. c) inhibin-alpha (original magnification x 200), d) SMA (x100), e) vimentin (x200), f) testosterone (x 200).

The two-component cell types are positive for inhibin-alpha, vimentin, and testosterone. Only the spindle cells are positive for SMA.
bilateral solid ovarian tumors presented maternal virilization [9]. The unique MRI imaging features of this case were reported by Kao et al. as follows; intermediate high signal and contrast enhanced on T1, and low signal on T2-weighed images [11]. Based on the clinical and MRI imaging, bilateral ovarian tumors of this case were diagnosed as pregnancy luteoma, and this case was subsequently avoided from surgical intervention at pre- or post-partum [9, 11]. Moreover, this woman conceived her second pregnancy with a female fetus of 46, XX karyotype. Her pregnancy was terminated at 14 weeks gestation because of suffering from pregnancy luteoma repeatedly (maternal serum testosterone level; 751 ng/ml), and fetal ambiguous external genitalia with clitoral hypertrophy was confirmed [12].

Differentiation of the female external genitalia occurs between the seventh and 12th week of gestation. Increased exposure to androgens during this critical period results in labial fusion. After the 12th week of gestation, labia and clitoral hypertrophy may be induced [13, 14]. Almost all previously reported cases of female pseudohermaphroditism caused by pregnancy luteoma have been Prader type I to III (Table 1) [7]. However, Mazza et al. reported a case with Prader type V fetal masculinization [6]. They identified the duration and timing of embryo-fetal androgen exposure, a deficit of protective factors, and fetal organ sensitivity as influencing the degree of fetal masculinization. The duration of embryo-fetal androgen exposure in the present case with maternal steroid cell tumors-NOS was longer than in those of cases with a pregnancy luteoma and likely explains the complete female masculinization.

Steroid cell tumors typically are solid and well-circumscribed and are rarely lobulated [15]. These tumors are bilateral in six percent of cases [2]. Steroid cell tumors-NOS occur at any age with average age of diagnosis of 43 years [2]. These tumors are larger than the other steroid cell tumors; with cases ranging from 1.2 to 45 cm in diameter [2, 16]. Histopathologically, steroid cell tumors-NOS can be...
differentiated from stromal luteomas, which are confined within ovarian stroma and commonly associated with stromal hyperthecosis. They are also distinguishable from Leydig cell tumors which contain cytoplasmic Reinke crystals [1]. Steroid cell tumors-NOS are composed of two types of cells: cells with abundant eosinophilic, slightly granular cytoplasm, and cells with vacuolated cytoplasm [2]. These cells are most commonly arranged in a diffuse pattern but are occasionally seen in nests and columns. The stroma is sparse, consisting of delicate connective tissue supporting a rich vascular network, and is occasionally fibrous or hyalinized [2]. These tumors are commonly positive for inhibin-alpha and vimentin, and negative for cytokeratin. They have recently been shown to be positive for calretinin and Melan A [17, 18]. In the present case, the histopathological findings were not typical for a steroid cell tumor-NOS. The tumor consisted of large cells with abundant pale or eosinophilic cytoplasm, as well as spindle cells. The former cells were consistent with those found in a steroid cell tumors-NOS. Inhibin-alpha and vimentin were positive and cytokeratin was negative for both cell types, whereas SMA and desmin were positive only in the spindle cells. These spindle cells were thought to be differentiating to smooth muscle cells, which may have been what prompted the diagnosis of leiomyoma for the previously resected left ovarian tumor in this patient.

The majority of steroid cell tumors-NOS are benign. Despite the majority being low-grade, approximately 25%-43% of these tumors are malignant in adults [2, 19]. In a review of 63 cases, the pathological features associated with malignant behavior are: two or more mitotic figures per ten high-power fields (92% malignant); necrosis (86% malignant); tumor diameter of more than seven cm (78% malignant); hemorrhage (77% malignant); and grade 2-3 nuclear atypia (64% malignant) [2]. The average age of patients with malignant steroid cell tumors-NOS is higher than that of patients with benign tumors, of 54 and 38 years, respectively [2]. The tumor in the present case showed benign pathological features with the exception of the tumor measuring seven by eight cm. Since the patient was treated with a cystectomy, she has been followed closely with monitoring of the serum testosterone level and has shown no evidence of recurrence.

Two of four masculinized females caused by pregnancy luteoma underwent feminizing genitoplasty, and one case was considering urogenital sinus repair at appropriate age in the literature (Table 1). As for the present case, the neonate died of her cerebellar tumor before the planning of postnatal medical care, gender assignment, and the timing of feminizing genitoplasty. The association between the cerebellar tumor and maternal testosterone excess is uncertain.

Steroid cell tumors-NOS produce the full spectrum of hormonal perturbations seen with other steroid cell tumors. Therefore, they frequently result in primary or secondary infertility. The present patient became pregnant by ICSI after a four-year history of infertility treatment. The serum testosterone level during infertility treatment of the present case was not available without the awareness of virilization in previous hospital. While the infertility was circumvented, the effects on the fetus remained. The present case also illustrates a potential pitfall of artificial reproductive technology.

References


Placenta accreta: conservative approach

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Summary

Placenta accreta refers to any abnormally invasive placental implantation. Diagnosis is suspected postpartum with failed delivery of a retained placenta. Massive obstetric hemorrhage is a known complication, often requiring peripartum hysterectomy. The authors report a case of placenta accreta in a primiparous patient with multinodular leiofibromomyomatosis of the uterus following failed manual removals of a retained placenta. They describe a conservative management in a stable patient desiring future fertility with a unilateral prophylactic uterine artery embolization, a multidose regimen of methotrexate, and a subsequent abdominal myomectomy.

Key words: Placenta accreta; Methotrexate; MTX; Multidose methotrexate; Conservative treatment.

Introduction

The reported maternal mortality for morbidity adherent placenta ranges from seven to ten percent worldwide. The incidence of morbidity adherent placenta has increased over the past 50 years, mirroring the increase in the rate of cesarean delivery [1]. Damage to the decidua basalis secondary to previous uterine injury, such as cesarean section, myomectomy, traumatic uterine curettage, and intrauterine septum has been implicated. Significant maternal morbidity may occur because of massive postpartum hemorrhage and its sequelae, which include loss of fertility, multiple blood transfusions, transfusion-associated acute lung injury, coagulopathy, sepsis, multiorgan failure, and even death. Many women experience psychologic effects owing to loss of fertility secondary to peripartum hysterectomy. Additional complications include damage to the urinary bladder, bowel, or ureters including fistulae or incontinence [2, 3]. A 15-year analysis of peripartum hysterectomy reported that the procedure was associated with a maternal mortality rate of 12.5% and a urinary tract injury rate of 7.5% [4]. Separation of the placenta from its highly vascular bed is likely to cause massive obstetric hemorrhage. It is mostly diagnosed after delivery when manual removal of the retained placenta fails. The conventional treatment is hysterectomy.

Case Report

A primigravida 28-year-old patient, presented at 38 weeks plus five days amenorrhea with a premature rupture of membranes (PROM). After few hours of labor, she vaginally delivered a healthy female baby, followed by retained placenta. She underwent two unsuccessful attempts of manual removal with surgical curettages. She was conscious, cooperative, weighing 68 kilograms, with moderate pallor, regular pulse rate of 90 beats per minute, blood pressure recording of 120/75 mm of mercury, afebrile, no cyanosis with clear chest, and nothing abnormal on circulatory system examination. On abdominal examination, her uterus was 22 weeks in size, well-contracted but with an irregular surface for the presence of a bulky node myoma. The pelvic examination showed scarce amount of bleeding per vagina. She was hemodynamically stable and her hemoglobin was 12.4 mg/dl. She was blood group B, Rh positive, with normal readings of routine urine analysis, platelet count, coagulation profile, and hepatic and renal function tests. Vaginal swab was sent for culture which later reported sterile. Transabdominal and vaginal sonography revealed uterus to be of postpartum size with endometrial cavity showing an echogenic mass of dimensions 8.11 cm x 7.0 cm, suggestive of placenta, with vascularity on colour Doppler confirming it to be adherent to the uterine wall (placenta accreta), but with no definite invasion, and a solid, inhomogeneous, poorly vascularized mass in the lower part of the anterior wall of the uterus showing typical features of an intramural fibroid measuring approximately ten cm. Supportive measures, like broad-spectrum antibiotics, were initiated. Considering the desire of the patient for retaining her uterus for future fertility, conservative management was planned. Modality adopted was: placenta left in situ and performance of a prophylactic selective right uterine artery embolization to reduce vaginal discharge, an injection of methotrexate given intramuscularly in the schedule of one mg/kg, using the multidose regimen that involves the administration of methotrexate calculated according to body weight, alternated with 0.1 mg/kg of leucovorin calcium per os after 30 hours in four doses, based on continuous monitoring of the dimensions and vascularity of the mass (representing adherent placenta) with serial sonographic and colour Doppler studies which regularly showed the reducing trend. Leucocyte counts were routinely performed on a daily basis which remained within limits. Size of the placenta decreased remarkably with a concomitant reduction by 30% in uterine myoma volume. With this conservative strategy, vaginal bleeding never became alarming and vaginal discharge never purulent. Patient was discharged, not breastfeeding, in a satisfactory condition, fulfilling her initial desire of conserving the uterus, after 12 days of hospitalization. On subsequent follow-ups, every seven days, patient remained afebrile with no history or evidence of infection. After two months she experienced her first period after childbirth. Vaginal sonography revealed uterus to be entirely occupied by the detached placenta, whose release was hampered by the myoma node. Thus, in agreement with the patient, it was decided to perform a myomectomy with concomitant removal of the placenta. She was discharged in a satisfactory condition after four days of hospitalization and the subsequent follow-up showed perfect clinical conditions of the patient.
Placenta accreta is a severe obstetric complication involving an abnormally deep attachment of the placenta, through the endometrium and into the myometrium. There are three forms of placenta accreta, distinguishable by the depth of penetration: accreta, increta, and percreta. Placenta accreta is the invasion of the myometrium which does not penetrate the entire thickness of the muscle. This form of the condition accounts for around 75% of all cases. The placenta usually detaches from the uterine wall relatively easily, but women who encounter placenta accreta during childbirth are at great risk of hemorrhage during its removal. This commonly requires surgery to stem the bleeding and fully remove the placenta, and in severe forms can often lead to a hysterectomy or be fatal.

One of the potentially catastrophic obstetric complications, placenta accreta is alarmingly on the rise in the developed as well as developing world given the current trend towards elective repeat cesarean sections [5]. The incidence of placenta accreta is considered between one in 7,000 to as high as one in 540 pregnancies [6]. It is a life-threatening condition associated with high maternal morbidity and mortality rate reaching as high as seven percent [7]. The risk factors for placenta accreta are previous uterine surgery (myomectomy or cesarean sections, multiple cesareans are present in over 60% of placenta accreta cases), previous dilation and curettage (which is used for many indications including miscarriage, termination, and postpartum hemorrhaging), placenta previa (placenta accreta affects around ten percent of cases of placenta previa), advanced maternal age, multiparity, smoking, Asherman’s syndrome, and presence of fibroids [8, 9, 10]. A thin decidua can also be a contributing factor to such trophoblastic invasion. Some studies suggest that the rate of incidence is higher when the fetus is female [11].

It is important to make an early and accurate diagnosis for appropriate management and reduction of associated morbidity, thereof, and prenatal diagnosis may be established by ultrasound, colour Doppler, and magnetic resonance imaging [7]. Premature delivery and subsequent complications are the primary concerns for the baby. Bleeding during the third trimester may be a warning sign that placenta accreta exists, and when placenta accreta occurs, it commonly results in a premature delivery. The placenta usually has difficulty separating from the uterine wall. The primary concern for the mother is hemorrhaging during manual attempts to detach the placenta. Severe hemorrhaging can be life threatening. Other concerns involve damage to the uterus or other organs (percreta) during removal of the placenta. Hysterectomy is a common therapeutic intervention, but the results involve the loss of the uterus and the ability to conceive. There is nothing a woman can do to prevent placenta accreta, and there is little that can be done for treatment once placenta accreta has been diagnosed. The safest treatment is a planned cesarean section and abdominal hysterectomy if placenta accreta is diagnosed before birth [12, 13]. Conservative treatment can also be uterus sparing but may not be as successful and has a higher risk of complications [13]. Though traditional management of this entity has centered upon hysterectomy, but there has been a gradual shift towards its management which involves uterine conservation and leaving the adherent placenta in situ with either a) adjuvant treatment with methotrexate [14] or b) by simply awaiting its spontaneous resorption [8], with the possibility to perform a complementary uterine artery embolization [15]. Percutaneous embolization was initially performed to control traumatic [16] or tumor bleeding [17, 18]. The first reported use of transcatheter arterial embolization of postpartum hemorrhage was described by Brown et al. [19] in 1979. The use of methotrexate in the conservative treatment of the placenta accreta left in situ was described for the first time by Arulkumaran in 1986: oral methotrexate allowed the expulsion of the placenta at a distance of 11 days after its administration [20]. Tong et al. [21] pioneered the conservative method by administering systemic methotrexate. The outcome varies widely ranging from expulsion at seven days to progressive resorption in roughly six months [22]. Courbiere et al. [15] conducted a study on conservative management in which placenta accreta was always left in situ with one of the following associated treatments: bilateral hypogastric artery ligation, medical treatment with methotrexate or uterine artery embolisation. Placental resorption occurred in the majority of their cases with no report of maternal mortality.

**Conclusion**

Conservative management appears to be a safe alternative to the extirpative management and is a logical option in well-selected hemodynamically stable patients with adherent placenta. Antepartum diagnosis should be improved among patients with a high risk profile for placenta accreta in order to optimize conservative strategy. Conservative treatment for placenta accreta can assist women to avoid hysterectomy and involves a low rate of severe maternal morbidity in centers with adequate equipment and resources.

**References**


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Rectus abdominal muscle endometriosis in a patient with cesarian scar: case report

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Summary

Endometriosis is the existence of endometrial tissue out of the intrauterine cavity. Abdominal wall endometrioma is a well-defined mass composed of endometrial glands and stroma that may develop after gynecologic and obstetrical surgeries. A cyclic painful mass at the site of a cesarean section scar is most likely due to an endometrioma, and wide local excision is the advisable treatment. The authors present a case of endometrioma in the abdominal wall, which was treated with local excision.

Key words: Endometriosis; Scar; Cesarean section.

Introduction

Endometriosis is the existence of endometrial glands and stroma outside the uterine cavity. Ectopic endometrial tissue is commonly found at pelvic region, but it can be found anywhere in the body [1]. It can be found in the extrapelvic areas such as the eyes, kidneys, adrenal glands, lungs, intestines, umbilicus, diaphragm, gall bladder, heart, liver, bones, and central and peripheral nervous systems [2]. There are several theories about the etiology of endometrial tissue outside the uterine cavity. These include metaplasia, retrograde menstruation, venous and lymphatic metastases, and mechanical implantation. Endometrioma is a well-defined form of endometriosis. Incisional endometriosis (IE) generally occurs after hysterectomy, cesarean section, episiotomy, tubal ligation, and trocar entry during laparoscopy and amniocentesis [3].

The authors present a case of endometrioma in the abdominal wall, which was treated with local excision.

Case Report

A 33-year-old woman had a cesarean section five years ago. She was admitted to this clinic complaining of left lower quadrant abdominal pain and swelling which was more severe during menstruation. A painful firm mass was palpated at the middle of the cesarean incision scar during a physical exam. Ultrasonographic examination showed a 23 x 20 mm hypoechoic solid lesion with irregular contours. Magnetic resonance imaging (MRI) was performed to delineate the relationship between the mass and other intra-abdominal organs. MRI showed a fibrous soft tissue component in the rectus abdominal muscle which was not related to the intra-abdominal organs and indistinguishable from muscle contours (Figure 1). Subsequently, surgical excision was performed and the mass was widely excised, forming a three-cm defect in the abdominal wall (Figure 2).

Microscopic examination revealed endometrial gland structures with endometrial stroma in adipose tissue in sections of specimens, indicative of endometriosis. During the pathological examination, the fibroadipose tissue was found with the neighboring hemorrhagic areas. The lumen of histiocytes and neutrophils in a single-row that contained the structure of the endometrial glands were lined by endometrial epithelium. Around the areas of hemorrhage showing an endometrial stromal structure in a single-row columnar epithelium lined by endometrial gland structures was observed (Figure 3). The patient recovered uneventfully and did not report any symptoms of recurrence without any medical treatment four months after surgery.

Discussion

Abdominal wall endometriosis is the most common form of extrapelvic endometriosis. It is seen most frequently in women 20–40 years of age, and generally detected two to five years after cesarean section [1]. In the present case, the patient was 33-years-old, and became symptomatic five years after the cesarean section. In a study of post-cesarean cases, 0.2% of the cases developed incisional endometrioma after two years or more [4]. Pathogenesis is thought to be due to implantation, direct invasion, and vascular/lymphatic invasion. The diagnosis of scar endometriosis can be difficult despite specific symptoms, such as pain and swelling during menstruation. Less frequently, it can be seen as a mass unrelated to menstrual cycles [5]. The differential diagnosis of IE includes hernia, hematoma, lymphadenopathy, lymphoma, lipoma, abscess, subcutaneous cyst, suture granuloma, neuroma, soft tissue sarcoma, and metastatic cancer [5]. Fine-needle aspiration biopsy, ultrasound, computed tomography (CT), and MRI are valuable for the preoperative diagnosis [6, 7]. In this case, the patient had pain and swelling during menstruation. Her work-up included imaging by ultrasound and MRI, and she underwent surgery after the determination of the differential diagnosis.

During the surgical excision for the treatment of IE, the mass must be removed with a ten-mm margin of healthy tissue, and without rupturing and leaving behind endometrioma tissue. Recurrence after resection is seen in 4.3% of cases and the possibility of malignancy should be considered if the mass grows rapidly or recurs [5]. Mesh or tissue graft...
may be utilized in order to repair the defect that may occur after the excision of IE [8]. In this case, the mass was excised with one-cm margins of healthy tissue. As the fascial defect was small and tension free, it was primarily repaired. Rarely, IE can be multifocal. Since the most common site of an incision lesion is at an end, to prevent direct inoculation, Evsen et al. suggested that while suturing the fascia at the end of the incision, the surgeon or assistant must use clean surgical equipment instead of their fingers to retract the subcutaneous tissue in the incision [9].

Conclusions

In order to make the preoperative diagnosis of incisional endometrioma, a detailed history should be taken, and a physical examination should be performed. Additionally, radiological investigation and fine-needle aspiration biopsy should also be performed. Endometrioma should be the top differential diagnosis in patients who have pain and swelling occurring every menstrual cycle on the scar following gynaecological surgeries.

References


Uterine multiple leiomyomas complicated by extensive mucoid degeneration: case report

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Summary

Uterine leiomyomas are the most common form of gynaecological tumours, and are exclusively benign. Only a few are associated with sarcomatous change. It is therefore important for the radiologist to be familiar with their range of appearances on magnetic resonance imaging (MRI) scans to distinguish them from other significant uterine pathologies, such as ovarian neoplasms, that require different management strategies. Here, the authors present the case of a 37-year-old Han woman, gravida 2, para 1 (cesarean section in 1996), who presented with a two-month history of lumbosacral swelling and pain. Physical examination revealed a pelvic mass and she was admitted with the presumptive diagnosis of an ovarian neoplasm. Laparotomy revealed multiple degenerated neoplasms that were benign in appearance, which was pathologically confirmed. A literature review was conducted to explore the natural history of uterine leiomyomas and their underlying etiopathogenesis. The optimal imaging modalities are also defined in the report, which enable the correct preoperative diagnosis to be made in order to optimize the care of women by multiple uterine leiomyomas.

Key words: Uterine leiomyomas; Gynaecological tumour; Aetiopathogenesis.

Introduction

Uterine leiomyomas are common, benign smooth muscle tumours of the uterus. They are found in nearly half of women over the age of 40 years and infrequently cause complications. Uterine leiomyomas, also colloquially known as fibroids, tend to grow under the influence of estrogen, and regress when estrogen levels are reduced. Thus, growth frequently occurs during pregnancy, followed by regression after delivery. Most uterine fibroids are asymptomatic, but some women develop heavy menstrual flow (menorrhagia), which often cause anemia, bleeding between periods, pain, infertility or subfertility, pelvic pressure, stress urinary incontinence, and ureteral obstruction. The diagnosis of uterine leiomyoma is usually based on the clinical findings of an enlarged, irregularly shaped, firm uterus, which may or may not be tender. Sometimes the diagnosis is unclear, and diagnostic tests are used to delineate fibroids and exclude other problems. Diagnostic techniques include ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT) scanning, laparoscopy, and histological examination.

A variety of degenerative changes may occur in leiomyomas. The larger the leiomyoma, the more likely it will be that a degenerative component will be present. Several mechanisms are likely to contribute to this phenomenon, including ischemia and hormonal effects. More than one pattern of degeneration may be observed in the same leiomyoma. These changes include hyaline, cystic, red, calcific, and fatty degenerations. The most common of these is hyaline degeneration, whereby expanded septa lose their fibrillar structures and assume a uniform, pale, eosinophilic, translucent appearance resembling ground-glass. Degenerative changes may be localized or affect extensive areas of the tumour, and occasionally even its entirety. Surviving muscle cells may orient themselves into lacework patterns that accompany degenerative changes in leiomyomas.

The terms mucoid and myxoid degeneration are used to describe changes that are similar to hyaline changes, with or without cystic formation. In mucoid degeneration, the matrix typically appears to be mucinous in nature. There is no difference in practical terms between mucoid and myxoid forms of degeneration, thus the two terms are often used interchangeably. However, extensive mucoid degeneration is rare among these changes. Here, the authors report a case of uterine multiple leiomyomas that were complicated by mucoid degeneration.

Case Report

A 37-year-old Han woman, gravida 2, para 1, with a history of one cesarean section in 1996, was admitted after presenting with lumbosacral swelling and pain for more than two months. These symptoms had worsened over the previous week. Gynaecological examination revealed a non-tender anteverted uterus, which was enlarged to the size of a two-month pregnancy, with moderate texture and mobility. A mass that was four cm in diameter was discovered in the left adnexal area, and another mass, six cm in diameter, was discovered in the right adnexal area. The two masses were moderate in texture with no clear borders but had infiltrated the uterus.

B-mode ultrasound examination revealed an antverted uterus that measured 6.4 × 4.6 × 5.0 cm, with a regular morphology. An intrauterine device was observed in the correct location. One enhanced echo image showed a mass that measured 4.8 × 3.1 cm in the left adnexal area, and another mass, six cm in diameter, was discovered in the right adnexal region. The wall of this cyst appeared

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to be thick and ill-defined, and a dense reflection to the right could be detected from the cystic wall. A fluid dark area, measuring 1.1 cm in diameter, was observed in the pouch of Douglas. The results of the following examinations and tests were within the normal range: routine blood tests; coagulation function; hepatic and renal functions; blood biochemistry; blood glucose; carcinoma antigen 125 (CA 125); alpha fetal protein (AFP); carcinoma embryonic antigen (CEA); and electrocardiography. No positive findings were detected with an ultrasound examination of the liver, gallbladder, pancreas or spleen. A barium enema check also yielded negative results.

The patient was admitted to hospital with the presumptive diagnosis of an ovarian neoplasm in November 2009. A laparotomy was performed under general anaesthesia. At laparotomy, the uterus was found to be slightly enlarged, and two soft masses, measuring three cm in diameter, were found in the bilateral uterine horns (Figure 1). There were multiple dark red bubbles, ranging in size from one to three cm, attached to the anterior uterine wall. A four-cm cyst was found in the left mesosalpinx, and another six-cm cyst was found in the right mesosalpinx. Both were soft in texture and were composed of multiple cysts with gelatinous contents. Similar neoplasms were also detected in the bilateral broad ligaments of the uterus. The neoplasm in the left mesosalpinx was stripped and sent for frozen section examination. The findings suggested the diagnosis of a benign soft tissue neoplasm. Both ovaries appeared to be normal in both morphology and size.

When the masses from the uterine horns were incised, soft and gelatinous tissue with indistinct boundaries was observed. Pathological examination suggested the diagnosis of an endometrial mesenchymal neoplasm, which was likely to be benign (Figure 2). Consequently, a total hysterectomy and bilateral salpingectomy were performed, and the neoplasms in the bilateral broad ligaments were stripped using blunt dissection. The features of these neoplasms were the same as those from the mesosalpinx. There was no obvious abnormality in the endometrium upon dissection. Gelatinous tissue with no observable envelope was scattered within the muscular layer of the uterus. Examination of the pathology of paraffin-embedded sections yielded a diagnosis of multiple uterine leiomyomas with conspicuous mucoid degeneration. The patient was discharged from hospital after rehabilitation. No recurrence was observed after a 12-month follow-up period.

Discussion

Molecular biologists have begun to probe the etiology of uterine leiomyomas, exploiting DNA methylation differences between polymorphic loci on both active and inactive X chromosomes to confirm that each leiomyoma is derived from a single transformation event [1]. Most importantly, these studies also suggest that each tumour is a distinct clone, which reinforces the notion that smooth muscle tumourigenesis is exceedingly common. The genetic mechanisms that initiate and promote the growth of leiomyomas must occur frequently, but are not fully understood. However, cytogenetic analysis of these benign smooth muscle tumours has already revealed some important clues. Almost half of leiomyomas have chromosomal rearrangements that are large enough to be seen in G-banded karyotypes. These chromosomal rearrangements are generally simple, which is in sharp contrast to the aberrations seen in leiomyosarcomas. To date, recurrent aberrations have allowed the definition of seven cytogenetic subgroups: t(12;14)(q14-15;q23-24); del(7)(q22q32); rearrangements of 6p21 and 10q22; trisomy 12; and deletions of 3q and 1p. Of these, the translocation between chromosomes 12 and 14 and the rearrangements involving chromosome 6 are perhaps best understood. Both rearrangements involve genes for two closely related non-histone chromatin proteins: HMGA1 at 6p21 and HMGA2 at 12q15 [2, 3]. There are few reports that describe the various mechanisms by which uterine leiomyomas degenerate.

In the present patient, the degeneration of multiple uterine leiomyomas led to the formation of variously-sized,
hollow masses filled with neoplastic tissue, which were liquefied due to the lack of a blood supply. The mucoid contents in the cavities modified the texture of the leiomyomas to soft masses. Given the soft texture of the tissue, malignant ovarian tumour and/or malignant uterine tumours had to be considered in the differential diagnosis. Intraoperative pathologic diagnosis was very helpful in the differential diagnosis.

Degenerated leiomyomas, especially those with larger volumes, often bring difficulties in differential diagnosis and corresponding clinical decision-making. A MRI scan is the most accurate technique for detecting and localizing leiomyomas. Degenerated leiomyomas have variable appearances on T2-weighted images and contrast-enhanced images. The common types of degeneration are hyaline (>60% of cases), cystic (approximately four percent), myxoid, and red. Edema is not a phenomenon of degeneration, but is a common histopathological finding (approximately 50% of cases). Hemorrhage, necrosis, and calcification (approximately four percent of cases) may also be observed. Specific types of unusual leiomyomas include lipoleiomyoma and myxoid leiomyoma, which may have MRI features that are sufficiently characteristic to allow differentiation from other gynaecological and non-gynaecological diseases. Intravenous leiomyomatosis, metastatic leiomyoma, diffuse leiomyomatosis, and peritoneal disseminated leiomyomatosis represent unusual growth patterns. Other unusual growth patterns are retroperitoneal growth, parasitic growth, and a pattern that may occur in cervical leiomyoma [4].

On T2-weighted MRI images, non-degenerated leiomyomas appear as well-circumscribed masses of decreased signal intensity; however, cellular leiomyomas can have relatively higher signal intensities on T2-weighted images and demonstrate enhancement on contrast material-enhanced images. The differential diagnosis of leiomyomas includes adenomyosis, solid adnexal mass, focal myometrial contraction, and uterine leiomyosarcoma [5]. For patients who are symptomatic, medical or surgical treatment may be indicated. MRI also has a role in treatment of leiomyomas by assisting in surgical planning and monitoring response to medical therapy. The use of 18F-FDG positron emission tomography/CT (PET/CT) may also play a role in the diagnosis of uterine leiomyoma and can sometimes be helpful in the evaluation of related degeneration [6].

As leiomyomas are the most common gynaecological tumours, and are almost exclusively benign, it is important to be familiar with the variety of MRI appearances of uterine leiomyomas in order to distinguish them from other significant diseases.

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Detection of unruptured ovarian pregnancy subsequently successfully treated by conservative laparoscopic surgery: a case report and review of the literature

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Summary

Early detection of ovarian pregnancy (OP) is essential for successful laparoscopic conservative surgery. However, early preoperative ultrasonography-based diagnosis is often difficult when fetal cardiac activity or the yolk sac is absent. The authors report a case of OP diagnosed at eight weeks gestational age in a natural pregnancy. The patient presented with amenorrhea and transient vaginal bleeding, and slight tenderness in the right ovary was noted during vaginal ultrasonography. Furthermore, ultrasonography showed a gestational sac (GS) without fetal cardiac activity or yolk sac, consistent with OP, and an adjacent compressible lutein cyst. The uterus, fallopian tubes, and left ovary were normal, and no cul-de-sac blood or ascites were found. Laparoscopy showed a two-cm mass partially covering the right ovary, which contained an unruptured GS. Subsequently, the mass was removed, and OP was histologically confirmed.

Key words: Laparoscopic surgery; Ovarian pregnancy; Ultrasonography.

Introduction

Ovarian pregnancy (OP) is a rare form of ectopic pregnancy, constituting approximately three percent of all ectopic pregnancies [1]. Assisted reproductive technologies have been associated with an increased incidence of ectopic pregnancy. In cases of ectopic pregnancy following in vitro fertilization-embryo transfer (IVF-ET), the prevalence of OP has been reported to be six percent [2]. Early diagnosis of OP is mandatory to ensure the success of life-saving laparoscopic conservative surgery.

Several reports of unruptured OP in IVF-ET patients attributed successful treatment by laparoscopic surgery to early ultrasonography diagnosis, close follow-up, and awareness of the high-incidence of ectopic pregnancy, including OP, compared with natural pregnancy [3]. Diagnosing OP in the case of natural conception is difficult, especially when the date of conception is not known. Although a low serum human chorionic gonadotropin (HCG) level facilitates early recognition of abnormal implantation [3], repeat measurement is usually needed.

Transvaginal ultrasonography (TVUS) is an important tool for early detection of OP. Comstock et al reported that an echolucent ovarian area with a wide echogenic ring was a diagnostic sonographic finding of OP [4]. Here, the authors present a case where a lesion with this typical ring appearance compressed an adjacent echolucent sol of a corpus luteum cyst in the ovary.

Case Report

A 31-year-old nulligravida woman presented to the present hospital with amenorrhea and transient vaginal bleeding. She was undergoing prednisolone therapy (three mg, daily) for treatment of rheumatoid arthritis but had no history of pelvic inflammation, surgery, or infertility. The chief complaint was a scant brownish vaginal discharge; there were no other symptoms. A rapid urine pregnancy test yielded positive results, and the HCG level on the following day was 400 mIU/ml. The menstrual age was eight 4/7 weeks. Vaginal ultrasonography showed no intrauterine gestational sac (GS) and a normal left ovary without a lutein cyst (Figure 1a). There was no evidence of cul-de-sac fluid or pelvic adhesions. The right ovary was slightly enlarged, with a maximum diameter of 47 mm, and the patient complained of slight tenderness when it was pushed by the ultrasonography probe. In the right ovary, there was a echolucent area (diameter, 20 mm) surrounded by an echogenic ring. The ring was more echogenic than the ovarian stroma or adjacent corpus luteum, an appearance consistent with GS. No fetal cardiac activity or yolk sac was evident. A 25-mm corpus luteum cyst was compressed by the adjacent ovarian mass (Figure 1b). The findings suggested a provisional diagnosis of OP, and the patient elected surgical treatment.

During laparoscopy, the uterus and both fallopian tubes appeared normal (Figure 2a), and no ascites or adhesions were seen. The right ovary was enlarged because of a lutein cyst and a dark bluish mass with a smooth external surface (Figure 2b). Wedge resection was performed with monopolar electroscissors (Figure 2c), and the mass was removed through a ten-mm trocar using a retrieval bag. The resected tissue was cut in half (Figure 2d). Macroscopic examination revealed chorionic villi in the mass and negative tissue margins. The lutein cyst was confirmed by cutting the surface. The remaining right ovary was not oversewn. Uterine curettage showed no chorionic villi in the endometrium. The operative time was 80 minutes, and intraoperative blood loss was minimal. The patient had an uneventful recovery and was discharged on postoperative day five. Subsequent pathological diagnosis confirmed the diagnosis of OP. She became pregnant four months later and has experienced no complications.

Discussion

Spiegelberg published the first report describing the diagnostic criteria of OP. He stated that the fallopian tube on the affected side must be intact and separate from the ovary, the gestational sac must occupy the position of the ovary, the ovary must be connected to the uterus by the utero-ovar-
ian ligament, and that ovarian tissue must be found in the gestational sac wall [5]. The risk factors for OP include history of prior gynecologic surgery, use of intrauterine contraceptive devices, assisted reproduction, or endometriosis [6]. The present case did not have known risk factors. Marcus and Brinsden suggested that implantation in the ovary occurs after reverse migration of the fertilized egg [3]. According to this theory, the fertilized egg may have adhered to the ruptured follicle and thus remained in the ovary. Laparoscopic resection of the GS and preservation of remain-
ing ovarian tissue is the preferred treatment for ovarian pregnancy [7-15].

Early diagnosis of OP is vital to prevent emergency invasive procedures, serious complications, or death. Raziel et al. reported that OP was diagnosed after laparoscopic examination or direct laparotomy in 20 patients from 1971 to 1989 [1]. Odejimi et al and Choi et al. reported that 75% and 16% of cases of OP, respectively, were diagnosed before surgery [6, 7]. These findings are summarized in Table 1.

Technical advances in ultrasonography and the development of more sensitive methods for HCG detection facilitate earlier diagnosis or direct laparotomy in 20 patients from 1971 to 1989 [1]. Odejimi et al. reported that OP was diagnosed after laparoscopic examination or direct laparotomy in 20 patients from 1971 to 1989 [1]. Odejimi et al. and Choi et al. reported that 75% and 16% of cases of OP, respectively, were diagnosed before surgery [6, 7]. These findings are summarized in Table 1.

Table 1. — Summary of findings in reports of OP. Studies published since 2000, excluding single case reports. Data reported by Choi and Koo for patients in the same institute during the same period. n, number of patients; IUD, intrauterine contraceptive device; OP, ovarian pregnancy; US, ultrasonography; NA, not available.

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Study period</th>
<th>IUD use</th>
<th>Prior surgery or endometriosis</th>
<th>Post IVF follow-up</th>
<th>No symptoms</th>
<th>OP diagnosed by US</th>
<th>Embryo or yolk sac detected by US</th>
<th>Unruptured</th>
<th>wedge resection</th>
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<tr>
<td>Comstok et al.</td>
<td>6</td>
<td>1990-2003</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>2 (33%)</td>
<td>4 (67%)</td>
<td>NA</td>
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<tr>
<td>Odejini et al.</td>
<td>12</td>
<td>2003-2008</td>
<td>2 (17%)</td>
<td>NA</td>
<td>NA</td>
<td>9 (75%)</td>
<td>NA</td>
<td>0</td>
<td>11 (92%)</td>
<td>NA</td>
</tr>
<tr>
<td>Var et al.</td>
<td>2</td>
<td>1999</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Priya et al.</td>
<td>2</td>
<td>2001-2005</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Tobiune et al.</td>
<td>3</td>
<td>1996-2009</td>
<td>2 (4%)</td>
<td>NA</td>
<td>5 (10%)</td>
<td>9 (18%)</td>
<td>8 (16%)</td>
<td>NA</td>
<td>39 (80%)</td>
<td>NA</td>
</tr>
<tr>
<td>Choi et al.</td>
<td>49</td>
<td>1996-2009</td>
<td>2 (7%)</td>
<td>18 (64%)</td>
<td>5 (18%)</td>
<td>3 (11%)</td>
<td>17 (61%)</td>
<td>26 (93%)</td>
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<tr>
<td>Koo et al.</td>
<td>28</td>
<td>1996-2009</td>
<td>2 (7%)</td>
<td>18 (64%)</td>
<td>5 (18%)</td>
<td>3 (11%)</td>
<td>17 (61%)</td>
<td>26 (93%)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

References

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Reversible posterior leukoencephalopathy syndrome in pregnancy: a case report

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Summary
Posterior reversible encephalopathy syndrome (PRES), is an acute, neurotoxic state. It is a very rare clinico-neuroradiological entity, and it is a complication of multiple clinical conditions. The association of PRES with toxemia in pregnancy is established. In this article, the authors discuss the case of a 22-year-old woman, gravida 1, 36-week pregnant, with extensive, bilateral white matter hypodensity, predominantly involving the parieto-occipital lobes region. These changes were highly suggestive of posterior reversible encephalopathy. This case report demonstrates that early treatment with control of blood pressure seizures can reverse this condition and also prevent progression to an irreversible damage, thus emphasizing the need for early diagnosis and treatment.

Key words: Reversible posterior leukoencephalopathy syndrome; Hypertension in pregnancy; Eclampsia; Brain edema.

Introduction
Reversible posterior leukoencephalopathy syndrome (RPLS) or posterior reversible encephalopathy syndrome (PRES) was first described by Hinchey in 1996 [1]. The syndrome is acute with diverse clinical presentations and characteristic computed tomography (CT) scan or magnetic resonance imaging (MRI) features.

This clinico-neuroradiological entity is a complication of multiple clinical conditions: hypertension, pre-eclampsia and eclampsia, renal failure, therapy with immunosuppressant or high dose of cytotoxic medications (cyclosporin A and tacrolimus) for autoimmune disease, and allogeneic bone marrow or organ transplantation. Other clinical conditions are characterized by uraemia and porphyria [2]. The association of PRES with toxemia of pregnancy is established [3].

The clinical hallmarks of this syndrome are: headache, altered mental functioning, seizures, and loss of vision associated with white matter changes. These changes are suggestive of edema mainly in the posterior regions of the cerebral hemispheres, but also involving the brainstem, cerebellum, and other cerebral areas [4]. The findings on neuroimaging in PRES include non-enhancing white matter abnormalities that appear as areas of low attenuation on CT scan and appear hypointense on T1-weighted imaging and hyperintense on T2-weighted MRI. The lesions are mainly seen in the posterior regions of the cerebral hemispheres. These abnormalities partially or completely resolve on follow-up scanning, thereby, suggesting subcortical edema without infarction [5].

The white matter is composed of myelinated-fiber tracts in a cellular matrix of glial cells, arterioles, and capillaries that makes it susceptible to the accumulation of fluid in the extracellular spaces [4]. It is suggested that vertebro-basilar territory, owing to its relatively sparse sympathetic innervation, may experience preferential disruption of autoregulatory mechanisms, leading to increased perfusion and edema [6].

Case Report
A 22-year-old woman, 36-week pregnant, weighing 63 kg, gravida 1, presented to the present department after she experienced headache, blurring of vision, and acute onset of generalized seizure.

The results of her general examination were unremarkable. Blood pressure was 140/90 mmHg with a heart rate of 95 beats per minute. Respiratory rate was 17 breaths per minute with an O₂ saturation of 99%. Body temperature was 36.3°C. Electrocardiogram was normal.

Her investigations included: haemochrome, serum electrolytes, serum calcium, serum magnesium, liver function tests, and coagulation profile were within normal limits. Biochemical values were: Hgb 11.7 g/dl, Htc 37.2%, WBC 13,130/mm³; PLT 220,000/mm³, AST 29 U/l, ALT 32 U/l, amylase 26 U/l, LDH 450 U/l; her coagulation parameters were: prothrombin time (PT): 113%, activated prothrombin time (APTT): 26 sec, INR 0.97. serum level of sodium was 138 mmol/l, potassium 3.6 mmol/l, and calcium 9.0 mmol/l. Renal function test and urine analysis were normal.

A diagnosis of eclampsia was made and the patient was transferred to the operating room where the patient underwent an emergency lower segment cesarian section under spinal anaesthesia. She gave birth to a healthy baby with a five-minute Apgar score of 9. Postoperatively she was transferred to the medical intensive care unit.

She underwent an invasive monitoring of vital parameters, assisted ventilation, neurological counselling, brain and thorax CT scans, and spinal tap. At the time, she was treated with nifedipine and fenobarbital.

Neurological examination, lumbar puncture, and thorax CT was normal. Brain CT showed extensive, bilateral white matter...
hypodensity, predominantly involving the parieto-occipital lobes region. These changes were highly suggestive of posterior reversible leukoencephalopathy. However atypical imaging findings can at times be misleading. On follow-up examination, patient showed marked clinical improvement with control of hypertension and was discharged in stable condition, as also confirmed by imaging. She was discharged from hospital on the nine post-operative day. At one month follow-up, the CT was completely normal.

Discussion

PRES is a very rare clinical entity. The differential diagnosis for seizures in pregnancy period includes: eclampsia, subarachnoid haemorrhage, intracerebral haemorrhage, thrombotic phenomena, intracranial neoplasm, head trauma, idiopathic epilepsy, infection (meningoencephalitis), and amniotic fluid embolism. PRES is still an under-recognised and untreated condition and the clinic-radiological hallmarks are to be established. There are no consensual guidelines to validate diagnosis of PRES [8].

Two theories have been proposed to explain the pathophysiology. The more popular theory suggests that hypertension leads to failure of autoregulation, subsequent hyper-perfusion, and vasogenic edema. The other theory suggests that vasoconstriction and hypoperfusion leads to brain ischemia and subsequent vasogenic edema [7].

PRES is a clinico-radiological entity. The combination of suggestive clinical manifestation and radiological criteria establishes the diagnosis of PRES.

PRES is reversible after appropriate treatment, which makes it important to recognize and treat the etiology to prevent its progression to irreversible damage.

This case report demonstrates that early treatment with control of blood pressure seizures can reverse this condition and also prevent progression to irreversible damage, thus emphasizing the need for early diagnosis and treatment [9, 10].

References


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Ovarian torsion associated with cessation of hormonal treatment for polycystic ovarian syndrome: a case report

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Summary

Torsion of an ovary or fallopian tube (adnexal torsion) usually occurs in ovaries with tumors or functional cysts. In polycystic ovarian syndrome (PCOS), the ovaries are bilaterally enlarged, but these enlarged ovaries rarely twist. Recently, the authors encountered a PCOS patient with ovarian torsion after the cessation of Kaufmann treatment. The etiological factors were unclear, but the authors suggest that the increase in ovarian volume was due to transient hypergonadotropic feedback. Thus, more attention should be paid to adnexal torsion that may arise subsequent to transient hypergonadotropic states, in relation to the cessation of hormonal treatment, and enlarged ovaries in PCOS patients.

Key words: Polycystic ovarian syndrome; Contraception; Ovarian torsion; Ovarian hyperstimulation syndrome; Ovarian cysts.

Introduction

The incidence of polycystic ovarian syndrome (PCOS) is reported to be six to ten percent of the female population [1]. The disorder is characterized by polycystic ovaries, hyperandrogenemia, and menstrual irregularity. Oral contraceptives that contain both estrogen and progestin constitute the most common form of therapy for adolescents with PCOS-related amenorrhea. Women with ovulatory dysfunction are treated with clomiphene or gonadotropin to induce ovulation [2]. Recently, ovarian hyperstimulation syndrome (OHSS) has been reported to be complicated by ovarian torsion [2, 3]. Thus, women with PCOS, who are undergoing ovulation induction, are at high-risk for OHSS. Hence, the cycle of ovulation induction should be carefully monitored to prevent the onset of OHSS. However, in general, adnexal torsion is a rare complication following ovarian enlargement due to hyperstimulation [3]. The authors recently treated a PCOS patient who had right ovarian torsion and had not undergone ovulation induction. The case has been presented here, together with a review of some of the literature regarding this subject.

Case Report

A 21-year-old nulliparous woman who complained of right, lower abdominal pain had been prescribed estrogen and progesterone to treat PCOS. She had stopped taking the medication without consulting her physician, two months before visiting this hospital. The physician considered that the patient’s abdominal pain was not caused by a digestive disorder. Ultrasonography (US) examination showed an enlarged right ovary, and the patient experienced pain in this region during the procedure. The patient was referred to the outpatient clinic.

The patient’s height was 145 cm and her body weight was 44 kg. Physical examination showed mild tenderness in her right lower abdomen. US examination showed normal uterine findings. However, her right ovary measured 66.2 × 41.5 × 51.4 mm and her left ovary measured 37.8 × 20.9 × 28.2 mm; she showed US features characteristics of PCOS. The patient’s blood tests showed a normal hemoglobin level of 13.8 g/dl, a serum CA125 level of 10.1 U/ml (normal range, 0 - 35 U/ml), a luteinizing hormone (LH) level of 12.62 mIU/ml, a follicle-stimulating hormone (FSH) level of 5.75 mIU/ml, and an estradiol (E2) level of 22 pg/ml. Magnetic resonance imaging (MRI) showed ischemic edema of the right ovary and a polycystic left ovary (Figures 1A, B). An emergency laparoscopy showed a necrotic right ovary that was purplish-black in color and had undergone a 540° torsion around the utero-ovarian ligament (Figures 1C).

Consequently, a right salpingo-oophorectomy (SO) was performed, and the diagnosis of PCOS with torsion was confirmed by the presence of edema and hemorrhagic foci. Signs of necrosis were visible during the subsequent histological examination.

One month after the operation, another US examination showed that the endometrium of the uterus exhibited the typical secretory changes accompanying spontaneous ovulation, as well as findings typical of menstrual cycle progression after ovulation (Figure 1D). During this examination, the left ovary was found to measure 40.4 × 31.5 × 36.1 mm. The LH level was 2.33 mIU/ml, FSH level was 0.73 mIU/ml, and E2 level was 230 pg/ml. Spontaneous ovulation was confirmed at six months after the operation.

Discussion

The PCOS criteria defined by the Japanese Society of Obstetrics and Gynecology (JSOG) consist of the presence of all of the following factors: chronic anovulation, LH hypersecretion and/or hyperandrogenism, and the presence of polycystic ovaries [4]. Moreover, an elevated LH level and an elevated LH/FSH ratio are typical findings in the ma-
The majority of patients with PCOS [1]. However, poor reproducibility of the elevated LH levels or LH/FSH ratios have been reported in PCOS patients. In addition, US images have shown that oral contraceptives suppress LH secretion and lead to a decrease in ovarian androgen production [5].

In this case, the patient met the diagnostic criteria; however, she had also discontinued her hormonal treatment. Hence, the authors thought that the effect of the hormonal treatment would have disappeared. The FSH and LH levels might have increased as part of the feedback interruption caused by the cessation of hormonal treatment. This concept was considered on the basis of the MRI finding of an enlarged right ovary; the right ovary was thought to have been enlarged to the same degree before the ovarian torsion. The mobility of the left ovary might have been limited by the sigmoid colon, allowing only the right ovary to twist in this case.

Torsion of the ovary or fallopian tube usually occurs in ovaries with tumors, functional cysts, or paraovarian cysts. Here, the authors have described a PCOS patient who had right ovarian torsion and underwent unilateral oophorectomy (UO). A beneficial side-effect of UO treatment was the development of spontaneous ovulation. PCOS patients have been reported to have good fecundity and have an ovarian reserve that is possibly superior to women with normal ovaries [6]. UO is a fertility-sparing procedure that allows the preservation of the functional ovary.

**Conclusion**

The authors recommend that PCOS patients should be carefully monitored for adnexal torsion after cessation of hormonal treatment.
References


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