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Brief Comunication

Amici-Larciprete knotless technique. An easy way to suture in laparoscopy

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The following technique has been developed from observation of the original Amici celioscopic suture.

Suturing and knot tying are basic skills for surgeons. Performing these tasks laparoscopically can be a tedious, time-consuming endeavor associated with much frustration [1].

Laparoscopic intracorporeal knot tying in minimally invasive surgery is an advanced skill and mastering this skill is an arduous process with a long learning curve. Recent advances in instrumentation have allowed easier suturing and tying, up to now.

Attempts have been made to modify the suture materials and instrumentation in order to facilitate this process. The Endo Stitch suture device was developed to facilitate sutures, reducing the amount of time needed for placement of stitches and knot tying in reconstructive laparoscopic procedures requiring multiple suture planes [2].

This tool has been shown to also be encouraging for inexperienced residents reducing the learning curve [3].

The pre-looped intra-corporeal knot was described as an interesting alternative to extracorporeal classic suturing [4] and a variety of combined instruments have been-reported with both needle-holding jaws and loop-forming members, in order to use a single tool to make an easy suture.

We evaluated a new method for performing the basic tasks of intracorporeal suturing and knot tying.

Basically we used a Vicryl 1-15 cm long stitch (Johnson & Johnson Int., St. Stevens-Woluwe, Belgium) with a curved atraumatic needle. Firstly we put a clip (Ligaclip MCA, Ethicon Endo-Surgery, LLC, Johnson & Johnson) at the end tip of the stitch, then we pass the needle within the tissue surfaces to be closed. Thus the suture is fixed at the beginning.

Then a continuous suture is used. After we pass the needle the last time at the end of the tied suture, we put the second clip on the stitch close to the tissue, tying the suture with the other hand, providing an adequate closure of the surgical margins of the wound (Figure 1).

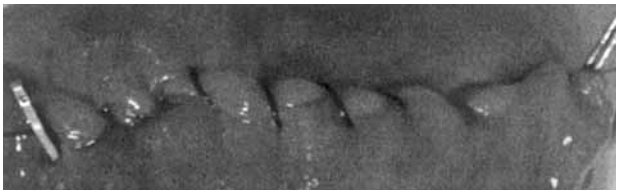

Figure 1. — Continuous suture with clips at the beginning and at the end tip.

This suture is suitable for myomectomy or supracervical hysterectomy, and in any circumstances in which it could be necessary to suture the myometrium.

The knotless suture provides significant time-saving for surgeons regardless of experience and thus reduces operating room costs. Less experienced surgeons and surgeons in training could benefit the most by the use of this procedure.

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Editorial Articles

The concept and treatment methodology for inducing ovulation in women in apparent premature menopause

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Summary

Purpose: To provide the concept and details of the methodology of inducing ovulation in women in apparent menopause. *Methods:* A recent case is discussed and other previous publications described illustrating how to induce ovulation and achieve pregnancies despite what appears to be menopause. The various methods of lowering serum follicle stimulating hormone (FSH) and restoring down-regulated FSH receptors in granulosa theca cells of the follicle are described. *Results:* The newly reported case had two successful pregnancies after having a trisomy 15 in her first pregnancy. *Conclusions:* Women aged 42 and younger in apparent menopause have a reasonably good chance of ovulation induction and pregnancy by adhering to the tenets discussed, especially including lowering the elevated FSH in some way (the easiest and cheapest with ethinyl estradiol), using minimal or no gonadotropins, and supporting the luteal phase with progesterone.

Key words: Ovarian failure; FSH receptor; Gonadotropin suppression.

A technique was described in 1984 in which women in apparent menopause based on amenorrhea with estrogen deficiency, failure to menstruate despite progesterone withdrawal, and resistance to follicular stimulation by gonadotropin stimulation were made to ovulate by lowering the elevated gonadotropins by using pharmacologic dosages of estrogen [1]. The theory of why the estrogen helps to induce ovulation even when high-dose gonadotropins or high endogenous gonadotropins fail is that the pharmacologic dosage of estrogen, by lowering the high serum level of follicle stimulating hormone (FSH), allows a restitution of down-regulated FSH receptors in the granulosa-theca cells which previously regressed related to the chronic high levels of FSH exposure.

Initially, this technique involved staying on a pharmacologic dosage of estrogen until the serum FSH fell into the normal range when it would be maintained while gonadotropins would be injected and follicular maturation would be monitored [1]. The main estrogen used was ethinyl estradiol because it does not measure in the assay for serum estradiol [2]. At that time gonadotropins were not reimbursed by insurance companies, in general, and thus those women who were not going to demonstrate follicular maturation would be spending a lot of money without even a chance for pregnancy.

Observation of follicular monitoring with ultrasound, serum estradiol and serum FSH demonstrated that women would recruit a follicle or follicles simply by lowering the serum FSH [2]. Sometimes, these follicles would progress to dominant follicles (defined as reaching an average diameter of ≥ 17 mm with a serum estradiol (E2) ≥ 175 pg/ml). Others did better with a small boost of gonadotropins [2]. For maximal cost effectiveness this modification is the predominant technique used to try to initiate ovulation in a woman who seems to be in menopause (usually prematurely).

One may question the technique as to whether it was the proposed mechanism that was operational, i.e., restoration of down-regulated FSH receptors in granulosa-theca cells, which was responsible for the ovulation or whether the ovulation was merely fortuitous and independent of therapy. There have been anecdotal cases of ovulation and pregnancy without any treatment [3] or just with estrogen replacement therapy [4-6]. However, the occurrence of premature menopause is estimated at 1% of all women during their reproductive years [7]. The study describing the modified technique evaluated 91 women with premature ovarian failure defined as having > 12 months of amenorrhea, failure to have withdrawal menses following ten days of 10 mg medroxyprogesterone acetate, a serum E2 < 25 pg/ml and a serum FSH > 35 mIU/ml. Yet despite these criteria using the technique described about 20% of the treated cycles resulted in ovulation and 38% of the women ovulated at least once [2]. Furthermore, about 20% conceived [2]. This is much higher than would be expected by chance alone (1 in 6,500) considering the paucity of publications related to spontaneous conception [3-6].

It may be questioned whether the eggs of these women with apparent premature ovarian failure, but who are made to ovulate, may be qualitatively different than women of advanced reproductive age in menopause. The possibility exists

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that the women responding to pharmacologic ethinyl estradiol therapy with or without mild stimulation with exogenous gonadotropins may not suffer from a paucity of eggs but in fact have a plethora of eggs that are resistant to gonadotropins and somehow the estrogen restores their sensitivity to FSH. However, in contrast to that theory, and in support that there is actually a paucity of eggs even in younger women with premature ovarian syndrome, is the demonstration of ovulation and pregnancy in some women who have basically only streaked gonads left as demonstrated in one woman at the time of C-section and another woman during a laparoscopy prior to treatment [8, 9]. In fact, the serum FSH for the woman who had the C-section was 124 mIU/ml [8]. Furthermore the ovaries generally appear to be small by ultrasound and show usually no antral sized follicles and few if any pre-antral sized follicles.

Even if there is a deficiency of follicles, the possibility exists that it is not the effect of ethinyl estradiol lowering the serum FSH which is the operating mechanism but perhaps estrogen somehow other than lowering the FSH and restoring FSH receptors restores the sensitivity of the few remaining follicles to FSH. This mechanism seems less plausible than the theory of the need to lower the serum FSH to attain the right circumstances for recruitment of the follicle. This is supported by the induction of ovulation in similar circumstances by merely lowering the elevated serum FSH by using the gonadotropin releasing hormone (GnRH) agonist leuprolide acetate [2, 10]. Similarly ovulation induction with hypergonadotropic amenorrhea has been achieved using the GnRH antagonist cetrorelix [11]. Furthermore, it has been demonstrated that one can create an apparent menopausal state by further raising the already elevated day 3 serum FSH in a menstruating woman and create an estrogen deficiency state resistant to endogenous gonadotropin or clomiphene citrate therapy only to restore ovulation even with multiple follicles simply by withdrawing clomiphene citrate [12].

More support for the consideration that these women do not have the gonadotropin resistant gonad with a plethora of follicles versus a paucity of follicles with acquired gonadotropin resistance is by watching the progression of the condition in the same patient. For example, one 37-year-old woman who came with regular menses of 26-30 days conceived the first month that she and her husband tried but had a miscarriage. Chromosome analysis of the fetus showed a trisomy 15.

Further evaluation by her consulting reproductive endocrinologist determined that her day 3 serum FSH was elevated over 15 mIU/ml. Though she had conceived the very first cycle that she attempted to conceive, she was advised by that reproductive endocrinologist that her eggs were from a quantitative and qualitative standpoint "old" and that conception again would be highly unlikely, and even if it did occur, a repeat trisomy would be likely. In support of this argument she was referred to a recent study by an excellent IVF facility that had failed to have any successful live deliveries at any age of the woman despite the transfer of normal appearing embryos following IVF-ET if the serum FSH was > 15 mIU/ml [13]. The woman was advised to consider using donated oocytes.

She came to us for a second opinion and I advised her that from our experience her eggs might be quantitatively similar to women over the age of 45 (an age where pregnancies rarely occur), but from a qualitative standpoint they should be more compatible with her age peers of age 37. She was advised that in contrast to data showing atrocious pregnancy rates following IVF-ET in women with elevated day 3 serum FSH given traditional controlled ovarian hyperstimulation [13-15], we have been able to achieve very high pregnancy rates when using much lower dose FSH protocols and to avoid adding exogenous FSH when the serum FSH is already elevated [16, 17].

Since she conceived the very first cycle that she tried I did not think in vitro fertilization was necessary. I advised her that based on her regular menses my approach would be to determine whether she attains a mature follicle (average diameter 18-24 mm associated with a serum E2 > 200 pg/ml) and if so to only use vaginal progesterone supplementation in the luteal phase. If the oocyte released before follicular maturation was attained, in the succeeding cycle small dosages of exogenous FSH (such as 75 IU) would be given from the mid to late follicular phase when the serum FSH level would be decreased by the rising level of endogenous estradiol [18, 19].

On her initial visit she was actually a couple of days late for her menses so serum beta-hCG and serum progesterone were obtained. The serum beta-hCG was positive at 433 mIU/ml and the serum progesterone level was appropriate at 55.1 ng/ml. However since her vaginal cytology showed an inadequate progesterone effect based on the number of superficial cells seen she was placed on vaginal progesterone support during the first trimester. She had a full-term live delivery by C-section.

She returned a few months after delivery at age 40 to consider having another baby. She began weaning and her menses had not resumed. By vaginal cytology she now showed predominantly parabasal cells and she was clearly estrogen deficient. Her serum E2 was < 10 pg/ml and her serum FSH was increased to 21 mIU/ml. An ultrasound failed to demonstrate any pre-antral or antral sized follicles.

She was started on 20 mcg of ethinyl estradiol every day and then changed to every other day when the FSH dropped too low to < 1. After 21 days there still were no pre-antral or antral follicles seen on ultrasound. After 41 days taking ethinyl estradiol, 20 mcg every other day, there were two follicles seen at 6 and 4 mm. The serum E2 was still < 10 pg/ml (note that ethinyl estradiol is not measured in the serum 17 beta estradiol assay). One week later the serum E2 rose to 40 pg/ml and finally on the 57th day the serum E2 reached 270 pg/ml with a 15.7 mm follicle. However two days prior the serum E2 was 153 pg/ml with a 12 mm follicle as the serum LH was 26 mIU/ml and the serum P was 0.8 ng/ml. However with the 15.7 mm follicle the LH rose to 39 mIU/ml and the serum P rose to 1.0 ng/ml. The follicle reached 23.7 mm but the serum P was 3.3 ng/ml. Eventually the follicle collapsed but she did not conceive. One

possible reason for failing to conceive on this cycle besides possibly not selecting a normal egg was premature luteinization [20].

During her preceding luteal phase the ethinyl estradiol was switched to oral estradiol to allow exposure to the potential conceptus to a natural estrogen. She was also supplemented with progesterone vaginal suppositories, 200 mg, twice daily. With the ensuing menses she had a baseline serum E2 on day 3 of < 10 pg/ml and her serum FSH was 4 mIU/ml having been kept down by the combination of endogenous and exogenous E2 and P during the preceding luteal phase.

The plan was to carefully observe her again and depending on how well she was responding she might be given a boost of 75 IU exogenous FSH and possibly started on cetrorelix if the serum E2 approached 100 pg/ml and a follicle approached 14 mm. Her only medication was ethinyl estradiol every other day. By day 11 there was a 15.7 mm follicle seen with a serum E2 of 165 pg/ml. However since the serum LH was only 5 mIU/ml and thus lower than the earlier level on day 6 of 11 mIU/ml, and because the serum P was only 0.5 ng/ml (less than the 0.7 ng/ml level on day 5) it was elected not to boost with exogenous FSH or start cetrorelix. On day 12 the serum E2 was 264 pg/ml and the follicle size averaged 18.7 mm. The patient was given 10,000 IU of human chorionic gonadotropin and a repeat ultrasound two days later showed egg release by demonstrating follicular collapse. The ethinyl estradiol was stopped and she was supplemented again with estradiol and progesterone. She conceived that cycle. Chorionic villis sampling was performed and no chromosome abnormalities were found. She has successfully completed the first trimester.

I selected this recent case to first illustrate that the women with apparent ovarian failure are not those with ovarian failure with gonadotropin resistant follicles but merely less follicles. Obviously when our patient had regular ovulatory cycles with a high serum FSH she did not demonstrate any gonadotropin resistance despite increased FSH. She clearly showed that she could respond to her own endogenous gonadotropins. Her case was selected to show the evolution of ovarian failure from regular menses in one year to estrogen deficiency and amenorrhea the next year.

She also demonstrated the technique of lowering elevated serum FSH with ethinyl estradiol with the theory that remaining follicles acquire a resistance to FSH by the chronically elevated FSH down-regulating FSH receptors in granulosa theca cells.

Pregnancy is a state where because of the high levels of estrogen and progesterone the serum FSH and LH are suppressed. Our patient's progression from regular menses to ovarian failure while she was pregnant shows that suppression of follicular development with oral contraceptives or GnRH agonists are probably ineffective in preventing further atresia of follicles. The fact that she conceived three of the four times she had unprotected intercourse despite elevated serum FSH lends credence to the fact that oocytes from women with elevated serum FSH are not qualitatively poor; their eggs will frequently result in pregnancy. Two of three pregnancies being chromosomally normal dispels the concept that even if she were to conceive it would most likely be a trisomy.

It could be argued that one cannot state for sure in the case described above that recruitment of the follicle could have been spontaneous and not related to the suppression of serum FSH by ethinyl estradiol. Whether it was related to the ethinyl estradiol therapy or not, careful monitoring allowed detection of the follicle and thus proper timing of intercourse, and proper timing of luteal phase support with progesterone. Other cases clearly demonstrate the need for lowering the serum FSH in some way, e.g., the 25-year-old with two years of amenorrhea and estrogen deficiency as evidenced by failure to menstruate upon progesterone withdrawal treatment whose first three serum FSH levels measured were 144.9, 145.6, and 164.2 mIU/ml. She ovulated in six of the next ten cycles treated exclusively with ethinyl estradiol and had a chemical pregnancy in cycle 9 and a live delivery resulted from cycle 10 [21]. Another case supporting the theory is a 37-year-old woman whose serum FSH had been 120 and 123 mIU/ml with a serum E2 of 20 pg/ml who had not had a menstrual period for almost three years. Her endometrial thickness was only 2 mm. She was made to ovulate with just ethinyl estradiol alone in two of three treatment cycles. She elected to try donor oocytes in her part of the country 3,000 miles away. She failed to conceive after four donor egg cycles yet had spontaneous ovulation again two years later at the age of 40 following estrogen suppression of the elevated serum FSH and conceived and delivered a healthy live baby [22].

Eventually the oocytes become depleted or at least no longer recruited. Two women in apparent ovarian failure were able to induce ovulation and successfully conceive with in vitro fertilization for tubal factor problems [23, 24]. However, neither were able to ovulate again after their deliveries.

It is difficult to gauge, however, how fast a woman will develop ovarian failure once the serum FSH is elevated. We previously reported the case of a woman with tubal factor and increased day 3 serum FSH who had successful pregnancies following three of four IVF-ET cycles over an 8-year time span [25]. Ten years after her first high FSH she still menstruates about once a month. Another unreported case with elevated serum FSH had three successful pregnancies among a couple of miscarriages with an 8-year span between pregnancies and did not require IVF-ET.

Previously we also reported successful pregnancies in two women 45 and 46 years old with elevated serum FSH and even reported a successful pregnancy in a 45-year-old woman in apparent menopause [26-28]. Nevertheless we have had only one successful pregnancy in over 200 egg retrievals in women 45 or older even with normal serum FSH. Advanced age possibly related to the natural selection over the years of the follicles with the most apoptosis inhibiting factor forebodes a much less optimistic chance of successful conception.

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A putative role of versican in uterine leiomyomas

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Summary

The extracellular matrix (ECM) has been thought to contribute to the pathogenesis of uterine leiomyomas. Uterine leiomyomas have abundant ECM components, including collagen, fibronectin, and glycosaminoglycans. Recent studies have demonstrated the overexpression of versican in uterine leiomyomas. Versican is a chondroitin sulfate proteoglycan that constitutes the main component of the ECM. However, the role of versican in the growth of uterine leiomyomas remains unknown. In this article a putative role of versican in uterine leiomyomas is discussed in association with cell proliferation and apoptosis..

Key words: Versican; Leiomyoma; Extracellular matrix.

Uterine leiomyoma is a fibrotic disease characterized by the accumulation of the abundant extracellular matrix (ECM) components such as collagen, fibronectin, and glycosaminoglycans. The deregulated ECM metabolism has been thought to play a pivotal role in the pathogenesis of uterine leiomyomas [1]. Versican is one of the main ECM components and is widely distributed in various tissues and cancers.

Versican belongs to the family of hyaluronan-binding proteoglycans that include aggrecan, neurocan, and brevican [2]. Versican modulates cell adhesion, proliferation, migration, and ECM assembly, and hence plays a central role in tissue morphogenesis and maintenance [2, 3]. An alternative splicing yields four isoforms, V0, V1, V2, and V3 [2]. Versican V0 isoform possesses two chondroitin sulfate carrying segments, GAG-α and GAG-ß, whereas V1 and V2 isoforms lack the GAG-α or GAG-ß domain, respectively, and the smallest versican V3 isoform has no GAG carrying modules [3].

Versican interacts with several ECM molecules. All versican isoforms interact with hyaluronan and form different sized versican-hyarulonan aggregates, thereby determining the tissue volume [2]. Versican also binds to the other ECM components such as collagen, tenascin-R, fibulin, fibrillin, fibronectin, P- and L-selectin, and cell surface proteins such as CD44, integrin ß1, and epidermal growth factor receptor (EGFR) [2, 3]. The diverse interaction of versican with its partners regulates cell tissue behavior [3].

A recent study has demonstrated that the versican gene is up-regulated in primary cell cultures of uterine leiomyomas compared with the myometrium [4]. Furthermore, versican V0, V1, and V3 isoform mRNAs are shown to be elevated in cultured leiomyoma cells as compared to myometrial cells [5]. However, the biological significance of versican in uterine leiomyomas remains unknown. Nevertheless, it is speculated that versican may act to promote cell proliferation and inhibit apoptosis of uterine leiomyoma cells. Because the biology of versican in uterine leiomyomas has never been explored so far, a putative role of versican in leiomyoma growth is discussed here based on the known actions of versican examined in various cells.

In addition to the role of versican in the ECM assembly, versican has recently been reported to regulate cell proliferation and apoptosis. Platelet-derived growth factor (PDGF) was shown to increase versican expression at mRNA and protein levels in arterial smooth muscle cells, leading to ECM expansion [6]. PDGF was demonstrated to be up-regulated in leiomyoma tissue compared with myometrial tissue [7]. This suggests that the PDGF-induced stimulation of versican may cause the expansion of the ECM in uterine leiomyomas.

Versican isoforms have different roles in the regulation of cell proliferation and apoptosis. Wu *et al*. [8] reported that versican V1 induced neuronal differentiation and promoted neurite outgrowth by enhancing EGFR and integrin activities in PC12 cells. Furthermore, they reported that stable expression of versican or its C-terminal domain protected astrocytoma cells from oxidative stress-induced apoptosis and enhanced cell attachment and the expression of integrin ß1 and fibronectin, suggesting that versican may promotes cell survival and cell adhesion [9]. Moreover, versican V1 isoform was shown to enhance cell proliferation and inhibit apoptosis of NIH3T3 fibroblasts [10]. It was demonstrated that V1 isoform activated EGFR expression, induced p27 degradation, and enhanced cyclin-dependent kinase 2 activity as well as down-regulated the expression of proapoptotic protein Bad, whereas V2 isoform inhibited cell proliferation and down-regulated EGFR and cyclin A expression [10]. A recent study has demonstrated that overexpression of versican V1 isoform in cultured fibroblasts increased proliferation and apoptotic resistance by down-regulating Fas

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expression [11]. By contrast, V3 isoform was shown to inhibit migration and reduce proliferation of arterial smooth muscle cells [12]. Thus, V1, V2, and V3 isoforms act differently on cell proliferation and apoptosis. The alternation of the balance among versican isoforms may determine the proliferative potential of the cells. Although versican V1 isoform mRNA was reported to be up-regulated in uterine leiomyomas [5], the expression of V2 isoform in uterine leiomyomas remains to be explored. The biology of each versican isoform in the growth of uterine leiomyomas remains to be clarified. However, it is tempting to speculate that the balance of versican isoform activities may be in favor of the promotion of cell proliferation and inhibition of apoptosis in uterine leiomyoma cells. Further study will be necessary to elucidate the effects of V1, V2, and V3 isoforms on the proliferation and apoptosis of uterine leiomyoma cells. The elucidation of versican action on leiomyoma growth would contribute to a better understanding of the novel role of the ECM in leiomyoma growth.

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Original Articles

Reproductive Biology Section

The effect of length of the follicular phase on pregnancy outcome following single embryo transfer (ET) in hypergonadotropic women

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Summary

Objective: To evaluate whether a short follicular phase adversely affects pregnancy rates following in vitro fertilization-embryo transfer in women with diminished egg reserve similarly to women with short follicular phases and normal egg reserve. *Methods:* A retrospective review of women with day 3 serum FSH > 12 mIU/ml having only a single embryo transfer. Pregnancy rates were determined according to length of follicular phase, i.e., until day of egg retrieval. *Results:* The ongoing/delivery pregnancy rates for women having oocyte retrievals on day 10 or earlier was 20.0% (20/63) compared to 16.1% (34/210) for those having retrievals on day 11 or later (p = NS). *Conclusions:* Either length of the follicular phase is not an important factor for achieving a pregnancy in women with diminished egg reserve or the use of ethinyl estradiol in the follicular phase negates the adverse effect of the short follicular phase even if it fails to lengthen this phase to at least ten days.

Key words: Diminished egg reserve; Length of follicular phase; Ethinyl estradiol.

Introduction

Even in the modern era of in vitro fertilization-embryo transfer (IVF-ET) using typical controlled ovarian hyperstimulation (COH) regimens, there are reports of extremely poor pregnancy outcome despite transfer of morphologically normal embryos of sufficient number when the day 3 serum follicle stimulating hormone (FSH) level is elevated [1, 2].

However a reasonable pregnancy outcome has been reported in couples with not only increased day 3 serum FSH levels but even less egg reserve when minimal or no gonadotropins were used [3, 4].

A short follicular phase has been found to be associated with a lower pregnancy rate [5, 6].

The present study had two purposes: 1) To evaluate the efficacy of single embryo transfers in women with high day 3 serum FSH levels in a larger series; 2) To determine if pregnancy outcome is affected by the length of the follicular phase.

Materials and Methods

A retrospective review of all single embryo transfer outcomes from January 1, 1997 to January 1, 2004 was performed in women whose day 3 serum FSH was > 12 mIU/ml.

Inclusion criteria included: Serum FSH > 12 mIU/ml, and age ≤ 39 years. The women were included if serum estradiol (E2) was > 50 pg/ml as long as serum FSH > 12 mIU/ml.

Careful frequent monitoring during follicular phase with pelvic sonography to measure follicle and endometrial growth was performed. Also, measurements of concomitant serum E2, progesterone (P), luteinizing hormone (LH), and FSH levels were made.

During pregnancy an ultrasound at eight weeks and 16 weeks was performed. The end of the follicular phase was considered as the day of oocyte retrieval.

Results

The pregnancy outcome for single embryo transfers according to length of follicular phase is shown in Table 1.

The majority of oocyte retrievals occurred between days 11-15 (160/273, 58.5%). Though there were only five retrievals on days 1-5, this may be spuriously low because the women were more likely to cancel because they were advised that our own studies suggest lower outcomes with very short follicular phases [4, 5].

There were no differences in clinical pregnancy rates or implantation rates in any of the five groups subdivided according to days of follicular phase (Table 1).

There were no differences in the ongoing/delivery pregnancy rates in women having the retrieval from day 6 to over 21 days. There were no delivered pregnancies in the day 1-5 group but there were only five transfers (Table 1).

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Table 1. — *Pregnancy outcome for one ET based on number of days of follicular phase.*

	1-5 ≤ 39	6-10 ≤ 39	11-15 ≤ 39	16-20 ≤ 39	≥ 21 ≤ 39
# of transfers	5	58	160	35	15
Mean E2 day hCG (pg/ml)	433.8	444.7	749.0	5546.0	390.8
Mean P day hCG (ng/ml)	0.90	0.83	0.90	0.91	0.76
# of follicles	6	218	759	109	37
# of eggs retrieved	7	117	511	91	32
# of inseminations	6	108	436	83	30
# fertilized	5	67	220	39	29
% fertilized	83.3	62.0	50.5	47.0	96.7
# of pregnancies	1	16	33	7	5
% pregnancy/transfer	20.0	27.6	20.6	20.0	33.3
# of clinical pregnancies	1	14	27	6	5
% clinical/transfer	20.0	24.1	16.9	17.1	33.3
# ectopic	0	1	0	0	0
# viable	0	13	25	6	3
% of ongoing/delivered	0.0	22.4	15.6	17.1	20.0
% of SAB: clinical pregnancy	100.0	7.1	7.4	0.0	40.0

SAB: spontaneous abortion.

The ongoing/delivery pregnancy rates for women having oocyte retrievals on day 10 or earlier was 20.0% (20/63) compared to 16.1% (34/210) for those having retrievals on day 11 or greater (p = NS).

Since there was only one embryo transferred the implantation rate equals the clinical pregnancy rate.

Discussion

Previous non-IVF studies in women with better oocyte reserves found a lower pregnancy rate in women who ovulated before day 11 [4, 5]. It was found that delaying the follicular phase with ethinyl estradiol to ≥ 11 days improved the pregnancy outcome to normal [5].

At least with IVF-ET, the present data do not suggest canceling the retrieval or freezing of the embryo and not transfering if the oocyte retrieval is from days 6-10. Many of the cases not included in this study whose retrievals were day 1-5 availed themselves of the option of retrieval and cryopreservation of the embryo or cancellation of the retrieval. The present data might make some reconsider this option since one of five women did achieve a clinical pregnancy.

The method of lengthening the follicular phase was with ethinyl estradiol. Many of the patients in this study, especially the ones with early retrievals, had been on ethinyl estradiol to lower elevated day 3 FSH in an effort to restore down-regulated FSH receptors and restore sensitivity to endogenous or exogenous gonadotropins [6].

The possibility exists that the improved outcome in the one study by lengthening the follicular phase could have been a direct effect of the ethinyl estradiol and not to lengthening the follicular phase, at least based on these IVF data [5].

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High doses of GnRH antagonists are efficient in the management of severe ovarian hyperstimulation syndrome

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Summary

Objective: To determine whether treatment of severe ovarian hyperstimulation syndrome (OHSS) with high-dose gonadotropin-releasing hormone (GnRH) antagonist, due to its luteolytic effect, is an effective method of management. *Methods:* Six infertile patients who had been scheduled for embryo transfer and developed early-onset severe OHSS with ascites and hemoconcentration were chosen for treatment with 3.0 mg of a GnRH antagonist (Cetrotide; Cetrorelix, Serono, Madrid, Spain). The response of these patients was compared with five patients with severe early-onset OHSS who received support therapy alone. All patients were evaluated clinically, echographically, and hematologically. *Results:* Estradiol (E2) levels dropped significantly a few days after treatment. Peritoneal fluid regression measured by ultrasound was faster on the study group compared with controls. Hematocrit remained comparable in both groups during follow-up. In two cases a second bolus of GnRH-antagonist was used due to clinical and biochemical findings during the four days of observation following the initial dose. None of the patients treated with GnRH antagonists required paracentesis. *Conclusions:* Treatment with high doses of GnRH antagonists seems to be effective in the management of severe OHSS.

Key words: Ovarian hyperstimulation syndrome; GnRH antagonists.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic, life-threatening disease that complicates in its most severe form 0.2-2% of *in vitro* fertilization (IVF) attempts. Characteristics of patients prone to develop OHSS are: young (< 35 years of age), thin women of short stature, women who produced multiple cysts or were high responders in previous IVF cycles, those exposed to aggressive stimulation regimens such as those using recombinant follicle stimulating hormone (r-FSH), women with hormonal and/or ultrasound (US) morphological signs of polycystic ovary syndrome, women who produce excessively high numbers of small follicles (> 15-30 in the present IVF cycle), and women who have a high E2 response (> 2000 - 6000 pg/ml) in the present IVF cycle.

Human chorionic gonadotropin (hCG) may promote ovarian secretion of vasoactive substances. Pregnancy, as a continuum of ever-increasing quantities of hCG, may aggravate early onset OHSS and may induce late onset OHSS. GnRH receptors have been shown to be present in granulosa-lutein cells [1, 2], the endometrium [3, 4], endosalpinx, and in ovarian structures [3-8]. Some studies suggest that steroidogenic activity of cultured granulosa cells may be affected by GnRH [6, 8, 9], suggesting that the ovary, the endometrium, and the embryo (3,4,7) could be targets for direct extrapituitary GnRH action in humans.

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There are reports [10, 11] on the efficacy of GnRH antagonists to induce luteolysis and elicit subsequent ovarian quiescence in IVF cycles. The purpose of our study was to determine whether high-dose GnRH antagonist treatment of patients with severe OHSS is effective after triggering ovulation with hCG.

Materials and Methods

Eleven patients who developed severe OHSS were selected for treatment with high doses of a GnRH antagonist and support therapy or for treatment with only support therapy. Six of these infertile patients scheduled for *in vitro* fertilization-embryo transfer (IVF-ET) who were afflicted with severe OHSS following ovulation induction with r-FSH and administration of 10,000 IU of hCG were treated with high doses of a GnRH antagonist and support therapy. The outcomes of these patients were compared with the outcomes of five other patients with severe OHSS who were treated only with support therapy. All patients provided written informed consent to participate in this study, which was approved by the Ethics Committee of The University of Valencia Hospital. OHSS was diagnosed as a patient having at least [12]: ovarian diameter > 10 cm; marked ascites, hematocrit > 45%; leucocytes > 15000/mm^3; serum creatinine > 1.0 mg/dl .

All patients who received GnRH antagonist treatment had ovaries greater than 10 cm in diameter, more than 25 follicles with diameters greater than 15 mm by US examination, and either E2 levels greater than 3500 pg/ml on triggering day, or greater than 25 oocytes retrieved. Embryo transfer was not performed in any of the patients. The six selected patients who fulfilled the criteria for diagnosis of early-onset severe OHSS following US examination received a subcutaneous bolus of 3.0 mg of Cetrotide® (Cetrorelix; Serono, Madrid-Spain) immedi-

ately after oocyte retrieval. All embryos (in the pronuclear stage) were subjected to freezing for transfer in subsequent cycles. Patients chosen for supportive therapy received intravenous fluids (1500 to 3000 ml saline solution or Ringer's lactate). Cases with resistant hemoconcentrations were treated with 1000 ml Voluven® 6% plasma volume expander (6% hydroxyethylstarch) in"Y" with 1500 ml physiologic serum over a 24-hour period. Fluid dose was adjusted every six hours according to hematocrit. All patients were heparinized using enoxaparin (Clexane®) at a dose of 40 mg subcutaneously every 24 hours and kept at bed rest. Patients were monitored every four days with a complete physical examination, transvaginal US examinations, and repeat endocrine studies. They remained hospitalized until there was objective evidence of clinical resolution.

Statistical analysis

Data were analyzed with a software package for Social Sciences (SPSS) v. 13 program. Data are expressed as mean ± standard deviation and in percentages when applicable. We employed the Kruskal-Wallis non-parametric test and chi square for proportions. Sample size calculation and statistical significance were not applicable due to the small number of patients.

Results

Patient characteristics did not differ significantly between groups (age, BMI, total dosage of rFSH, follicular size, estradiol levels, the triggering of ovulation, and number of oocytes retrieved). The average age of the case patients was 28.8 ± 1.7 years and of the control patients 30.4 ± 3.6 years. The BMI was 21.4 ± 1.7 for cases and 23.0 ± 2.3 for controls (Table 1).

Table 1. — *General data.*

	Study group	Controls
No. of patients	6	5
Age	28.8 ± 1.7	30.4 ± 3.6
Total dose of FSH (IU)	1420 ± 585	1200 ± 396
Follicles > 15 mm	42.2 ± 9.7	28.4 ± 10.1
E2 triggering day	3694 ± 334	4871.2 ± 1196
Oocytes retrieved	30.6 ± 6,2	36.2 ± 10.4
BMI	21.4 ± 1.7	23.0 ± 2.3

Values are means ± SD unless otherwise stated.

Estradiol concentration

On retrieval day E2 levels were comparable in both groups. Four days after treatment there was a lowering in E2 levels in cases compared to the levels in controls (Figure 1). However, two patients in the study group needed a second dose of the antagonist because of persistently high estradiol levels and persistent symptoms of ovarian hyperstimulation after four days of initiation of treatment. The decrease in estradiol levels in cases became significant on subsequent control dates and was associated with clinical recovery.

Hematocrit

Although there was a hematocrit improvement trend in cases following treatment (Figure 2), the difference with controls was not statistically significant.

Peritoneal fluid estimation

Ascites was evident in patients in the treatment group at the first control evaluation (fourth day after oocyte retrieval). Two patients in the study group had a large amount of peritoneal fluid. This finding along with the presence of high estradiol levels was the reason for the administration of a second dose of GnRH antagonist four days after the initial antagonist administration. During subsequent evaluations better improvement was noticed in the cases, but the difference with controls was not statistically significant (Figure 3). Paracentesis, however, was not performed in any of the cases, while two patients among the controls required paracentesis. The physicians who decided whether paracentesis was necessary were not aware whether these patients were in the antagonist treatment group or in the control group.

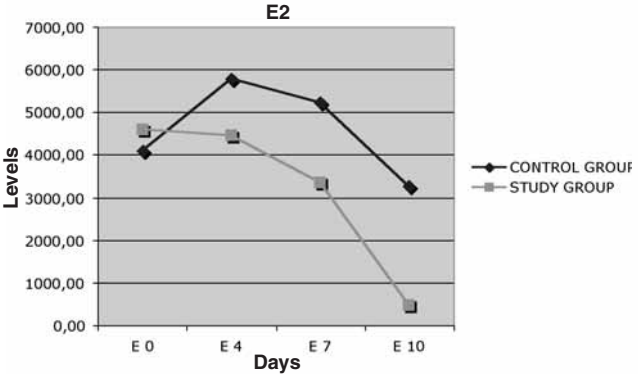
Two patients in the treatment group who were discharged after evaluation on day 4 required hospital readmission on day 8 due to US and clinical evidence of serious hyperstimulation (ascites, hemoconcentration, hypoproteinemia, hyperocoagulability, and weight gain). These two patients had a history of severe hyperstimulation in two previous ovulation induction cycles. They were treated on those occasions with paracentesis with retrieval of five to seven liters of fluid each time, and required hospitalization for 15 and 21 days, respectively. One of these patients also had pleural effusion (data not shown). Two other patients in the treatment group were discharged following the second scheduled evaluation. Ovarian enlargement was the only remaining abnormality. The ovaries of these patients were completely normal when examined one month later.

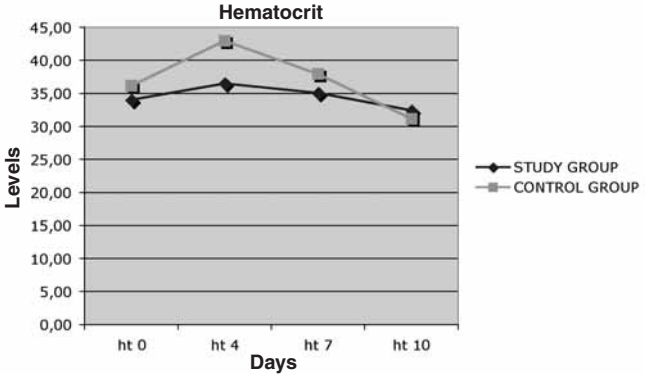
Patients in the treatment group needed a mean of nine days hospitalization. Two patients in the control group needed 21 days hospitalization. The mean hospitalisation time for the control group was 12.3 days (data not shown). One month after discharge all the patients had resumed menstruation and their ovarian sizes were of approximately 5 cm. Three patients from the treatment group have already received transfer of frozen embryos. One of these transfers was unsuccessful.

Discussion

Consensus about the management strategy for OHSS is lacking due to the imperfect understanding of pathogenesis of this disease. Today it is thought that OHSS results from an acute change in vascular (especially intraovarian) permeability secondary to arteriolar dilatation and extravasation or leakage of protein-rich intravascular fluid into the peritoneal, pleural, and pericardial cavities and a consequent reduction in circulating volume. All these changes seem to be influenced (or induced) by several local ovarian vasoactive mediators such as vascular endothelial growth factor (VEGF) and many others.

Severe OHSS lacks reliable predictive criteria. Although as has been described, stimulated patients presenting a large number of small-sized follicles, especially





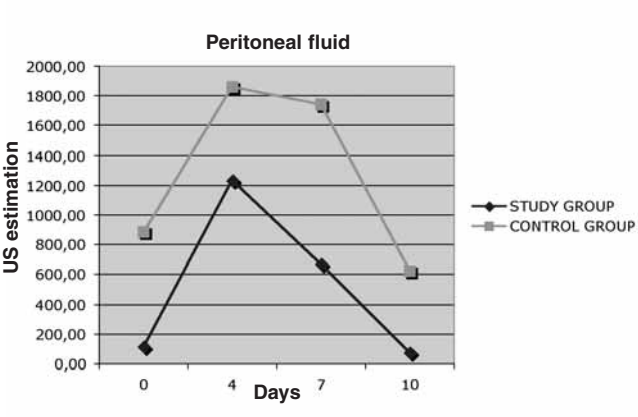


Figure 1. — Comparative graphic showing estradiol levels in OHSS patients after treatment with a bolus of GnRH antagonists (study group) versus support therapy alone (historical controls).

Figure 2. — Comparative graphic showing hematocrit levels in OHSS patients after treatment with a bolus of GnRH antagonists (study group) versus support therapy alone (historical controls).

Figure 3. Comparative graphic showing peritoneal fluid estimation by ultrasound (ml) in OHSS patients.

with a high degree of perifollicular and medullary vascularization [13], are more prone to develop the syndrome, neither of these findings are a guarantee thereof. Elevated E2 values on the day of hCG administration are not a reliable criterion. Although there is an 80% risk of developing severe OHSS when there are elevated estradiol levels on the day of hCG administration, this severe syndrome has also been observed in patients who have conceived spontaneously, as well as in patients with low serum E2 levels on the day of hCG administration. It is well known in practice that high E2 levels do not always lead to ovarian hyperstimulation [12, 14, 15].

In contrast to GnRH agonists, GnRH antagonists elicit an immediate effect by competitive blockage of GnRH receptors. Since late follicular phase growth of follicles and subsequent estradiol production are dependent on stimulation by both LH and FSH, prolonged use of high-doses of GnRH antagonists may effectively arrest further development of follicles through pronounced suppression of pituitary gonadotropin release in cases of imminent OHSS [16, 17]. GnRX (embryonic GnRH) plays a fundamental role in the development of neoangiogenesis in terms of the maternal decidua necessary for correct implantation and subsequent development of a gestation. This is carried out via modulation of VEGF and KDR, FLT-1 and sFLT-1 receptors [6, 7]. It probably produces a similar effect in the ovary in order to maintain vascularization of the corpus luteum. Once again, this process is blocked by GnRH antagonists [4]. It has yet to be determined whether GnRH antagonists act solely at the ovarian level. The optimal dose and length of treatment is not yet established.

In view of the increased risk of OHSS associated with pregnancy, it may be wise to have cryopreservation of all embryos and plan for transfer during a subsequent natural cycle. One may also consider avoidance of hCG administration in the luteal phase and substitution thereof with progesterone, coasting, GnRH agonist for triggering ovulation, and administration of anti-VEGF antibodies as other methods of prevention. However, effective preventive measures remain controversial [14].

To the best of our knowledge there is only one other recently published article focusing on this protocol [18]. In this study the antagonist was reintroduced in small doses of 0.25 mg three days after oocyte pickup showing a decline in OHSS symptoms. Surprisingly none of the patients needed hospitalization. Beside the differences between these protocols the results were positive in both trials showing that the use of GnRH antagonist combined with cryopreservation of embryos is associated to clinical recovery of severe cases of OHSS.

In conclusion, clinical, ultrasonographic, and hormonal results with our small group of patients suggests that treatment with high doses of GnRH antagonists shows promise as an effective regimen for the treatment of severe OHSS [13]. It is worth highlighting that despite

serious symptoms (severe ascites), none of the patients in the treatment group required paracentesis. The principal weakness of this study is the small number of patients included. Although the results suggest that OHSS treatment with high doses of GnRH antagonists seems to be effective, a larger double-blinded study is needed to determine whether this impression is valid.

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Repeated intracyclic clomiphene citrate therapy can be more effective than hMG therapy in inducing ovulation: case report

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Summary

Purpose of investigation: When clomiphene citrate is ineffective in the treatment of anovulation, hMG administration is typically selected. However, high-dose hMG therapy is associated with a variety of adverse events. We describe the use of a modified clomiphene citrate regimen that was successful in increasing the effectiveness of ovulation induction. *Case report:* A patient who did not initially respond to clomiphene citrate therapy required a total dose of 2400 IU hMG to prodeuce mature follicles. However, because of the physical and emotional burdens on the patient, and the possibility of multiple pregnancy and ovarian hyperstimulation syndrome, re-treatment with clomiphene citrate was then selected. Two courses of clomiphene citrate administered at a fixed interval during the same cycle safely induced ovulation. After initial induction of ovulation, her ovulatory failure improved and natural ovulation occurred. *Conclusions:* Repeated intracycle clomiphene cirate therapy may be more effective than hMG therapy in inducing ovulation in some patients.

Key words: Clomiphene citrate; hMG; Multiple pregnancy; Amenorrhea; Anorexia nervosa.

Introduction

Clomiphene citrate is the first-line treatment for anovulation. If a favorable response to clomiphene citrate is not obtained even after the dosage is increased, human menopausal gonadotropin (hMG) therapy is typically selected. In such cases, high-dose hMG therapy may be necessary to induce ovulation. However, a high-dose hMG regimen is associated with higher incidences of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). In recent years, improved hMG preparations have been developed to minimize such adverse reactions [1]. Nevertheless, hMG therapy places considerable physical and emotional burdens on patients and requires a substantial time commitment due to the need for frequent hospital attendance.

Although clomiphene citrate therapy is free from these problems, the standard regimen [2-5] may not be effective for inducing ovulation. In patients who require high-dose hMG citrate for the induction of ovulation, favorable results may not be possible if standard clomiphene citrate therapy is re-administered.

Because our patient had not responded to standard clomiphene citrate therapy, hMG therapy was administered. High-dose hMG therapy did result in the formation of mature follicles, but the patient had great anxiety about the treatment (especially concerning the daily injections). Due to the patient's concerns and risk of adverse events, administration of hMG was not continued. Instead, treatment with a modified clomiphene citrate regimen was chosen, with the hope of improving the results of the standard treatment. This report was written with the informed consent of the patient.

Case Report

Past history

The patient was a 29-year-old married woman who presented to the clinic of one of the authors with a 2-year history of infertility associated with anovulation and amenorrhea; she had become amenorrheic due to anorexia nervosa while working as a model. However, at the time of her clinic visit, she had stopped modelling and her eating habits had returned to normal. At the first hormonal examination, low gonadotropin and E2 levels were detected (Table 1-a). As she showed no response to clomiphene citrate therapy at a dose of 50 mg/day for five days, the dose was increased to 100 mg/day for five days. However, her ovarian function still did not respond, and an additional 100 mg/day for five days (a total of 1000 mg) was administered during the same cycle. After she did not respond to this regimen, hMG was prescribed for four days at 150 IU/day. However, her ovaries still did not react. Then, hMG was administered at a dose of 300 IU/day for eight days (total dose: 2400 IU). Two mature follicles were observed on the ninth day of treatment; approximately ten small follicles were also detected. The hCG injection was subsequently stopped due to the development of these small follicles. Luteinzing hormone (LH)-kid analysis on the ninth day showed that her urine was negative for presence of the LH surge, indicating that she had not ovulated. Natural withdrawal bleeding occurred seven days later. After her ovaries did not respond to two subsequent cycles of clomiphene citrate therapy at a dose of 100 mg/day for five days, she was referred to our Department of Gynecology.

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Table 1. — *Hormone levels at each treatment phase.*

Stage of treatment	LH (mIU/ml)	FSH (mIU/ml)	E2 (ng/ml)	P (pg/ml)
a Initial medical examination at previous clinic	0.3	1.3	10	-
b After first WB in our hospital	0.53	2.8	23.6	0.11
c Final day (Day 27) of the second course of CC therapy	6.9	5.3	203	0.36
d Mid-luteal phase (Day 34) after first ovulation	0.89	0.75	86.5	15
e Day 48 (December)	3.2	6.9	21.3	0.18
f About two months after WB (next year)	2.9	6.8	28.1	0.3

WB: withdrawal bleeding; CC: clomiphene citrate.

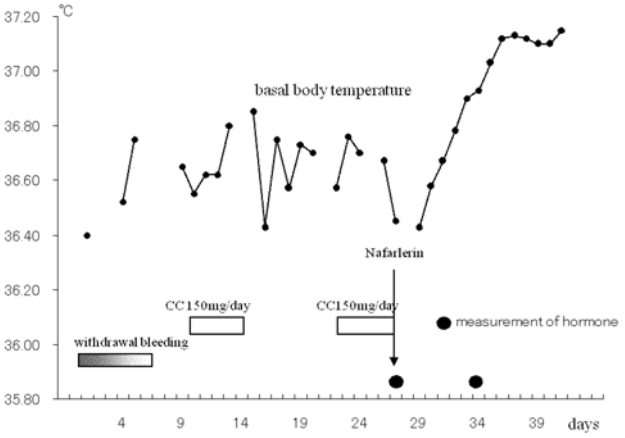
Table 2. — *Patient response during hMG therapy and repeated CC therapy.*

	hMG therapy	Repeated CC therapy
Before the treatment		
Daily life	good	good
Hormonal level	LH: 0.3, FSH: 1.3, E2: 10	LH: 0.53, FSH: 2.8, E2: 23.6
Cycle of ovulation	anovulation	anovulation
CC 100 mg x 5 days	no response	no response
During the treatment to induce ovulation		
Mature ovarian follicles	2	1
Small follicle	10 or more	1
LH surge	(–)	(+) (Gn-Rha)
OHSS		(–)
After the treatment		
CC 100 mg x 5 days	no response	ovulation

CC: clomiphene citrate.

Clinical course

At the time of the patient's first visit to our hospital, there were few follicles in either ovary and amenorrhea was still present. Hormone levels (Table 1-b) measured after a withdrawal bleed did not appreciably differ from the baseline values obtained at the previous clinic. Although she remained hopeful of successful conception, it was felt that physically and emotionally taxing treatments should be avoided because of the patient's considerable anxiety about her ovulatory failure and the treatment (especially the daily injections). Treatment with clomiphene citrate was therefore selected. At this time, approximately seven months had passed since the hMG treatment. The growth of follicles was not noted when clomiphene citrate was administered at a dose of 150 mg/day for five days, beginning on the tenth day after withdrawal bleeding. Clomiphene citrate was re-administered at a dose of 150 mg/day for five days after an 8-day drug holiday. On the final day of the second course of clomiphene citrate therapy, one mature follicle was noted in the left ovary and one small follicle was seen in the right ovary. Hormone levels at this time were appropriate for ovulation (Table 1-c), so Gn-RHa (Nafarelin: 1200 mg) was administered in the evening. One week after administration, ovulation was confirmed by ultrasonography and hormone levels (Table 1-d) (Figure 1). After ovulation, the patient's response to clomiphene citrate improved. The improved function of the pituitary and ovaries was confirmed by the hormone levels measured 48 days after menstruation (6 months after admission) (Table 1-e) and two months after withdrawal bleeding (10 months after admission) (Table 1-f). One year later, it was possible to induce ovulation by a single course of clomiphene citrate therapy at a dose of 50 mg/day for five days. Two years later, natural ovulation and emmenia were confirmed although they were slightly irregular.



CC: clomiphene citrate.

Figure 1. — Repeated intracyclic clomiphene citrate therapy protocol.

Discussion

As our case illustrates, patients with amenorrhea may have considerable anxiety [6] due to the great physical and emotional burdens associated with daily injection of hMG. For this reason, hMG therapy is not immediately indicated after patients show no response to clomiphene citrate. In addition, hMG therapy may result in multiple pregnancy and/or OHSS, leading to additional hardship. In recent years, improved hMG preparations have been developed to minimize such adverse reactions [1]. However, it has not been possible to markedly decrease the frequency of administration or the dosage of hMG [7]. Moreover, hMG therapy imposes a heavy economic burden on patients [8].

Clomiphene citrate continues to be administered in almost the same manner as that detailed in the first report of its clinical use by Greenblatt *et al.* in 1961 [9]. The usual dose of clomiphene citrate is 50 to 100 mg/day. If a response is not obtained, the daily dose is increased to 150 to 250 mg [3]. Using the standard protocol, the response to clomiphene citrate therapy is not much better than the response to hMG therapy. The duration of clomiphene citrate administration is usually five days, although durations of three days [4, 5] and ten days [10, 11] have been reported. Apart from reports describing combination therapy with other drugs [12-14], few reports have shown that the effect of clomiphene citrate was enhanced by methods other than an increase in dosage.

In the present case, daily injections of hMG were required to obtain a mature follicle. However, this regimen could not be sustained due to the physical and emotional strain it placed on the patient. Re-treatment using a modified clomiphene citrate regimen was therefore chosen, as it was less stressful for the patient. Because her ovulation disorder had not substantially improved since the time of her hMG therapy, clomiphene

citrate therapy was necessary to obtain effects similar to those of high-dose hMG treatment. Repeated intracyclic clomiphene citrate therapy was initiated at the dose of 150 mg/day for five days. This had no effect, so a second round of clomiphene citrate treatment was performed during the same cycle. Fortunately, ovulation was stimulated without any adverse effects.

A modified clomiphene citrate protocol was reported by Hamada *et al.* in 1976 [15]. In this regimen, clomiphene citrate is administered a second time without any withdrawal bleeding if the induction of ovulation by standard clomiphene citrate therapy cannot be confirmed after approximately one month. The authors believed that gonadotropin secretion might be activated and follicles might partially grow during the first course of clomiphene citrate therapy, and that follicles might grow further and ovulation might be induced during the second course. This regimen could enhance the sensitivity of the ovaries to gonadotropin and therefore result in ovulation. On the basis of this hypothesis, we developed a reported clomiphene citrate regimen with a recovery period. In this regimen, the duration of the first and second courses of treatment is five to seven days [16]. In standard clomiphene citrate therapy, clomiphene citrate is re-administered after withdrawal bleeding if the ovary activity is poor. In repeated clomiphene citrate therapy, the second and third courses of treatment are added according to the response of the patient, so that an additive effect is obtained. With this method, the day clomiphene citrate therapy is initiated (50-200 mg/day for 5 days), and the interval between the first and second course of treatment (range: 5-10 days) can be scheduled at the patient's convenience.

In this study, hMG and clomiphene citrate were administered to the same patient and the conditions just before both administrations were almost identical. We evaluated changes during and after the administration of hMG and clomiphene citrate (Table 2). Our results in the present case illustrate the advantages of repeated intracyclic clomiphene citrate therapy as compared to hMG therapy, which can lead to considerable stress due to the need for daily treatment and to possible multiple pregnancy and OHSS. Our findings show that when ovulation is induced in repeated intracyclic clomiphene citrate therapy, the hormonal environment may improve resulting in a better response to treatment during the next cycle. If a patient does not respond to repeated intracyclic clomiphene citrate therapy, the possibility of successful subsequent hMG treatment would not be adversely affected. Although the cervical mucus and/or endometrium may be affected by clomiphene citrate, it is important to initially select the treatment method that is least burdensome for patients with chronic amenorrhea.

Conclusion

In the treatment of anovulation, it is obviously important to induce ovulation that results in pregnancy. However, it also essential to avoid multiple pregnancy and/or OHSS. Our results indicate that repeated intracyclic clomiphene citrate therapy may be more effective in inducing ovulation than hMG therapy for some patients.

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No association found between decreased ovarian reserve and low thyroid function

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Summary

Purpose: To determine if women with diminished egg reserve are more likely to have in addition diminished thyroid reserve compared to women with normal egg reserve. *Methods:* Serum thyroid stimulation hormone levels and history of thyroid hormone replacement therapy was determined according to three ranges of elevated serum follicle stimulating hormone (FSH) in donor egg recipients with diminished egg reserve, and comparisons were made to women having embryo transfers on the same day. *Results:* No difference or trends were found of diminished thyroid function in egg recipients vs controls in women aged 39 or under. *Conclusions:* Since autoimmune damage to an endocrine gland is more commonly associated with damage to other endocrine glands because of sharing of common proteins, autoantibody damage to the ovaries does not seem to be a common cause of diminished ovarian egg reserve.

Key words: Ovarian failure; Hypothyroidism; Autoimmunity.

Introduction

One theoretical cause of premature diminished egg reserve is autoimmune destruction of the ovaries, similar to the common cause of hypothyroidism as seen in Hashimoto's disease. Due to the existence of common proteins among various endocrine glands, autoimmune damage to the ovaries could theoretically be part of a generalized autoimmune endocrine destruction process involving multiple endocrine glands.

One study found ten cases of hypothyroidism in 119 women with normal karyotypes with premature ovarian failure [1]. The same group found a 3.2% incidence of adrenal insufficiency detected by using the commercially available assay for measuring adrenal antibodies to the 21-hydroxylase enzyme, the primary adrenal autoantigen [2].

Hypothyroidism is a common problem. Thus the question arises as to whether the aforementioned 8.4% incidence of hypothyroidism in a population of women with idiopathic diminished egg reserve is greater than what would be expected in women with normal egg reserve or not [1].

Materials and Methods

A retrospective cohort analysis was performed on donor oocyte recipients aged ≤ 40 years of age. Participants were divided into three groups based upon serum FSH (mIU/ml) levels: FSH 11-15, FSH 16-30, and FSH > 30. Serum thyroid stimulating hormone (TSH) levels were recorded at the time of initial visit and it was determined if they were taking any thyroid replacement therapy.

Controls included all women having embryo transfers on the same day as the recipients, who were using their own eggs, and had a serum FSH ≤ 5 mIU/ml.

A TSH level > 5 uIU/ml was considered elevated, and if a woman was currently taking thyroid hormone replacement, her TSH level was excluded in the determination of mean TSH levels.

Results

TSH levels according to recipient groups are given in Table 1. The ranges of day 3 serum FSH (mIU/mL) in the three groups were 6.9-15 (median 11.2), 17-28 (median 20.6), and 36.7-90 (median 50.3). The average age in the three groups were 36.3 ± 2.7, 32.7 ± 2.7, and 35.1 ± 4.4.

No association was seen between elevated FSH and elevated TSH (Table 1). None of the recipients were receiving thyroid hormone replacement. Only two of 30 (66%) had a TSH > 5 uIU/ml.

For the controls, the average age was 36 ± 5, with 11 controls (24%) aged ≥ 40. Six of the 46 controls (13%) were taking L-thyroxin, with three of the six in the age ≥ 40 group (thus they had a history of elevated serum TSH levels). There were three additional women with TSH > 5 uIU/ml, yielding a percentage of 19.5% (9/46) with elevated TSH levels. If one eliminates the six control women ≥ age 40 there were three of 40 or 7.5% with diminished thyroid reserve.

Discussion

These data do not support a correlation between high day 3 serum FSH levels and a high TSH level. Though the 6.6% frequency of diminished thyroid reserve is consistent with the findings of 8.4% by Kim *et al.* [1], control women with normal ovarian reserve less than age

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Table 1. — *Comparison of TSH levels by FSH levels in oocyte recipients < 40 years of age.*

TSH levels (uIU/ml)	Group 1 FSH ≥ 15 (n = 11)	Group 2 FSH 16-30 (n = 8)	Group 3 FSH > 30 (n = 11)
Minimum	.77	.6	.65
Maximum	8.10	4.3	7.9
Median	1.3	1.4	2.1
Mean + SD	2.1 + 2.1	1.7 + 1.2	2.6 + 2.0
95% confidence interval for mean	(.7 - 3.5)	(.7 - 2.7)	(1.2 - 3.9)
% with elevated TSH (> 5)	9.1% (1/11)	0.0% (0/8)	9.1% (1/11)

p = NS.

40 also had a similar frequency of 7.5%. Thus the trend for a higher incidence of diminished thyroid reserve in women with normal egg reserve seems to have been age-related.

Specific anti-ovarian antibodies may exist, but the lack of an association with damaged thyroid glands makes autoimmune damage to the ovaries a less likely etiology for premature ovarian failure.

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Factors affecting bone mineral density of young women and predictive factors of low bone mineral density

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Summary

Purpose of investigation: We investigated various factors affecting bone mineral density (BMD) in young women and predictive factors of low BMD. *Methods:* Subjects were 105 nursing school students aged from 19 to 24 years old. Body weight (BW), pituitary hormones, sex steroid hormone and bone turnover markers were selected as factors. BMD was measured at the lumbar spine at L2-L4 by dual-energy X-ray absorptiometry (DXA). *Results:* BW (p = 0.002), serum N-terminal telopeptide of type 1 collagen (NTx) (p = 0.006) and bone specific alkaline phosphatase (BAP) (p = 0.02) were significantly correlated with BMD. For identification of the low BMD group, all subjects were divided into four groups on the basis of BW and NTx concentrations. In the group with BW under 51 kg and Ntx concentrations over 11 nMBCE/l, BMD was significantly (p = 0.0013) decreased compared with the other three groups. In this group, the ratio of women with a low BMD was significantly higher (p = 0.004) than the other groups. *Conclusion:* In young women, BW and bone turnover markers significantly affected BMD. Low BMD can be indicated using BW and NTx concentrations without measurement by DXA.

Key words: Young women; Osteoporosis; Bone mineral density; Body weight; Serum N-terminal telopeptide of type 1 collagen; Predictive factor.

Introduction

As the average life span in Japan continues to increase, the society will continue to become a more aged one. Due to these conditions, osteoporosis is becoming a serious social problem. Current strategies have focused on identifying postmenopausal women who have low bone mineral density (BMD) and who are thus already at risk for fracture. This approach is problematic, because intervention with proven efficacious therapies, while resulting in reduction in fractures over time, cannot eliminate fracture risk entirely if BMD is already significantly reduced at the time of first assessment.

An alternative approach is to focus on premenopausal women. An increase in peak bone mass may contribute to a decrease in the incidence of osteoporosis. Recent reports show that the peak bone mass in Caucasian females and Japanese females occurs at the age of 18 years [1, 2]. For prevention of osteoporosis, young women with a low BMD are identified and offered interventional health education.

We have already reported that body weight and age of menarche are well correlated with BMD [3]. The objective of this study was to evaluate the osteoporosis risk factor among young women. We evaluated the influence of body weight, pituitary hormones, sex hormones and bone turnover markers on BMD. Further, we attempted to identify factors that might indicate low bone mass in young females without measurement of dual-energy X-ray absorptiometry (DXA).

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Subjects and Methods

Subjects

The subjects were 105 female students enrolled at the nursing school of Wakayama Medical University. Characteristics of the subjects are shown in Table 1.

Table 1. — *Demographic and clinical characteristics of the 105 young women in this study.*

	Age 1	BMD 2	BW 3	PRL 4	FSH 5	E2 6	NTX 7	BAP 8
Mean	19.5	1.10	51.6	26.2	5.91	77.8	10.2	26.2
SD	0.878	0.117	7.54	6.48	3.06	61.3	2.52	6.48
Range	19~ 24	0.828~ 1.41	36.1~ 93.9	6.90~ 44.6	1.50~ 18.7	10.0~ 26.4	6.90~ 20.4	6.90~ 20.4
Median	19	1.104	50.2	25.8	5.90	55.7	9.60	9.60

1 year old; 2 g/cm^2; 3 kg; 4 ng/ml; 5 mIU/ml; 6 pg/ml;7 nM BCE/L; 8 U/l.

Measurements of BMD

The BMD of the posterior-anterior lumbar spine at L2-4 was measured by DXA (DPX-NT, GE Co. Utah, USA).

Measurements of sex steroids, pituitary hormones and bone turnover markers

Serum estradiol (E2) levels were measured by electrochemiluminescence immunoassay (ECLIA) and serum prolactin and serum follicular stimulating hormone (FSH) concentrations were measured by chemiluminescent immunoassay (CLIA). We selected serum bone-specific alkaline phosphatase (BAP) and serum N-terminal telopeptide of type 1 collagen (NTx) as bone turnover markers. Serum NTx concentration was measured by an enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody directed against the N-telopeptide intermolecular cross-linked domain of type 1 collagen of bone (Osteomark; Mochida Pharmaceutical Co., Tokyo, Japan).

Serum BAP concentration was measured by the ELISA kit (Osteolinks-BAP; Sumitomo Pharmaceutical Inc., Tokyo, Japan). Blood samples were collected once on a given day for each subject at 12 a.m.

Identification of the low BMD group

For selection of the low BMD group, we divided the subjects into the following four groups on the basis of the mean of BW and NTx concentrations. A group: BW over 51 kg, NTx under 10 nMlBCE/l; B group: BW over 51 kg, NTx over ten nMlBCE/l; C group: BW under 51 kg, NTx under ten nMlBCE/l; D group: BW under 51 kg, NTx over ten nMlBCE/l. BMD was compared among the four groups. Further, the ratio of young women who had a low BMD in the various groups was evaluated. Low BMD was identified as being under 0.986 g/m^2 (mean-1SD).

Statistical analysis

Data analysis was performed using Stat View software (ver. 5, Hulinks, Tokyo, Japan) and a p value of less than 0.05 was considered statistically significant. Correlations between BMD or NTx concentrations and various factors were assessed by univariate analysis using Pearson's correlation coefficients. Independent variables related to BMD or NTx were analyzed by multivariate analysis. Comparison of the four groups (A, B, C, and D) was analyzed by one-way factorial ANOVA and multiple comparison tests. Comparisons between the D group and A, B, and C groups were analyzed by Fisher's exact method.

Results

The correlation between BMD and various factors by univariate analysis is shown in Table 2. A scatter gram and regression between BW and BMD and NTx and BMD are shown in Figures 1 and 2. BW was significantly ($p < 0.005$) positively correlated with BMD and Ntx was significantly ($p < 0.05$) negatively correlated with BMD. Independent variables significantly related to BMD were evaluated by multivariate analysis and were as follows: BW ($p < 0.005$), NTx ($p < 0.01$) and BAP ($p < 0.05$). Correlation of NTx with various factors by univariate analysis is shown in Table 4. Only prolactin was significantly ($p < 0.05$) negatively correlated with NTx. However, we could not find various factors significantly related to NTx by using multivariate analysis (Table 5).

A significant difference ($p < 0.005$) was found when comparing BMD among the four groups (A, B, C, and D) by using one-way factorial ANOVA. Using a multiple comparison test with Fisher's PLSD, A vs D ($p < 0.001$), B vs D ($p < 0.005$) and C vs D ($p < 0.05$) groups were found to be significantly different. Therefore, the BMD of the D group was significantly lower than the BMD of the other groups (Figure 3).

The ratio of young women with a low BMD was evaluated among the four groups (Table 6). Using Fisher's exact method, the D group had a significantly higher ratio ($p < 0.005$) compared with the other groups. In the C and D groups, 87.5% of young women had a low BMD.

Table 2. — *Correlation between BMD and various factors in univariate analysis.*

Factors	r*	*p* value
BW	0.28	< 0.005
FSH	0.096	n.s.
Prolactin	0.077	n.s.
E2	0.13	n.s.
NTx	0.28	< 0.005
BAP	0.179	n.s.

*r: correlation coefficient; n.s. = non significant.

Table 3. — *Correlation between BMD and various factors in multivariate analysis.*

Factors	*p* value
BW	< 0.005
FSH	n.s.
Prolactin	n.s.
E2	n.s.
NTx	< 0.01
BAP	< 0.05

n.s. = non significant.

Table 4. — *Correlation of NTx and various factors in univariate analysis.*

Factors	r*	*p* value
BW	0.060	n.s.
FSH	0.008	n.s.
Prolactin	0.20	< 0.05
E2	0.14	n.s.
BAP	0.048	n.s.

*r: correlation coefficient; n.s. = non significant.

Table 5. — *Correlation of NTx and various factors in multivariate analysis.*

Factors	*p* value
BW	n.s.
FSH	n.s.
Prolactin	n.s.
E2	n.s.
BAP	n.s.

n.s. = non significant.

Table 6. — *Ratio of young women with low BMD in the various groups.*

BMD g/m^2	Group			
	A	B	C	D
≤ 0.986 (%)	1 (4.5)	1 (4)	5 (14)	9 (43)
> 0.986	21	24	32	12
	22	25	37	21

Discussion

In order to prevent osteoporosis in old age, maintenance of a high BMD is very important. We have previously reported that maintaining an appropriate body weight is crucial for maintaining a high BMD [3].

In this study, serum factors such as pituitary hormones, sex hormone and bone turnover markers were evaluated to determine their association with BMD. E2 is known to be a factor involved in increasing BMD. BMD loss had been attributed to relative estrogen deficiency in postmenopausal women [4], a view reinforced by studies of bone loss after oophorectomy [5] and minimization of

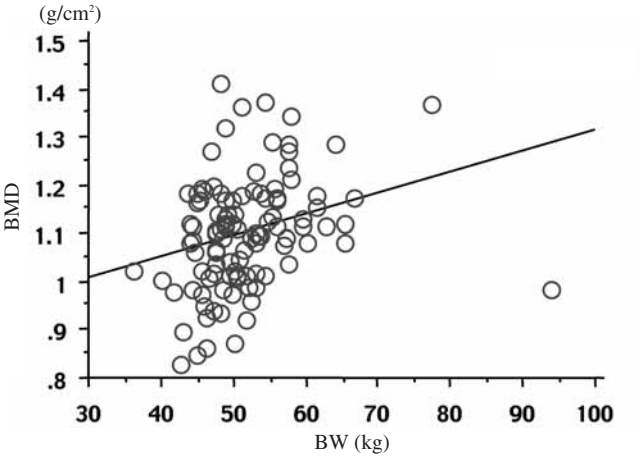

Figure 1. — Correlation between BMD and BW in univariate analysis ($p < 0.005$, R = 0.28).

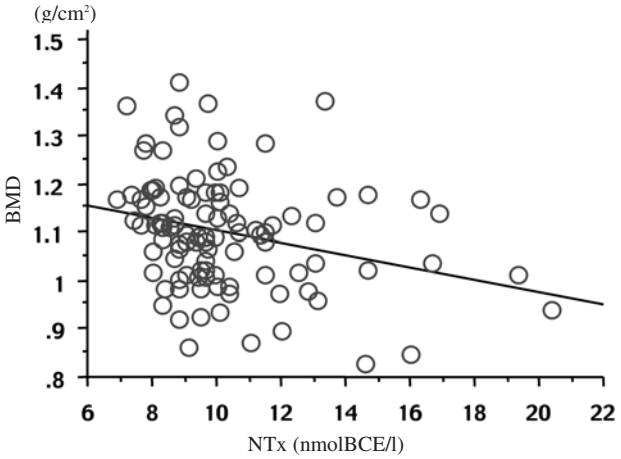

Figure 2. — The correlation between BMD and serum NTx in univariate analysis ($p < 0.005$, $R^2 = 0.28$).

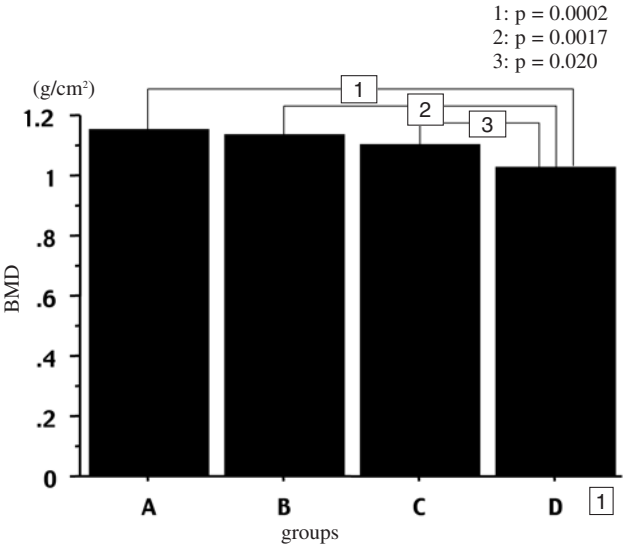

Figure 3. — Comparison of BMD among the four groups.

bone loss with estrogen replacement [6]. On the other hand, prolactin is associated with decreasing BMD; high prolactin levels have been shown to be correlated with a low BMD [7]. FSH is also related to BMD. FSHβ (ligand) and FSH receptor null mice have normal bone mass despite severe hypogonadism, and therefore, FSH might have direct negative effects on bone [8]. However, in our study, FSH, prolactin and E2 were not correlated to BMD by univariate and multivariate analysis. The highest level (44.1 ng/ml) of prolactin in this study did not affect BMD. FSH levels above 26 mIU/ml have been reported to decrease BMD [9]. Because the highest FSH level in our study was only 18.7 mIU/ml, this could be the reason why FSH did not affect BMD in our subjects. BMD loss was reported to be detectable in women whose E2 levels were less than 35 pg/ml [10]. In these women, E2 levels of 28 cases were less than 35 pg/ml. However, in our study, E2 did not influence BMD, which could have been due to the short exposure period to E2.

Serum BAP and NTx were selected as bone turnover markers in our study. BAP levels correspond well with osteoblast activity. The half life of BAP is 3.5 days. Therefore, no diurnal variation has been observed, and it is considered to be a very stable marker. NTx is produced by osteoblasts during bone resorption. Therefore, it is considered to be a bone resorption marker. Previously, NTx was measured in the urine; however, recently, measurement of NTx in serum has become possible. Urinary NTx and serum NTx concentrations are well correlated [11]. In this study, E2, prolactin, FSH and BAP were measured in serum, and therefore, NTx was also measured in serum. The concentration of serum NTx has a diurnal variation, which is highest at 8:00 a.m. and lowest at 12:00 a.m. to 8:00 p.m. [11]. The time of collection of blood in our study was fixed at 12:00 noon when morning school lessons were finished.

The normal concentrations of serum NTx are 7.5-16.5 nM BCE/L in 40-44-year-old women (premenopausal age) and 10.7-24.0 nM BCE/L in 45-79-year-old women (postmenopausal age). However, normal concentrations of NTx in young adult females have not been reported. In this study, we found that NTx concentrations in young females were 5.26 ± 15.1 nM BCE/L (mean, ± 1.96). These NTx concentrations are similar to concentrations in premenopausal females. Serum NTx concentrations are high in patients with Paget's disease and primary parathyroid disease, except in those patients with osteoporosis. In this study, NTx was significantly ($p < 0.005$) negatively correlated to BMD by univariate analysis. However, BW ($p < 0.05$), serum NTx ($p < 0.01$) and BAP ($p < 0.05$) were significantly correlated to BMD by multivariate analysis. The correlation between BMD and NTx in postmenopausal females has been previously reported [12]. To our knowledge, this is the first report of a good correlation of both BMD and NTx in young women. We also investigated factors, which correlated with NTx. Using univariate analysis, a significant correlation ($p < 0.005$) was found between NTx and prolactin.

However, there was no correlation between prolactin NTx by multivariate analysis. Unfortunately, we could not find any factors that affected NTx in this study.

For identifying the low BMD group, we selected two factors, BW and NTx, which show a good correlation with BMD. In this study, BAP was excluded for the sake of simplicity of clinical use. All subjects were divided into four groups according to the mean values of two factors. In subjects whose BW was less than 51 kg and NTx concentrations were over 11 nM BCE/L, BMD was significantly decreased. Further, the ratio of women who had a low BMD in the four groups was evaluated. Low BMD was identified as a BMD reduction of 1 SD from the mean of the study group, because this value is believed to represent a 2-3 fold increase in fracture risk [13]. Women with a low BMD made up 43% of the D group. In addition, in the C group and D group, 88% of women had a low BMD. Measurement of BMD by DXA was required in these groups (C and D) for precise evaluation of BMD. On the other hand, measurement of DXA for the A and B groups was not necessary. In health examinations at school, measurement of BMD for all students (young women) is very difficult. The factors we found in this study are considered to be useful predictors for the selection of students who need measurement of BMD. Even if measurement of BMD is not available, assessment of these factors accurately identifies a group of young women at potentially high risk for subsequent development of postmenopausal osteoporosis. For these young women, health education about osteoporosis, such as lifestyle intervention and risk factor modification, should be given.

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Activity of telomerase in ovarian cancer cells. Clinical implications

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Summary

Estimation of telomerase activity in cell nuclei of ovarian malignant tumours may provide an independent prognostic index. The test for telomerase activity in tumour cell nuclei may be accepted as a useful diagnostic test with application for differential diagnoses of benign ovarian tumours vs tumours of a borderline or malignant character.

Key words: Ovarian cancer; Telomerase; Telomeres.

Introduction

Ovarian cancer accounts for 4% of all female tumours and represents one of main causes of death due to female genital tumours [1]. In 2004 in Poland the malignancy was the sixth most important tumour if morbidity was considered and the fourth if tumour-induced mortality of women was considered. In the year ovarian cancer developed in 364 women and resulted in death of 2,273 women. The standardised coefficient of its incidence was 10.93 per 100,000 women [2]. Comparable values of the standardised coefficient of its incidence are also detected in highly developed countries such as the United States of North America (13.5 of new cases per 100,000 females annually), Australia (12 new cases per 100,000 females annually), Canada (12 new cases per 100,000 females annually) [3, 4]. Despite the increasing trend of incidence in the disease a growing percentage of 5-year survival is observed, linked both to growing health awareness of patients and to improved detectability of the tumours. The search continues for new tumour markers which would allow for a rapid and reliable diagnosis of ovarian cancer.

In recent years an increased interest in telomeres and telomerase has been seen. Muller and McClintock were the first to isolate redundant sequences of purine and pyrimidine bases at the ends of chromosomes in eukaryotic cells and they termed them telomeres (Greek: telos = end; *meros* = part) [5, 6]. It was not until the 1980s that human telomeres were found to be formed by double-stranded 5' TTAGGG 3' sequences, rich in guanine single-stranded sequences, in TRF 1, TRF 2, Rap 1p, sir proteins, in Cdc 13p and Est 1p [7]. The number of telomere repeats varies among species and in humans it amounts to around 2000. The number decreases with the unavoidable process of cellular senescence. Shortening of the telomeres provides a specific type of clock of cellular senescence, counting down a specific score of cell divisions before the cell stops dividing in the resting phase [8]. The repetitive telomeric sequences are most numerous in young dividing cells, i.e., in germinal, foetal cells, in the stratum basale of epidermis, and in haemopoetic as well as neoplastic cells.

The role of telomeres involves protection of chromosomes from activity of exonucleases and, thus, maintenance of genomes in an intact form. In the absence of telomeres random recombinations might lead to various pathological processes. Telomeres function as stabilizers of chromosomal structure and in this way they prevent formation of abnormal ring and centromeric forms in terminal segments of chromatin [9].

In cases of neoplastic cells telomere length depends on the activity of telomerase, the enzyme capable of telomere synthesis. The enzyme was isolated for the first time by Greider in 1985 while four years later Morin described it in human neoplastic cells, linking the fact with cell immortality [10, 11]. In 1997 Meyerson *et al.* [12] cloned the gene of telomerase reverse transcriptase while Bodnar with his research team introduced it to human cells and demonstrated that this was followed by augmented synthesis of telomerase and the cells acquired neoplastic traits [13].

Telomerase consists of template RNA *(TR)* formed by approximately 395-450 base pairs, the catalytic unit of reverse transcriptase *(hTERT),* and by TP1, p23, HSP 90 proteins. The extent of TP1 expression manifests a significant correlation with telomerase activity in neoplastic cells although factors which induce cell differentiation (retinoic acid, phorbol esters) decrease expression of hTERT but exert no effect on TP1 expression.

In 1994 Counter *et al.* [14] in their studies on neoplastic cells isolated from ascites sampled from patients with advanced ovarian cancer discovered the potential for detection of telomerase activity in cells *in vivo*. The authors demonstrated that short stable telomere sequences

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were present in cells of ovarian cancer but not in cells of ovarian surface epithelium. Such observations pertained also to the activity of telomerase, which was markedly higher in cells fractionated from ascites of patients with ovarian cancer than in cells of healthy women.

High interest in telomerase is linked to its involvement in processes of cell senescence and neoplasia [15-17]. In tumour cells telomerase prevents shortening of telomeres. It is assumed to be capable of influencing the neoplastic process: its activity is supposed to appear when the cells fully lose control over cell proliferation [18].

Materials and Methods

Our studies were conducted on a group of 34 patients treated for ovarian cancer. The mean age of the patients was 57.4 years (the youngest patient was 33 years old and the oldest was 78 years old).

The patients were qualified for the studies when ovarian cancer was histologically confirmed, the patient carried no other coexisting malignancies in the past, was subjected to primary surgical treatment according to standard medical procedures defined for ovarian cancer and to first lapse chemotherapy in line with therapeutic standards based on *paclitaxel* and platinum derivatives. On the day when the observation ended all patients completed their first lapse therapy.

The studies aimed at evaluating telomerase activity in tumour cell nuclei in patients treated for ovarian cancer and examining the relationships between the obtained results and clinical variables (extent of tumour clinical advancement, grade of cell differentiation, histological type of the tumour, size of tumour remains following primary surgery, presence of ascites, results of treatment following primary surgery and first lapse chemotherapy, results of *second-look* operation, relapse of the tumour and death due to ovarian cancer). The relation was also examined between activity of telomerase in ovarian cancer cell nuclei and concentration of CA 125 tumour marker, determined at four time points and presence of mutations in the *BRCA1* gene in patients. Activity of telomerase in tumour cell nuclei was also examined in five patients treated for marginal forms of ovarian cancer (mean age of the patients: 48.4 years; the youngest patient was 34 years old and the oldest 76 years old) and in a group of the same size including benign ovarian tumours, such as endometrial cysts (mean age of the patients: 36 years; the youngest patient was 23 years old and the oldest 50 years old).

Telomerase activity was estimated using in situ hybridization with DNA/RNA probe (Telomere PNA FISH Kit/FITC Code No K5325, DAKO Polska Sp. z o.o.).

Results

Activity of telomerase was demonstrated in cancer cell nuclei in 24 patients. Taking into account telomerase activity in tumour cell nuclei the patients were recruited to three groups. Patients in group 1 manifested no telomerase activity in cell nuclei (n = 10 patients), patients in group 2 showed telomerase activity in less than ten cell nuclei per visual field of a microscope (n = 17 patients), patients in group 3 manifested telomerase activity in 10-50 cell nuclei per microscope field (n = 7 patients). Telomerase activity was demonstrated in two patients with a marginal form of ovarian cancer (in one patient the activity was detected in 10 tumour cell nuclei and in the other in 10-50 tumour cell nuclei per microscope field), while in three patients with this form of ovarian cancer no telomerase activity could be shown in tumour cell nuclei. In none of the five patients with endometrial cysts could activity of the enzyme be demonstrated in cell nuclei of cells in the cyst wall.

In 27 patients with ovarian cancer an elevated level of CA 125 marker was documented. The mean value of the marker level, tested at four time points amounted to:

1) 1301.0 U/ml before the primary surgery (minimum value of 35.8 U/ml/maximum value of 4875.6 U/ml);

2) 778.9 U/ml before the first course of chemotherapy, administered up to ten days following the surgery (minimum value of 3.9 U/ml/maximum value of 4875.6 U/ml);

3) 28.9 U/ml before the fourth course of chemotherapy (minimum value of 3.2 U/ml/maximum value of 119.4 U/ml);

4) 18.1 U/ml following the last course of chemotherapy (in a single case the patient received just four courses of chemotherapy and in three cases the patients received five courses of chemotherapy, thus the estimation was conducted following the fourth and following the fifth chemotherapy course, respectively) (minimum value of 2.2 U/ml/maximum value of 132.8 U/ml).

Mutations in *BRCA1* gene were detected in 20 patients, including two patients with 5382*insC*.

A detailed analysis of data resulted in the following conclusions:

1) Activity of telomerase in tumour cell nuclei could be detected more frequently in patients treated for ovarian cancer in high stages of clinical advancement (FIGO Stages II and IV), of low tumour cell differentiation (G2, G3) and of serous adenocarcinoma type;

2) Augmented activity of telomerase was detected in tumour cell nuclei in patients treated for ovarian cancer with remnants of tumour masses below 1 cm following the primary operation and with presence of ascites detected during the surgery;

3) No relationship could be detected between activity of telomerase in tumour cell nuclei in patients treated for ovarian cancer, and results of treatment following primary surgery and a standard first lapse chemotherapy.

Estimation of telomerase activity in cell nuclei of ovarian malignant tumours may provide an independent prognostic index. Elevated activity of telomerase develops in patients with a higher number of tumour relapses and in patients who die due to ovarian cancer. A markedly higher probability of survival is manifested by patients demonstrating no telomerase activity in cancer cell nuclei as compared to patients with detectable activity of the enzyme. This has been confirmed by statistical analysis using the survival probability test of Kaplan-Meier (p = 0.015) (Figure 1).

The test for telomerase activity in tumour cell nuclei may be accepted as a useful diagnostic test with application for differential diagnoses of benign ovarian tumours vs tumours of a borderline or malignant character. Coex-

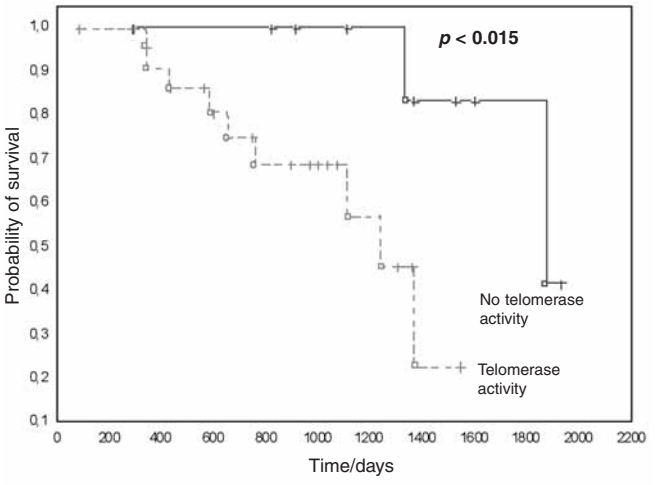

Figure 1. — Probability of survival in patients demonstrating no telomerase activity in cancer cell nuclei in comparison to patients with detectable activity of the enzyme.

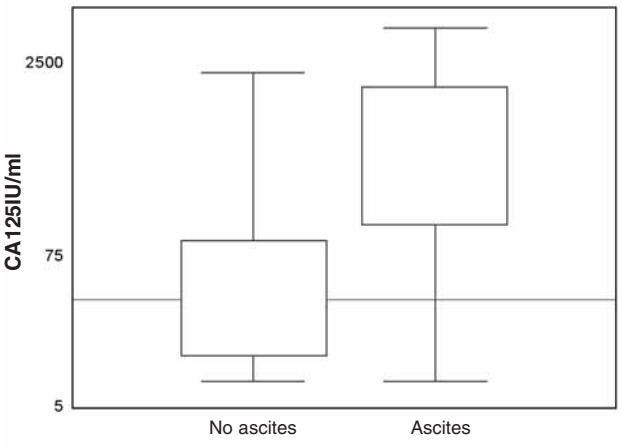

Figure 2. — Level of CA 125 in patients with ascites vs without ascites.

isting mutations in the *BRCA1* gene and telomerase activity in tumour cell nuclei provide a positive prognostic factor since no relapses of the neoplastic process or deaths due to ovarian cancer have been detected in patients with such characteristics of tumour genome.

In the Mann-Whitney U test, in which the level of CA 125 tumour marker served as a variable, significant differences were detected between patients with vs those without ascites ($p < 0.05$) (Figure 2).

Disscusion

In their report of 1995, Kim *et al.* [19] reported that telomerase activity was detected in the sensitive TRAP test in 85% of examined cases of malignant tumours (in 100 biopsies in 12 variable locations). A similar conclusion was drawn also by Unate *et al.* [20] following their analysis of telomerase activity in tumour cell nuclei. In the analysis the authors reported that 90% of tumours manifested activity of the enzyme but a proportion of tumour cells lacked such activity. Studies conducted on patients with breast cancer demonstrated telomerase activity in 95% of breast cancer cases, 65% of cases with non-malignant adenofibromas but not in a normal tissue [21, 22].

Similar observations were made in prostate cancers: telomerase activity was detected in 90% prostate cancer cases, in approximately 40% cases of benign prostatic hypertrophy but not in the normal tissue of prostate [23]. The investigators suggest that telomerase may provide a tumour marker used for early detection of cancer cells, when they cannot yet be detected by routine histopathology. Despite such an optimistic view on telomerase, doubts may appear whether the marker can differentiate malignant lesions from benign hypertrophies.

Similar data originate from other studies on tumours of the liver [24, 25], urinary bladder [26, 27] and thyroid gland [17].

In ovarian cancer telomerase activity is elevated but in most cases the elevation is not particularly pronounced. This can be illustrated by the group of patients studied in the present investigation. The group is not very numerous but analysis of the clinical data and results on the activity of the studied enzyme provides a few significant pieces of information.

In the studied group of 34 patients telomerase activity was demonstrated in 24 cases (70.6%). In parallel, activity of the enzyme was demonstrated in 40% patients with the borderline form of ovarian cancer while lack of this activity was detected in patients with benign endometrial tumours. Therefore, telomerase activity may be accepted as a diagnostic marker applicable in the differential diagnosis of benign ovarian tumours, preneoplastic lesions and tumours of the organ.

Development of sensitive tests for detection of telomerase activity, e.g., the TRAP test has provided hope for new potential in diagnosis and prognosis in oncological diseases. Intense studies have also started on mechanisms which control the activity of the enzyme, including the potential for blocking its activity. The search has started for telomerase inhibitors, which might find application in the therapy of tumours. The anti-sense technology has also been applied [28]. Currently, also other possibilities of switching off telomerase are known. One of them involves elimination of tumour cells with telomerase activity linked to induction of the specific immune response of cytotoxic T lymphocytes, activated following immunisation with telomerase reverse transcriptase [29, 30].

New reports which appear in the world literature make it mandatory to treat with a distance the new therapeutic solutions. Also this study confirms the need for moderate optimism in this question. First, demonstration of telomerase activity in cell nuclei of ovarian cancer only in 70.6% patients does not allow us to use the enzyme as a tumour marker specific for the tumour (specificity of a

marker is defined by a proportion of individuals with fully negative result among persons in whom no tumour is detected) since it is manifested in many tumours [31]. For the second, this is not a very sensitive marker (sensitivity of a marker is defined by the number of patients with a positive result to the number of patients suffering from a given type of tumour) and it provides no chances to detect it in patients with a low mass of neoplastic tumour. For the third, the marker carries no predictive value (the value is defined by the ratio of patients suffering from a given type of tumour with a truly positive result to the number of all individuals with a truly or falsely positive result) and it cannot be used in screening studies. Thus, telomerase does not exhaust the principal requirements posed to neoplastic markers and its activity should not be routinely estimated in cell nuclei of malignant tumours of the ovary.

A similar conclusion was drawn by Nagai *et al.* [32] in their report of 1998. The authors analyzed patients with malignant tumours of the uterine cervix, uterine body or ovary. Telomerase activity in tumour cells was detected using the TRAP test. The activity was detected in 91.7% of patients with cancer of the uterine cervix, in 85.2% of patients with cancer of the uterine corpus and in 90.9% of patients with ovarian cancer. The authors confirmed trace activity of the enzyme in 52.9% of cases of preneoplastic lesions in the vagina of the *high grade SIL* type and in 15.4% *low-grade SIL* type lesions. No relationship could be detected with the histological type of the studied tumours and clinical advancement stage, and no significant differences could be disclosed between patients with cancer of the uterine corpus and those with ovarian cancer. Significant differences were confirmed between malignant tumours and preneoplastic lesions of the uterine cervix, and between cancers and benign tumours of the ovary. In view of the above, the authors concluded that determination of telomerase activity might prove useful in diagnoses of cancers and in identification of groups with a high risk of developing a tumour.

In 2002 Sapi *et al*. [33] analysed the relationship between CA125 marker level and activity of telomerase in cell nuclei of ovarian cancer cells isolated from blood using an immunomagnetic procedure. The authors found a significantly higher concentration of CA125 in patients manifesting telomerase activity in cell nuclei (100% of patients with Stage IV tumour advancement according to FIGO and 35% of patients with Stage III tumour advancement according to FIGO) as compared to patients who manifested no activity of the enzyme in cell nuclei (65% patients with Stage III tumour advancement according to FIGO and 100% of healthy women). The authors concluded that telomerase provides a potential marker for detection of circulating cells of ovarian cancer.

In our hands, analysis of the group of 34 patients with ovarian cancer has demonstrated that significant differences, detected by the ANOVA rank test of Kruskal-Wallis at the level of $p < 0.05$, could be detected only for estimations of CA125 concentrations tested before surgery and for activity of telomerase in tumour cell nuclei. No significant differences have been detected between CA125 concentrations tested before the first, fourth and after the last course of standard first lapse chemotherapy or in telomerase activity in cell nuclei. Thus, the reduced tumour mass due to the primary surgery coexistent with the decrease in CA125 concentration in serum is significantly correlated with activity of telomerase activity in cell nuclei. However, the above does not justify the statement that telomerase represents a sensitive, specific tumour marker with prognostic potential since telomerase fails to exhaust criteria for a good marker and may be useful only in certain time points (it may be estimated before primary surgery), as mentioned in the earlier discussion.

As many as 75% of the studied group of patients have demonstrated telomerase activity in tumour cell nuclei in advanced stages of the disease (Stage III and IV of clinical advancement according to FIGO). When grade of tumour cell differentiation is concerned, higher activity of the enzyme has been observed in poorly differentiated ovarian cancers: such activity has been detected in 66.7% patients with G3 cancers and 29.2% patients with G2 cancers. It is also worth noting that 76% of patients with telomerase activity in tumour cell nuclei have ascites.

In 1998 Duggan *et al.* [34] claimed that evaluation of telomerase activity in cell nuclei of tumour cells contained in ascites in ovarian cancer patients represents a more sensitive test than cytological evaluation of the ascites fluid. Therefore, it is worthwhile considering application of the test in analyses of the advancement of neoplastic disease.

Considering the fact that following the adjuvant treatment of ovarian cancer with cytostatic drugs the *second-look* procedure for evaluation of results of treatment is performed with decreasing frequency [35], evaluation of telomerase activity could be applied for detection of the remains of subclinical neoplastic disease, as mentioned by Nouriani *et al.* in 2004 [36]. In parallel, the authors suggested that evaluation of the enzyme activity in cell nuclei of cells obtained from washings during *second-look* surgery in patients with ovarian cancer may increase sensitivity of the surgical procedure in detection of residues on the neoplastic disease.

In this study no evaluation of telomerase activity was performed in cell nuclei of tumour cells obtained from washings resulting from the *second-look* operation but the relationship was evaluated between telomerase activity estimated in the material from primary surgery obtained before the adjuvant treatment with cytostatic drugs and results of *second-look* surgery. Activity of telomerase in cell nuclei of ovarian cancer cells has been detected much more frequently in the group of patients with negative results of second-look operation. The conclusion follows that activity of the enzyme should be estimated in cell nuclei of tumour cells obtained from washings resulting from *second-look* surgery, as was done by Nouriani *et al.* [36].

As far as histological type of ovarian cancers is concerned, most involve tumours of a serous structure

(serous adenocarcinoma) and Rosai *et al.* [37], in a 2004 publication, claimed that such tumours comprise 60% to 80% of diagnosed ovarian tumours. Mucous and endometrial cancers are much less frequent. Also this study provides evidence that most of detected ovarian cancers involve serous cancers, which comprised 53% of studied cases (18 patients of the examined group). In analysis of the material, tumours of serous adenocarcinoma type have been shown to carry higher telomerase activity in cell nuclei of tumour cells. Thus, a relationship could have been noted between histological type of ovarian cancer and telomerase activity in cell nuclei of neoplastic cells.

The polish and world literature contains few reports on the clinical fate of ovarian cancer patients in whom a coexistence was documented of mutations in the *BRCA1* gene with telomerase activity in cell nuclei in tumour cells. In one recent report an inhibitory effect was detected in the *BRCA1* gene on the catalytic subunit of human telomerase, hTERT [38].

In this study the relationship between mutations detected in *BRCA1* gene age activity of telomerase in cell nuclei of ovarian cancer cells was examined. Mutations were detected in two cases of studied patients and both of the patients also manifested activity of the enzyme in cell nuclei of tumour cells. Analysis of survival in the cases has justified the conclusion that coexistence of mutations in the *BRCA1* gene and telomerase activity in tumour cell nuclei represents a positive prognostic index since both patients with a mutation in the *BRCA1* gene have reached complete clinical remission following surgical treatment and standard first lapse chemotherapy, and the neoplastic process has shown no relapse nor has it resulted in patient death.

It is also worth drawing attention to the simplicity of the applied technique. Most estimations used in contemporary diagnosis are based on the TRAP (Telomere Repeat Amplification Protocol) technique involving amplification of telomerase products. The method is sensitive permitting detection of telomerase activity in cell nuclei from one to ten cells, which may comprise not more than 0.01% of the studied cell population [39], but preparation of the procedure involves the use of an expensive polymerase chain reaction procedure. In this study the estim microscope was used to detect the reaction in tumour cells, equipment accessible in any pathology lab. The economy of the test clearly facilitates access to tumour diagnosis.

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Comparison of the number of uterine myomas detected by in-office transvaginal ultrasonography removed by laparotomic myomectomy: preoperative work-up concerns*

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Summary

Purpose of investigation: To assess the ability of detecting the number of uterine myomas by transvaginal ultrasonography (TVS) performed supporting the clinical examination of general gynecologists' office practice. *Methods:* A retrospective comparison of the number of myomas revealed by preoperative in-office TVS and documented after laparotomic myomectomy was conducted in 110 consecutive premenopausal patients referred for surgery. *Results*: The sensitivity of TVS in revealing the exact number of myomas was 59.4% in the whole series. In the subgroup of 88 patients with a preoperative diagnosis of three or fewer myomas TVS missed at least one myoma in 31 (35.2%) cases, achieving a 64.8% sensitivity. Among the 72 women diagnosed with one myoma at preoperative TVS, 19 (26.4%) resulted to have two or more myomas at the end of surgery, reaching a 73.6% sensitivity of TVS in revealing the exact number of myomas. *Conclusions*: In-office TVS reinforces the clinical diagnosis of uterine myomas but it often fails in the detection of their number, resulting in a poor preoperative characterization of patients. The fact that one myoma may be overlooked in one-third of patients theoretically eligible for laparoscopic conservative surgery may motivate the implementation of US diagnosis when laparoscopic myomectomy is considered.

Key words: Myoma; Ultrasonography; Myomectomy.

Introduction

Uterine myomas affect more than 20% of reproductive-aged women [1]. They are a frequent cause of pelvic pain and abnormal uterine bleeding and are thought to be involved in infertility [2].

At present symptomatic uterine myomas represent the most frequent reason for hysterectomy in the United States [3]. However, for patients who desire future pregnancies or wish to preserve their anatomic integrity, minimally invasive procedures are now available to perform conservative surgery. Mini-laparotomic and laparoscopic myomectomy have already resulted to be safe, reliable and reproducible techniques and uterine artery embolization may also be considered a promising approach [3-5].

With the advent of minimally invasive conservative treatments an accurate preoperative assessment of myomas has become a point of utmost importance. The presence or absence of uterine myomas, their number and size, their exact location and their differentiation from adenomyosis, are parameters that should be assessed before treatment.

Transvaginal sonography (TVS) with higher-frequency probes is the most cost-effective procedure to confirm the clinical diagnosis of myomas. TVS has revealed high accuracy in diagnosing number, size and location of myomas if performed by skilled operators using high-quality instruments [6-9].

Since TVS accuracy is highly operator-dependent and influenced by different available machines, surgical treatment often has to deal with a poor-quality preoperative evaluation made by in-office TVS during the clinical examination in general gynaecologic practice. This diagnostic work-up may even underestimate the number of myomas representing the main parameter that should be achieved in preoperative assessment, especially when minimally invasive procedures are planned [10].

The aim of the present study was to evaluate the ability of routine TVS during gynaecologic examination in detecting the correct number of myomas by comparing preoperative ultrasonographic diagnosis with removed myomas in an unselected group of premenopausal patients referred for laparotomic myomectomy.

Patients and Methods

Clinical data of all consecutive premenopausal patients submitted to laparotomic myomectomy at the authors' institution from June 2006 to August 2007 were collected and retrospectively evaluated.

The only sonographic inclusion criteria used in the study was a preoperative TVS, utilized for planning surgery, which included a written report clearly indicating the number of identified myomas.

To evaluate a group of subjects representative of a general gynaecologic practice we intentionally included office TVS evaluations performed by operators with variable experience, using multifrequence probes 5.5-7.5 MHz of different commercially available scanners.

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Age, indications for surgery, number of myomas identified by TVS and removed after abdominal myomectomy, their site and size, and latency between TVS and surgery were recorded for each patient.

Body mass index (BMI) and previous abdominal surgery were not investigated since TVS is thought not to be affected by these variables.

Preoperative bimanual gynaecological examination confirmed uterine enlargement in all patients scheduled for surgery. The number of myomas detected at TVS was compared with the number of myomas removed during surgery.

Although conservative surgery was performed in all cases by transverse soprapubic incision, several expedients were used to detect and remove all myomas. When there were uterine adhesions extensive lysis was the first endoabdominal surgical step to obtain complete uterine mobilization. When possible the uterine body was pulled toward and through the abdominal wall to individualize subserosal or partially intramural myomas by visual inspection. To succeed in this, anterior or fundal myomas were grasped with a Collins/Pozzi tenaculum or, in absence of reachable nodes, the fundal myometrium was deeply sewed by an absorbable cross-stitch and the uterus was pulled keeping it under tension.

After the visual inspection of the exteriorised part of uterus, all the uterine regions were accurately explored by finger touch to detect intramural or submucosal myomas.

Whenever the uterine body was too large for immediate exteriorization, intraabdominal enucleation of the larger myomas was performed with eventual morcellation by cold-knife to avoid enlargement of the opening. After enucleation of the intramural myomas, myometrial *fovea* was accurately explored to individuate eventual small adjacent myomas.

Surgical techniques to remove the myomas and suture the myometrium have been described previously [5, 11].

Definitive histological analyses of excised specimens were available for all patients.

Results are expressed as mean ± SD or mean and range, or percentage in some cases. For statistical analysis the *t*-test was used. Probability values of < .05 were considered significant.

Results

One hundred and thirty-three consecutive patients with clinical and TVS diagnoses of uterine myomas underwent conservative laparotomic surgery during the study period.

One hundred and ten patients comprised the study group because the TVS report and/or surgical description did not document the number of single myomas in 23 cases.

The mean age of treated patients was 37.8 years (range 25-52). The main indication for surgery was abnormal uterine bleeding in 39 cases (35.4%), pelvic pain or abdominal pressure in 52 (47.3%), and infertility in 19 (17.3%).

A total of 346 myomas were removed with a mean of 3.1 myomas for patient. Definitive histological analysis of excised specimens confirmed the diagnosis of uterine myomas in all cases. In 57 patients (51.8%) multiple myomas were excised during surgery (max 20). The size of the largest myoma ranged from 15 mm to 180 mm (mean 52 mm) (Table 1).

Table 1. — *Size, and location of removed myomas (n = 346).*

	N	%
Size (mm)		
< 20	37	10.7
20-50	199	57.5
> 50	98	28.3
Unknown	12	3.5
Site of myomas with respect to uterine body		
Fundal	139	40.1
Anterior	68	19.7
Posterior	73	21.1
Lateral	25	7.2
Isthmic	12	3.5
Unknown	29	8.4
Site of myomas with respect to uterine wall		
Intramural	240	69.4
Subserosal	58	16.7
Submucosal	27	7.8
Unknown	21	6.1

The mean latency ± SD between TVS and myomectomy was 67.8 ± 39.6 days (range: 2-184).

The number of myomas removed during surgery agreed with that detected by TVS in 63 cases (57.3%).

In four cases of multiple myomas diagnosed by TVS (4 myomas detected in 3 cases and 5 in one case), one was not found during surgery, resulting in a false sonographic finding. Only one of these, cleared by histological analysis, turned out to be adenomyosis, while the surgeon could not find any pathological aspect matching the diagnosed myoma in the other three cases.

In 43 patients (39.1%) there was at least one myoma missed by TVS. The sensitivity of TVS in diagnosing the exact number of existent myomas was 59.4% in the whole series.

Considering the subgroup of 88 patients with a TVS diagnosis of three or fewer myomas the number of the detected and removed myomas corresponded in 57 cases whereas in the remaining 31 (35.2%) there was at least one missed by TVS. The sensitivity of TVS in diagnosing the exact number of existent myomas in this cohort of patients was 64.8%.

Finally, among the 72 women diagnosed with only one myoma at preoperative TVS, 19 (26.4%) resulted to have two or more myomas during surgery, achieving a 73.6% sensitivity of TVS in diagnosis of the exact number of myomas.

The mean latency between TVS and surgery was 67.1 days (range: 2-178) in the group with the correct number of myomas evaluated by TVS and 68.1 days in the other (range: 5-184). There was no statistical difference in latency comparing the two groups (difference -1.0; C.I. 95% from -15.2 to 13.2; t = -0.140; $p = 0.889$).

Figures 1 and 2 display the mean number of myomas missed by TVS, respectively, versus the number of myomas preoperatively detected and those removed during surgery.

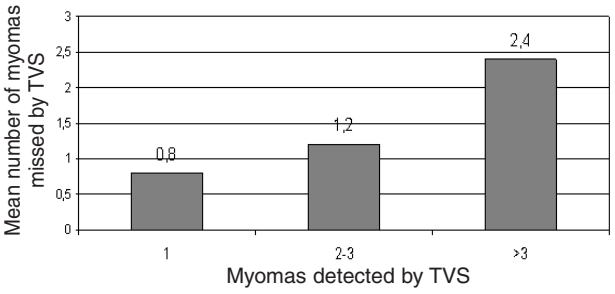

Figure 1. — Mean number of myomas missed by TVS in relation to number of myomas detected by TVS (106 patients, excluding 4 false-positives).

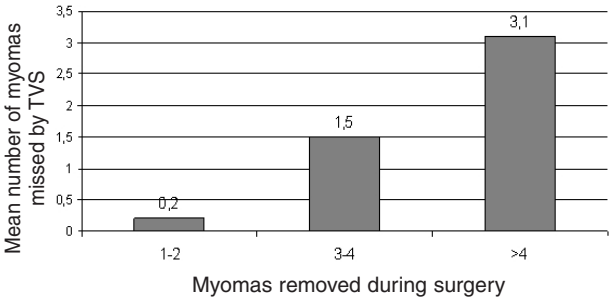

Figure 2. — Mean number of myomas missed by TVS in relation to the number of myomas removed during surgery (106 patients, excluding 4 false-positives).

Discussion

The standard treatment of uterine myomas is surgical removal. At present different therapeutic approaches already validated or under investigation are available but a preoperative diagnostic pathway has to be worked-up when considering the chosen surgical option.

Obviously when hysterectomy is planned an accurate preoperative characterisation of number, site and size of myomas is not required.

Similarly precise uterine mapping is not necessary when a patient is referred for laparotomic myomectomy since identification of all existent myomas by inspection and finger touch during surgery is possible.

In recent years new minimally invasive surgical techniques have been developed, resulting in effective conservative treatment of myomas [3-5].

Among all options, laparoscopy is considered of utmost interest since it produces minimal surgical trauma with reduced postoperative pain, fast recovery and optimal aesthetic results [12-14].

From a technical point of view laparoscopic illumination and magnification can reveal subserosal or intramural myomas able to distort the uterine profile but, conversely to open surgery, they do not allow localisation of smaller deep intramural myomas, denying direct palpation of the uterine walls.

Thus if all myomas are not documented during the preoperative work-up a considerable risk of leaving them *in situ* exists which may result in subsequent recurrence.

The above-mentioned issues outline the need for accurate preoperative identification and mapping of all myomas every time laparoscopic surgery is planned.

TVS represents the most cost-effective procedure to confirm a clinical diagnosis of myomas [6-9].

The presence or absence of uterine myomas, their number and size, their exact location relative to the endometrial cavity, and their differentiation from adenomyosis are parameters that can be accurately assessed before treatment when TVS is performed by skilled sonographists using advanced equipment. The low-cost and non-invasiveness are further recognized advantages of TVS which, for these reasons, is considered the first method of choice to preoperatively investigate uterine myomas.

At present the majority of obstetrician-gynaecologist practitioners have introduced TVS at the completion of routine clinical examination of patients [15, 16].

Unfortunately the technique remains highly operator-dependent and there is considerable variability in image quality between commercially available sonographic equipment [17, 18]. As a consequence the indication for surgical intervention is more often established on the basis of a poor-quality in-office TVS supporting a clinical examination where the number and characterisation of myomas are inaccurate [19].

The aim of the present study was to evaluate the usefulness of in-office TVS for detection of the correct number of myomas during the preoperative clinical-sonographic work-up for patients scheduled for conservative surgery.

The number of myomas was the leading sonographic variable analysed in our study as it should be the main outcome of preoperative TVS uterine mapping.

Our data confirm in-office TVS as a useful tool to confirm the existence of uterine myomas but, at the same time, describe it as often failing to correctly detect the number with a 39.1% underestimation rate in all patients.

Previous papers [20] and our results (Figures 1 and 2) revealed that the increment in number of myomas makes it hard to distinguish them singularly by TVS with a rapid decrease in the ability to detect their real number.

For this reason we intentionally focused on the subgroup of patients with three or less myomas at TVS examination which, moreover, could represent a reasonable indication for a laparoscopic approach. However, in our experience, even among this cohort of patients, TVS missed at least one myoma in more than one-third of cases and revealed a sensitivity of 64.8% in diagnosing the exact number of existent myomas.

Considering our results some aspects of the study should be interpreted as methodological bias and deserve particular considerations.

To the best of our knowledge, this is the first study in the literature that considers the number of myomas removed by laparotomic myomectomy as the standard of comparison. We suppose, in fact, that laparotomic exploration with meticulous and systematic palpation of the

uterine walls during surgery could lead to localisation and removal of all myomas, obtaining similar outcomes to authors who used pathological analysis after hysterectomy to establish the number of existent myomas.

The lack of standardisation with regard to ultrasound diagnosis of myomas could be indicated as another methodological bias in the present series. Truly, as previously outlined, we intentionally included TVS performed by several operators with different equipment to create an unselected group of patients representative of general gynaecologic practices where often a poor-quality in-office TVS supporting clinical examination represents the basis for surgical intervention.

The above-mentioned considerations along with the overlooking rate in TVS detection of myomas observed in our experience might explain some previous studies where the recurrence rate after laparoscopic myomectomy was higher compared with the laparotomic procedure. For example Doridot *et al.* [21] reported that the cumulative rate of myoma recurrence within five years appears to be greater after laparoscopy than after laparotomy. In a retrospective review of 114 laparoscopic myomectomies, Nezhat *et al.* [22] reported a 33.3% recurrence rate after an interval of 27 months and suggested that smaller intramural fibroids are hard to visualise and may be overlooked resulting in a higher recurrence rate respect to laparatomic removal.

The only randomised study in the literature comparing laparotomic and laparoscopic myomectomy showed no statistically significant differences in the recurrence rate between the two groups [23]. Given the randomised nature of the study, we believe that a single skilled or a small group of operators were entitled to perform both follow-up and preoperative work-up of patients to reduce the well noted operator-influenced bias of TVS.

In our opinion, in-office TVS supporting clinical examinations remains a reliable first-hand method to confirm clinically suspected uterine myomas and should be considered acceptable in case of planned hysterectomy or laparotomic myomectomy.

On the contrary the fact that one myoma may be overlooked in one-third of patients theoretically eligible for conservative laparoscopic surgery may motivate the implementation of US diagnosis through skilled specialists with proper equipment when laparoscopic myomectomy is considered.

In young patients with multiple myomas or large volume uteri who are scheduled for conservative advanced laparoscopic procedures, myoma mapping is mandatory and MRI should be taken into consideration since in some reports it clearly outperforms TVS [20, 24, 25]. Dueholm *et al.* [20] in a double-blinded study found that the sensitivities of MRI and TVS were equally accurate in the detection of myomas but MRI was superior in the mapping, when uterine volume exceeded 375 ml, or when the number of myomas increased.

However the elevated costs of MRI and the high prevalence of the pathology are not cost-effective for routine use in all patients scheduled for laparoscopic myomectomy.

In conclusion today the use of in-office TVS supporting clinical examination is implemented in the diagnosis of uterine myomas but it is far from being considered a reliable diagnostic tool to propose conservative laparoscopic surgery, adding poor information to clinical evaluation of uterine enlargement.

In our opinion patients scheduled for laparoscopic surgery should be preoperatively evaluated by experienced sonographists and offered counseling to clarify the technical aspects of conservative myomectomy managed by the laparoscopic route, including the possibility of recurrence.

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Catalase activity, serum trace element and heavy metal concentrations, vitamin A, vitamin D and vitamin E levels in hydatidiform mole

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Summary

Purpose of investigation: In this study we aimed to measure the activity of catalase, which is an antioxidant enzyme, the concentrations of some trace elements and heavy metals, and vitamin A, D and E levels in serum samples of patients with hydatidiform mole, normal pregnancies and healthy non pregnant women. *Methods:* Seventy-two women were enrolled in this study. Of these, 24 were healthy women in the first trimester of pregnancy (HP), 24 were healthy non-pregnant women (NP) and 24 were patients with complete hydatidiform mole (CHM). *Results:* Serum levels of catalase, Zn, Co, vitamin A, D and E were significantly lower in the CHM group when compared with the HP and NP groups ($p < 0.001$). Serum levels of Cu, Fe, and Cd were significantly higher in the CHM group when compared with the HP and NP groups ($p < 0.001$). *Conclusion:* The assessment of oxidant/antioxidant imbalance in pregnant women could be useful in the early determination of molar pregnancy and supplementation with antioxidants may be useful in the treatment of CHM, and may prevent recurrent molar pregnancy.

Key words: Catalase activity; Heavy metal; Trace element; Vitamin levels; Hydatidiform mole.

Introduction

Hydatidiform mole (HM) is a disorder of fertilization. It is characterized by a conceptus of hyperplastic trophoblastic tissue attached to the placenta [1]. Broad variations in the distribution of gestational trophoblastic disease (GTD) exist worldwide, with higher frequencies in some parts of Asia, the Middle East and Africa, with Turkey ranking in the middle of these countries [2]. The etiology of molar pregnancy is not completely understood [3]. Preeclamptic patients are exposed to increased oxidative stress [4, 5]. Placental hypersecretion of lipid peroxides or decreased placental antioxidant enzyme production may lead to endothelial dysfunction [6]. Placental abnormality and associated metabolic changes cause increased oxidative stress [7]. Similar metabolic changes are present in molar pregnancy [8]. The antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (GPx) are synthesized in the body. Catalase is an effective scavenger of aqueous peroxide radicals [9]. The maternal blood levels of vitamin C, vitamin E, vitamin A, and β-carotene which has a strong provitamin A activity are decreased [10, 11]. Vitamin D might exert subtle oxidative stress, which could stimulate the detoxification mechanisms to protect cells from the subsequent stress challenges [12]. The studies on the roles of trace elements in health and disease over the past 50 years have led to a good understanding of their mode of action and why they are essential for life [13]. Some metals are essential to maintain the metabolism of the human body, whereas they can lead to poisoning because they tend to bioaccumulate [14]. Lead (Pb) and cadmium (Cd) increase oxidative stress [15].

In this study, we aimed to measure the activity of catalase which is an antioxidant enzyme; the concentrations of some trace elements and heavy metals (Cu, Zn, Cd, Co and Fe); vitamin A (retinol), vitamin D (cholecalciferol) and vitamin E (α-tocopherol) levels in serum samples of patients with hydatidiform mole, with normal pregnancies and healthy non-pregnant women.

Material and Methods

Seventy-two women were enrolled in this study. Of these, 24 were healthy women in the first trimester of pregnancy with a single viable fetus (HP). Twenty-four healthy non-pregnant women also participated as controls (NP). The remaining 24 subjects had complete hydatidiform mole (CHM). Written informed consent was obtained from all subjects.

None of these patients were using any drugs at the time of collection of the blood samples. Venous blood samples were drawn from each patient during the 10^{th}-19^{th} week of gestation after a fasting overnight period. Serum was separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionized polyethylene tubes and kept in a deep-freeze at -20ºC (without thawing) until the study day.

Biochemical analysis of catalase activity in erythrocytes was determined according to the method defined by Aebi, in short, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol l^{-1} H_2O_2 solution prepared in potassium phosphate buffer (0.05M, pH 7.00) [16]. The change in absorbance was monitored at 240 nm over a 5-min period using a spectrophotometer (Shimadzu UV-1201, Japan). Determination of serum concentrations of Cu, Zn, Co, Cd, and Fe was performed by atomic absorption measurements, in which a

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UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK) was used. In addition, vitamin E, vitamin D and vitamin A levels were determined by a high-performance liquid chromatography (HPLC) method [17]. Data were expressed as mean ± standard error (SE) and analyzed using one-way repeated measures of analysis of variance (ANOVA). Duncan's test was used to test for differences among means for which one-way ANOVA indicated a significant ($p < 0.01$) F ratio.

Results

Demographic data of the subjects are shown in Table 1. There were no differences in mean age, gestational age, gravidity and parity between patients with CHM and HP ($p > 0.05$).

Table 1. — *Characteristics of complete hydatidiform mole (CHM), healthy pregnant (HP) and healthy non pregnant (NP) groups.*

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)	*p* value
Age (years)	28.88 ± 1.71	29.17 ± 1.11	28.83 ± 0.87	NS
Gestational age (weeks)	15.71 ± 040	15.33 ± 0.56	–	NS
Gravidity	4.46 ± 0.74	4.00 ± 0.49	–	NS
Parity	3.13 ± 0.67	2.67 ± 0.50	–	NS

NS: nonsignificant.

Serum levels of catalase, vitamin A, D and E were significantly lower in the CHM group when compared with the HP and NP groups ($p < 0.001$). The mean values of serum levels of catalase, vitamin A, vitamin D, and vitamin E are given in Table 2.

Table 2. — *Serum levels of catalase and vitamins (A, D, E) of complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)
Catalase EU/(gHb)$^{-1}$	3.01 ± 0.33^a	10.22 ± 1.12^b	11.86 ± 1.61^b
Vitamin A (mmol/l)	0.50 ± 0.04^a	0.72 ± 0.02^b	0.74 ± 0.03^b
Vitamin D (mmol/l)	0.02 ± 0.00^a	0.03 ± 0.00^b	0.03 ± 0.00^b
Vitamin E (mmol/l)	4.72 ± 0.34^a	6.26 ± 0.32^b	6.36 ± 0.20^b

Values are means ± SE; different letters represent significant differences ($p < 0.01$).

Serum levels of Cu, Fe, and Cd was significantly higher in the CHM group when compared with the HP and NP groups ($p < 0.001$). Serum levels of Zn and Co were statistically lower in the CHM group compared with the HP and NP groups ($p < 0.001$). Mean values of serum levels of Cu, Zn, Fe, Co and Cd are given in Table 3.

Table 3. — *Serum levels of Cu, Zn, Fe, Cd and Co of complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)
Cu (µg/dl)	3.49 ± 0.16^a	2.82 ± 0.09^b	1.81 ± 0.08^c
Zn (µg/dl)	1.25 ± 0.08^a	1.71 ± 0.07^b	1.88 ± 0.09^c
Fe (µg/dl)	4.48 ± 0.71^a	1.79 ± 0.32^b	1.30 ± 0.07^c
Cd (µg/dl)	0.26 ± 0.03^a	0.02 ± 0.00^b	0.03 ± 0.00^c
Co (µg/dl)	0.03 ± 0.00^a	0.26 ± 0.03^b	0.24 ± 0.04^c

Values are means ± SE; different letters represent significant differences ($p < 0.01$).

Discussion

Reactive oxygen species (ROS) are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms. In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage [18, 19]. There is increasing evidence that oxidative stress is an important contributing factor in the pathogenesis of preeclampsia [9]. Similar increasing oxidative stress is present in molar pregnancy [8]. We found that catalase was significantly lower in the CHM group when compared with the HP and NP groups. The importance of vitamin E and β-carotene in preventing free radical damage is well known and their levels have been reported to be significantly decreased in preeclampsia [11]. Maternal vitamin D deficiency may be an independent risk factor for preeclampsia. Vitamin D supplementation in early pregnancy should be explored for preventing preeclampsia and promoting neonatal well-being [12]. There is no study evaluating vitamin A, D and E levels in hydatidiform moles in the English literature. In the present study, we observed significantly lower levels of vitamin A, vitamin D and vitamin E in CHM patients when compared with the HP and NP groups. Significant changes in serum trace metal concentrations, particularly zinc and copper, have been documented during normal pregnancies. Harma *et al.* reported that levels of zinc in serum were found to be significantly higher and levels of copper in serum were significantly lower in hydatidiform mole patients than controls [20]. On the contrary, Wang *et al.* reported that the contents of Cu in erythrocytes were found to be significantly higher and the contents of Zn significantly lower in patients with hydatidiform mole than those in normal females [21]. In our study, we found that zinc was significantly lower and copper was significantly higher in the CHM group when compared with the HP and NP groups.

Some metals are essential to maintain the metabolism of the human body, whereas they can lead to poisoning because they tend to bioaccumulate [14]. The Industrial Revolution, which increased opportunities of occupational and environmental exposure to metals among women, revealed their adverse effects on pregnancy [22]. Lead and cadmium are established toxic and carcinogenic metals [23]. Lead and cadmium increase oxidative stress [15]. Iron is a redox-active transition metal and can participate in single electron reactions and catalyse formation of free radicals, including the undesirable hydroxyl radicals [24, 25]. There is no study evaluating heavy metal levels in hydatidiform moles in the literature. Serdar *et al.* observed a significant increase in serum iron levels in mild and severe preeclampsia patients when compared to normotensive healthy women [26]. In our study we found that cadmium and iron were significantly higher and cobalt was significantly lower in the CHM group when compared with the HP and NP groups.

The assessment of oxidant/antioxidant imbalance in pregnant women could be useful in the early determination of molar pregnancy. Supplementation with antioxidants in pregnant women with low antioxidant status may

be useful in the treatment of CHM and may prevent recurrent molar pregnancy. However, more studies are needed to verify and clarify the relationship between antioxidant levels and heavy metal levels and hydatidiform mole.

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The contribution of anaesthesia modus on reducing blood loss during caesarean section

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Summary

The purpose of this study was to compare the effects of general anaesthesia (GA) and regional anaesthesia (RA) on the blood loss during caesarean section. We report on 161 patients undergoing both elective and emergency caesarean section at the Obstetrical Department of Democritus University of Thrace. In the majority (113 women, 70.2%) GA was used, while in 48 women (29.8%) RA was used. No significant differences were found in the demographic characteristics of the study women in the two groups. Although the preoperative Hgb and Hct levels did not differ significantly in the two groups RA vs GA, the postoperative Hgb and Hct levels were significantly lower in women who were subjected to GA compared to those who were subjected to RA ($p < 0.05$). The study showed that there is greater reduction in blood loss with RA compared to GA during caesarean section.

Key words: Regional anaesthesia; General anaesthesia; Caesarean section; Blood loss.

Introduction

The World Health Organisation estimates that the rate of caesarean section is between 10% and 15% of all births in developed countries compared to 22% (2003) in the United Kingdom, 23% in the United States and 21% in Canada [1, 2]. Anaesthetic practice for caesarean section has changed during the last decades. There is a world-wide shift in favour of regional anaesthesia (RA) [3]. A survey conducted in 2002 revealed that the rate of RA for elective caesarean section had increased to 73.5% from 39% six years previously [4]. General anaesthesia (GA) was considered the technique of choice because it was quick, not because it was thought to be particularly good. GA was the only real choice for emergency sections indicated by foetal distress, and most women undergoing elective caesarean section preferred the idea of being asleep [5]. Spina anaesthesia (SA) and epidural anaesthesia (EA) techniques have been found to provide effective anaesthesia for caesarean section. Both techniques allow the mother to be awake, minimise maternal aspiration problems and significantly prevent neonatal depression associated with GA [6]. However in contrast to the RA, GA has the advantage of less hypotension, less cardiovascular instability, and better control of the airway and ventilation [6]. Although both GA and neuraxial anaesthesia (SA or EA) are generally equally efficacious for intraoperative anaesthesia, some data have indicated that the use of neuraxial (SA or EA) might be of benefit in reducing intraoperative blood loss, which could theoretically lead to a reduction in blood transfusions and associated complications [7-10]. The purpose of this survey was to evaluate the possible consequences of different anaesthetic techniques in caesarean sections concerning intraoperative blood loss.

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Method

This study was conducted on 161 pregnant women who underwent elective or uncomplicated caesarean section between January 1, 2004 and December 31, 2007. All pregnant cases were uncomplicated singleton, cephalic, no breech, term pregnancies estimated to be equal to 37 weeks of gestation. Pregnancies with serious obstetric or medical complications or foetal malformations were excluded.

The majority of caesarean sections were elective, while the rest had indicative pathology such as prolonged labour, beginning of foetal distress, and placenta position anomalies. Information collected included the number of blood units crossmatched preoperatively, type of surgery (emergency or elective), type of anaesthesia, parity of the patient, estimated blood loss by both anaesthesists and obstetricians, intraoperative and postoperative transfusion within 48 hours and pre- and postoperative haemoglobin (Hgb) and haemocrit (Hct). Intraoperative or postoperative salvaged red blood cell (RBC) transfusion appropriateness was determined using the recommended guideline of transfusing RBCs if the hemoglobin is < 7 g/dl in a patient with continuous bleeding. Efforts should be made to reduce the blood transfusion without increasing maternal morbidity and mortality. Maternal request for GA and/or refusal of regional anaesthesia was the main reason for GA. GA was also performed when intraoperative haemorrhage and/or prolonged surgery was anticipated. The immediate foetal and neonatal effects were assessed by cord blood gas analysis and the infant's Apgar scores. Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS, Inc., Chicago, IL, USA). The normality of quantitative variables was tested with the Kolmogorov-Smirnov test. Normally distributed quantitative variables were expressed as the mean ± standard deviation, while non-normally distributed variables were expressed as the median and range. Qualitative variables were expressed as frequencies and percentages. The Student's t-test and Mann-Whitney U-test were used to determine differences in demographic, clinical and laboratory characteristics between the two groups of women according to the type of anaesthesia. The chi-square test was used to evaluate any potential association between qualitative variables. To

assess the independent effect of the type of anaesthesia on with the change of Hgb and Hct levels and the presence of high reductions of Hgb and Hct levels, multivariate stepwise linear and logistic regression models were constructed, respectively. Womens's age, smoking status, presence of diabetes, history of abdominal surgery, ASA, emergency, eating and the duration of surgery and anaesthesia were the major confounders in the multivariate models. Beta regression coefficients and adjusted odd ratios (aOR) were estimated as the measure of association of the type of anaesthesia with the change of Hgb and Hct levels and the presence of high reductions of Hgb and Hct levels, respectively. Two-way mixed ANOVA was performed to assess the interaction between the two types of anaesthesia and the change of Hgb and Hct levels over time. All tests were two-tailed and statistical significance was considered for p values less than 0.05.

Results

The study population was comprised of 161 women who underwent caesarean section (CS), with a mean age of 29.19 ± 5.24 years (range, 15-37 years); the majority (113 women, 70.2%) were subjected to GA while 48 women (29.8%) were subjected to RA. The demographic and clinical characteristics of women according to the kind of anaesthesia used are compared in Table 1. There were no significant differences in age, smoking status, incidence of diabetes, previous abdominal surgery, American Society of Anesthesiology (ASA) status, emergency status, and the duration of surgery and anaesthesia between the two groups of women. On the contrary, preoperative (4-6 hours before surgery) eating was more frequent in women who were subjected to GA ($p < 0.001$).

Both, Hgb and Hct levels were statistically significantly reduced after surgery for 12.2% and 10.0%, respectively, among women who were subjected to RA ($p < 0.001$), and for 17.2% and 15.2%, respectively, among those who were subjected to GA ($p < 0.001$) (Table 1). Women who were subjected to RA exhibited significantly lower blood loss compared to GA, since the two-way mixed analysis of variance showed a statistically significant interaction between the kind of anaesthesia and the change of Hgb and Hct levels over time ($F_{1,159} = 10.562$, $p < 0.001$ and $F_{1,159} = 10.380$, $p < 0.005$, respectively). While the preoperative Hgb and Hct levels did not differ significantly between women who were subjected to regional and general anaesthesia, the postoperative Hgb and Hct levels were significantly lower in women who underwent RA compared to those who underwent GA ($p < 0.05$) (Figure 1). To assess the effect of the kind of anaesthesia on blood loss, we also performed multivariate stepwise linear regression analysis on the change of Hgb and Hct levels, including all other women's characteristics as possible confounders. Our results suggested that the use of GA was a significantly independent determinant of greater postoperative reduction of Hgb and Hct levels (unstandardised beta coefficient = –0.70, $p < 0.001$ for Hgb; beta = –1.76, $p < 0.01$ for Hct), explaining a large proportion of their variance (6.2% and 6.1%, respectively). Furthermore, emergency status showed significant independent associations with increased blood loss (beta = –0.10, $p < 0.001$ for Hgb; beta = –3.41, $p < 0.001$ for Hct), explaining 7.0% and 6.6% of the variance of the Hgb and Hct changes, respectively. Women's age also showed significantly independent but clearly weaker associations with the change of Hgb and Hct levels (beta = –0.04, $p < 0.05$ for Hgb; beta = –0.10, $p < 0.05$ for Hct), explaining another 2.8% and 2.0% of their variance, respectively, while eating was also associated with greater postoperative reduction of Hct levels (beta = –1.23, $p < 0.05$), explaining a 2.2% of their variance.

Table 1. — *Demographic and clinical characteristics of the pregnant women.*

	Regional anaesthesia N = 48	General anaesthesia N = 113	*p* value
Age (years)	29.08 ± 5.18	29.24 ± 5.24	n.s.
Smoking status			n.s.
Non-smokers	26 (54.2)	69 (61.0)	
Occasional smokers	19 (39.6)	35 (31.0)	
Daily smokers	3 (6.2)	9 (8.0)	
Diabetes	3 (6.3)	3 (2.7)	n.s.
Previous abdominal surgery	9 (18.8)	34 (30.1)	n.s.
Preoperative eating	24 (50.0)	92 (81.4)	< 0.001
ASA status	1 (1-4)	1 (1-4)	n.s.
Emergency status	6 (12.5)	9 (8.0)	n.s.
Duration of surgery (min)	45 (30-80)	50 (30-130)	n.s.
Duration of anesthesia (min)	65 (40-100)	65 (35-150)	n.s.
Hgb (before)	12.30 ± 1.41	12.50 ± 1.23	n.s.
Hct (after)	10.80 ± 1.18	10.35 ± 1.43	< 0.05
Hgb (before)	36.93 ± 3.54	37.61 ± 3.48	n.s.
Hct (after)	33.22 ± 2.91	31.90 ± 3.27	< 0.05

Data are expressed as means ± standard deviation for normally distributed quantitative variables and as medians and range for non-normally distributed quantitative variables. Qualitative variables are expressed as frequencies and percentages; n.s.: non significant.

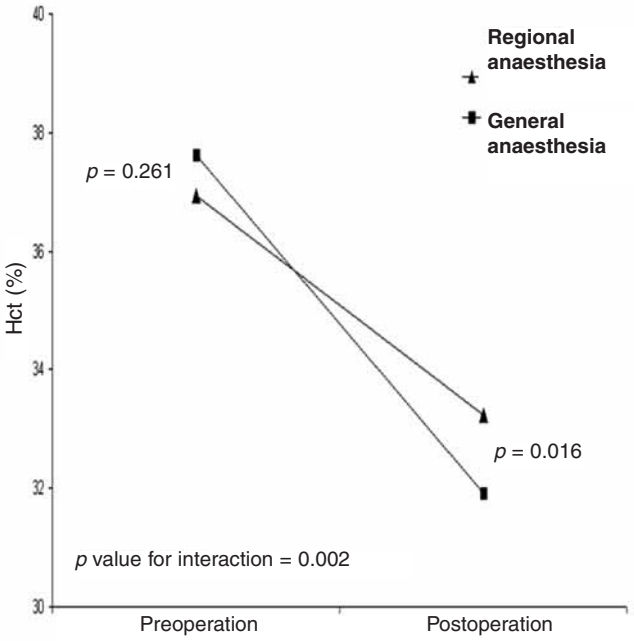

Figure 1. — Effect of the kind of anaesthesia on blood loss to.

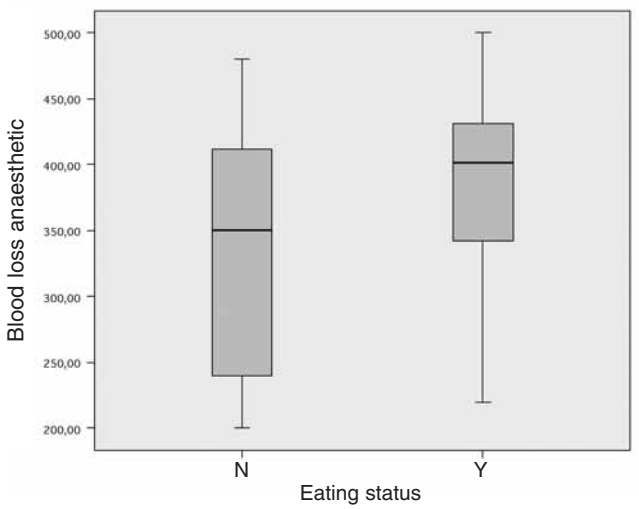

Figure 2. — Prior to operation effect of eating status on blood loss.

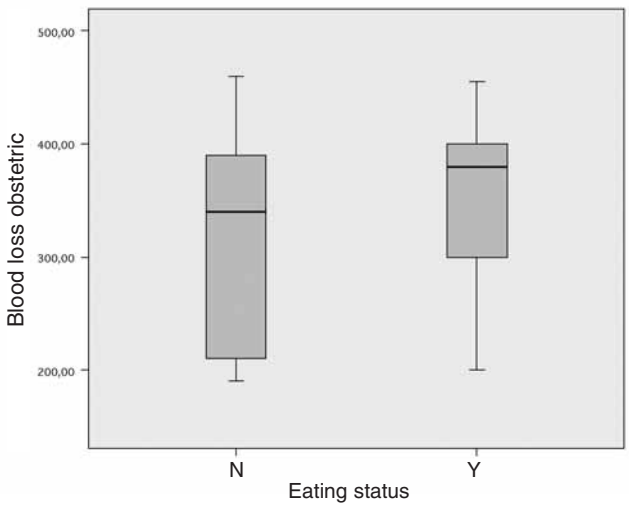

Figure 3. — Prior to operation effect of eating status on blood loss.

Among the entire cohort, the change in Hgb levels ranged from 0.00 to –6.70 with a median value of –1.80 and the change of Hct levels ranged from 0.40 to –23.00 with a median value of –4.10. In the sequence, the median value of the Hgb and Hct changes were selected as the cut-off points to subdivide women into groups with low reduction of Hgb or Hct levels and women with a high reduction of Hgb or Hct levels. High reduction levels were significantly more frequent among women who were subjected to GA compared to those who where subjected to RA (58.4% vs 27.1%, $p < 0.001$ for Hgb; 58.4% vs 31.3%, $p < 0.005$). Multivariate logistic regression analyses were performed separately to evaluate the role of potential confounders on the relationship observed between the type of anaesthesia and the reduction of Hgb or Hct levels. The use of GA remained a strong independent risk factor for high reduction of both levels (aOR = 3.93, 95% CI = 1.85 to 8.35, $p < 0.001$ for Hgb; aOR = 2.93, 95% CI = 1.33 to 6.41, $p < 0.01$ for Hct).

Blood loss at elective or emergency lower segment caesarean section was usually less than 500 ml and was estimated with reasonable accuracy. The mean blood loss was estimated to be more by anaesthetists compared to obstetricians. The mean measured blood loss estimated by obstetricians was 336.7764 ml (SE: 6.33186, range 270 min: 190, max: 460 ml) and by anaesthetists it was 366.9130 ml (SE: 6.38180, range 300 min: 200, max: 500 ml). A small number (6.5%) were transfused intraoperatively and 3.5% postoperatively. Our study shows that intraoperative blood loss was strongly related to whether patients had eaten or not prior to their surgery.

Women who ate prior to the caesarean section experienced larger amounts of blood loss (Figures 2 and 3).

Significant risk factors for blood transfusion were intraoperative during caesarean section and postopearative blood loss and some indications for caesarean section included: prolonged cephalic presentation, cephalic presentation anomalies, placenta position anomalies, previous caesarean section and foetal distress.

We did not observe any intra-or postoperative anaesthesia complications irregardless of anaesthesia mode. Especially in cases of RA no maternal hypotension was noted. Infants whose mothers received GA compared to RA had similar birthweights (median 3650, range 1950, min 2590, max 4500) but lower Apgar scores at one and five minutes (Table 2). By applying Spearman's correlation

Table 2. — *Correlations between kind of anaesthesia and neonatal results.*

			Birthweight	Apgar score	Kind of anaesthesia	Respiratory problems
Spearman's rho	Birthweight	Correlation Coefficient	1.000	–.034	.066	–.230(**)
		Sig. (2-tailed)		.666	.409	.003
		N	161	161	161	161
	Apgar score	Correlation Coefficient	–.034	1.000	–.820(**)	.021
		Sig. (2-tailed)	.666	–	.000	.789
		N	161	161	161	161
	Kind of anaesthesia	Correlation Coefficient	.066	–.820(**)	1.000	–.058
		Sig. (2-tailed)	.409	.000	–	.462
		N	161	161	161	161
	Respiratory problems	Correlation Coefficient	-.230(**)	.021	-.058	1.000
		Sig. (2-tailed)	.003	.789	.462	–
		N	161	161	161	161

** Correlation is significant at the 0.01 level (2-tailed).

coefficient test we can conclude that there was a statistically significant negative correlation between the kind of anaesthesia (RA vs GA) used and the Apgar score (r = –0.82 with $p < 0.001$) (Table 2). Mothers who received RA delivered newborns with higher Apgar scores. Statistical analysis was performed with SPSS 15. Variables were checked as to whether they were normally distributed. Quantitative variables (birthweight) were expressed with median value and range, while qualitative variables with frequency. The Mann-Whitney test was used to calculate whether there was a difference in birthweight in relation to the kind of anaesthesia and with the presentation of respiratory problems. Spearman's correlation coefficient test was used to prove the existence of correlations.

Discussion

The decision to proceed with general or regional anaesthesia depended on the underlying reason for caesarean section. While GA was administered in foetal distress and in cases where vaginal delivery was not feasible and turned into an emergency caesarean section, regional anaesthesia was preferred for elective or uncomplicated obstetric cases or when maternal disease existed. Neonatal outcome was assessed using Apgar scores and need for respiratory assistance at birth. Apgar score is a subjective measurement and its diagnostic value in foetal asphyxia is not significant [11]. Maternal outcome was assessed using the difference between pre- and postoperative packed cell volumes (PCV), need for blood transfusion and estimated blood loss [12]. Anaesthesia mode for caesarean section does not influence short-term outcomes in neonates although differences in the acid-base status of both the mother and especially the newborn recommend careful use of spinal anaesthesia [13]. Other authors have included GA as a risk factor for poorer immediate neonatal outcomes. Kolatat *et al.* reported that the Apgar scores of infants whose mothers received GA were lower than for infants whose mothers received RA [14, 15]. In contrast to these authors, Mueller *et al.* found that maternal arterial hypotension is by far the most common problem during RA and may be responsible for the higher incidence of foetal acidemia as hypotension has a deleterious effect on uteroplacental blood flow [16]. The complication of maternal hypotension occurs in 30% to 60% of cases, while significant foetal rate abnormalities and acidemia occur with a 15% to 20% reduction in maternal systolic blood pressure [17-19]. The results of our study do not confirm these findings although our cohort of women was not large enough to draw definite conclusions about neonatal respiratory morbidity. We reached the same conclusions with previous studies suggesting that the anaesthesia mode does not influence the short-term outcome [20]. To prevent maternal arterial hypotension during surgery, a 500 ml/IV infusion of Ringer's lactate solution was administered to all study women before the caesarean section and 1500-2000 ml during surgery. According to our results we found statistically significant differences in estimated intraoperative blood loss in correlation with the anaesthesia mode. The effect of RA is generally associated with a decrease in intraoperative EBL, which is clinically meaningful. The theoretical benefits of decreased intraoperative blood loss might include a lower incidence of blood transfusion, which is associated with possibly increased costs and many potential risks [21, 22]. However, RA introduced new problems, such as delays in inducing anaesthesia in emergency situations, postoperative immobility and urinary retention [23]. The increase in anaesthetic choices has led to inconsistencies in practice between individual anaesthetists, and between regions and nations [24-28]. Many hospitals surveyed were unable to provide basic data as they do not have these in retrievable form. An international consensus discussion and recommendations as well as comparable European instruments of quality control in obstetric anaesthesia are desirable. The trend from general to regional anaesthesia for caesarean section is continued, as is the trend from local infiltrative techniques to epidural anaesthesia for vaginal delivery. Switzerland was in the forefront for these developments [29]. Compared to our data from 1998, anaesthetic practice for caesarean section has changed with an increase in RA. This study confirms that the current practice of RA for caesarean section at Democritus University Hospital is good, but further studies need to be done to assess other outcome variables.

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Lipid peroxidation and antioxidant activity in complicated pregnancies

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Summary

Aims and Objectives: Preeclampsia and eclampsia are pregnancy complications with serious consequences for mother and infant. Uncontrolled lipid peroxidation may play an important role in the pathophysiology of preeclampsia and eclampsia by causing vascular endothelial cell dysfunction. Antioxidants serve to control lipid peroxidation. We attempted to ascertain whether antioxidant protective mechanisms are diminished in women with preeclampsia and eclampsia. *Materials and Methods:* Lipid peroxidation and antioxidant markers were assayed in 25 healthy non-pregnant women as a control group, 25 third trimester normal pregnant women, 25 preeclamptic and 25 eclamptic patients of the same trimester by standard spectrophotometer methods. *Results:* In preeclampsia and eclampsia malondialdehyde, a product of lipid peroxidation, was significantly increased while enzymatic antioxidants like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were significantly reduced as compared to normal pregnant women and non-pregnant controls. *Conclusion:* Lipid peroxidation is an important factor in the pathogenesis of preeclampsia and eclampsia. The decrease in antioxidants is probably due to a compensatory nature responding to the increased lipid peroxide load in preeclamptic and eclamptic patients and may indicate the severity of the disease.

Key words: Lipid peroxidation; Malondialdehyde; Preeclampsia; Eclampsia.

Introduction

Preeclampsia and eclampsia are leading causes of feto-maternal morbidity and mortality, accounting for more than 40% of iatrogenic premature deliveries. In developing countries they account for 10-15% of maternal deaths [1, 2]. Increased risk of death for women at 20-32 weeks of gestation is higher than for those at 36-40 weeks. Worldwide, preeclampsia and eclampsia contribute to the death of a pregnant woman every three minutes (200,000 maternal deaths worldwide per year) and have a significant implication for the ongoing health of both mother and infant [3]. The age-specific mortality ratios for preeclampsia and eclampsia reflect the trends observed in other studies - slight risks for younger women (under the age of 20 years) and markedly increased risks for older women [4]. Women who develop preeclampsia and eclampsia during pregnancy are at an increased risk of abruptio placentae, acute renal failure, cerebrovascular and cardiovascular complications, and maternal death [5]. Vascular endothelial cell dysfunction may be caused by uncontrolled lipid peroxidation [6]. Lipid peroxidation is an oxidative process that normally occurs at low levels in all cells and tissues. Under normal conditions, a variety of antioxidant mechanisms serve to control this peroxidative process [7]. However, a diminution of normal antioxidant function will allow increased peroxidative activity to occur at the expense of oxygen and polyunsaturated fatty acids. In disease states such as toxemia in pregnancy, an imbalance between lipid peroxidation and antioxidant mechanisms could impair normal endothelial function. Sera lipid peroxidation products are increased in pregnant women and this increase is further augmented in toxemic patients with decreased antioxidant levels [8].

In this study, we investigated whether the normal balance between lipid peroxidation and antioxidant activity observed during normal pregnancies is impaired in preeclamptic and eclamptic pregnancies. To test this hypothesis, we measured antioxidant activity levels relative to lipid peroxide levels in normal pregnant, preeclamptic, eclamptic and non-pregnant healthy controls and compared results in each group.

Materials and Methods

The present study was carried out jointly by the Department of Biochemistry and Obstetrics and Gynecology from July 2000 to June 2004. The study was approved by the ethical committee of J.N. Medical College and District Civil hospital of Belgaum. Informed written consent was given by all subjects. The study included 100 cases: 25 normal healthy controls, 25 normal healthy pregnant women in the third trimester, 25 third trimester preeclamptic patients and 25 eclamptic patients in the same trimester. The subjects selected for the present study were attending and/or admitted to the District Civil Hospital. Age ranged from 20-40 years. The diagnosis of preeclampsia was based on the definition of ACOG [8]: 1) Systolic blood pressure greater than 140 mm Hg or a rise of at least 30 mm Hg or 2) diastolic blood pressure greater than 90 mm Hg or a rise of at least 15 mm Hg (occurring on two occasions at least 6 hours apart), and 3) proteinuria of 300 mg or greater in a 24-hour urine collection or protein concentration of 1 g/l (on two occasions at least 6 hours apart). Eclampsia was defined as the occurrence of seizures in women with preeclampsia. Women with normal pregnancies were normotensive throughout the gestation and had no proteinuria. Preeclamptic and eclamptic

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Table 1. — *Malondialdehyde (MDA) enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and catalase) levels in non-pregnant women, 3rd trimester normal pregnant women and 3rd trimester toxemic (preeclamptic and eclamptic) patients.*

	MDA n mol/ml	SOD IU g/Hb	GSH-Px IU g/Hb	GSH-Rx IU g/Hb	Catalase IU g/Hb
Non-pregnant women (n = 25)	1.19 ± 0.09	683.90 ± 155.25	31.08 ± 4.45	10.52 ± 4.67	8.13 ± 2.21
3rd trimester normal pregnant women (n = 25)	1.79 ± 0.14	542.64 ± 142.86	23.45 ± 4.79	7.78 ± 3.40	6.20 ± 1.69
3rd trimester preeclamptic patients (n = 25)	2.93 ± 0.54	452.07 ± 106.05	18.58 ± 4.46	6.86 ± 2.33	5.07 ± 1.31
3rd trimester eclamptic patients (n = 25)	4.80 ± 0.61	397.82 ± 108.99	14.55 ± 3.67	5.47 ± 2.41	3.99 ± 1.22
F-value	347.85	25.22	63.75	9.95	27.45
p value	0.0005	0.0005	0.0005	0.0005	0.0005

patients did not receive any antihypertensive medication until the study samples were taken. Blood pressure and proteinuria levels were determined at the time of sampling.

The subjects were of low socio-economic status based on income. Women were excluded if they were obese, had diabetes mellitus under medication or untreated diabetes, suffered from alcoholism, severe anemia (< 6.0 g% of Hb) or any other systemic disorders.

Blood samples (5 ml) were drawn by venipuncture and collected in heparinized tubes. Malondialdehyde, a product of lipid peroxide detectable in blood, was used as an indicator of lipid peroxidation. Malondialdehyde concentrations were determined by using thiobarbituric acid [10]. Hemolysate was prepared to determine antioxidant acivities like superoxide dismutase [10], glutathione peroxidase, glutathione reductase and catalase [11], and hemoglobin [11-13].

Statistical data were expressed as mean ± SD and statistical significance was determined by ANOVA and the Bonferroni multiple comparison test.

Results

The characteristics of the non-pregnant and pregnant women and preeclamptic and eclamptic patients are shown in Table 1. Statistically significant increased levels of circulating malondialdehyde was observed in the third trimester normal pregnant women, preeclamptic and eclamptic patients as compared to non-pregnant controls. Preeclamptic and eclamptic patients had further increases when compared to normal pregnant women. Activation of PGH synthase can generate oxygen radicals that could act on lipids to generate increased lipid peroxides in normal pregnant women, which would be aggravated during pregnancy-induced hypertension [14].

Antioxidants oppose the toxic actions of lipid peroxides and oxygen free radicals by limiting the amount of lipid peroxides that are formed. Enzymatic antioxidants like superoxide dismutase, glutathione perxidase, glutathione reductase and catalase significantly differed in each group by analytical variance.

Discussion

In the present study oxidative stress was evaluated in normal pregnant patients, and preeclamptic and eclamptic patients by analyzing pro-oxidant and enzymatic antioxidants. Lipid peroxidation was considered as a marker for pro-oxidant, whereas superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were considered as enzymatic antioxidants. Free radicals are difficult to measure directly due to their unstable and transient nature. Their tendency to cause lipid peroxidation has been used as an indirect measurement [15].

Markers of lipid peroxidation (MDA) are increased during the progression of normal pregnancies and further aggravated in pregnancy-induced hypertension patients [15].

Consistent with previous reports [16] we also noted the significant increase in MDA levels in the third trimester of normal pregnancies compared to nonpregnant women, and further increases were observed in preeclamptic and eclamptic patients when compared to normal pregnant women as well as non-pregnant controls. Our findings clearly indicate that lipid peroxidation may be an important factor in the pathogenesis of preeclampsia and eclampsia.

The protective antioxidant mechanisms are complex and multifactorial. The susceptibility of cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and antioxidant defense capability. Previous studies have demonstrated that superoxide dismutase activity was reduced in the gestation period of normal pregnancies and was lowest in pregnancy-induced hypertension with proteinuria. This could be due to reduced enzyme activity and production of enzyme inactivation by lipid peroxides [17, 18]. However in this study, significant decreased activity of superoxide dismutase and catalase was found in normal pregnant women compared to non-pregnant controls. Further decreases were observed in preeclamptic and eclamptic patients compared to normal pregnant and non-pregnant women.

A study by Pathak *et al.* [19] demonstrated a progressive fall in the superoxide dismutase and glutathione peroxidase activity in normal pregnancies. Decreased activity of glutathione peroxidase and significantly increased levels of malondialdehyde were observed in women with preeclampasia compared to women with normal pregnancies. Glutathione peroxidase is one of the primary antioxidants present in tissues and it inactivates lipid peroxides thereby limiting their levels. However in this study significant decreased activity was seen in toxemic patients when compared to normal pregnant and non-pregnant controls.

Increased levels of lipid peroxidation and decreased

activity of antioxidants in women with preeclampsia and eclampsia, as compared to normal and non-pregnant women, indicates that imbalance in oxidant and antioxidant systems may be impaired. This impairment could result in vascular endothelial dysfunction. The primary reaction sites of lipid peroxidation involve membrane-associated molecules. Polyunsaturated fatty acids and cholesterol alters the structural and functional integrity of biological membranes [20], and can affect normal vascular endothelial cell activity. Rodgers *et al.* [21] reported that preeclamptic sera contain cytotoxic factors that damage endothelial cells; their study results correlate with our data.

It is evident from our study that oxidative stress in preeclampsia and eclampsia leads to decreased activity of antioxidants. Supplementation of natural antioxidants like vitamin E may be of some benefit in the prevention of impending complications like preeclampsia and eclampsia.

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The presence of Helicobacter pylori in cervical preinvasive lesions

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Summary

Purpose: Helicobacter pylori (H. pylori) is believed to play a role in several gynecological and obstetric pathologies since the cervical mucosa resembles the gastric environment. The microorganism is expected to infect the upper genital tract via the oral-genital and fecal-genital routes. *Methods:* We studied 35 cases with benign, ASCUS, ASC-H, LSIL and HSIL pap-smear results. The presence of *H. pylori* in the uterine cervix and active infection were investigated with the H. pylori stool antigen test. Biopsy specimens were stained with hematoxylin-eosin and Warthin-Starry stains to find *H. pylori* in cervical tissue. Seroprevalence was investigated by using ELISA for *H. pylori* IgG and IgA. *Results:* The *H. pylori* seroprevalence was 65.7%; further, 17.1% of the cases had an active infection. *H. pylori* was not found in the cervix or the cervicovaginal secretions. *Conclusion:* The cervix is not a reservoir for *H. pylori*, and the microorganism does not appear to be transmitted through the fecal-genital route.

Key words: Helicobacter pylori; Cervix.

Introduction

Helicobacter pylori (H. pylori) infects more than half the human population all over the world [1]. Its prevalence differs according to socioeconomic status, geography, ethnicity, age, and hygienic precautions [2, 3]. In developing countries the rate of infection among children under eight years of age is 10% per year and 70-90% of adults are already infected with the bacteria. Population studies in Turkey revealed that *H. pylori* seropositivity is 60% among the age group of six months to two years and 86% among the two to five year-old age group [4].

H. pylori is detected within the gastric fluid, saliva, feces, dental plaques, and nails [5-9]. Animals, food, and water are the other sources of infection [10]. Bacteria are transmitted through the oral-oral and iatrogenic (e.g., endoscopy, laryngoscopy) routes and due to environmental factors [11, 12].

Gürbüz *et al.* observed 90% *H. pylori* positivity in dental plaque and 86% positivity in the stomachs of chronic dyspepsia patients [13]. The persistence of the bacteria in dental plaque following the eradication from the stomach was an important finding of this study.

H. pylori seropositivity at an early age is one of the major factors in the etiopathogenesis of peptic ulcers, gastric adenocarcinoma, and gastric primary B-cell lymphoma [14-17]. The Eurogast study has revealed a correlation between the prevalence of *H. pylori* seropositivity and the incidence and mortality of gastric cancer [14].

H. pylori locomotes with its flagella and can pass through the mucosa to colonize under this layer. This new environment with a high pH level protects the microorganism from the bactericidal effect of gastric acid. High amounts of urease produced by the bacteria lead to bicarbonate and ammonium accumulation, which facilitate the colonization by further increasing the pH of the environment.

The female genital tract has been investigated for the presence of *H. pylori* a few times previously. Because it has structural similarities to the stomach, we aimed to investigate the presence of *H. pylori* in cervical preinvasive lesions in this study.

Materials and Methods

This study was performed at the Uludag University Medical Faculty, Department of Obstetrics and Gynecology, and approved by the ethical committee of the university. Informed written consent was obtained from the patients. Thirty-five patients with indications for colposcopy were included in the study. The presence of *H. pylori* in the cervix was investigated by brush cytology and histopathology. The *H. pylori* stool antigen test (Premier Platinum HpSA; Meridian, Bioscience, USA) was used to determine the presence of active infection, and the standardized test was used to investigate the presence of bacteria in cervicovaginal secretions for possible fecal transmission. Serum *H. pylori* IgA EIA and IgG EIA (IBL, Hamburg) antibodies were measured by ELISA to determine the status of bacterial infection.

Colposcopy was performed for all patients and biopsy specimens were taken from suspicious areas. Hematoxylin-eosin and Warthin-Starry stains were used for histopathological diagnosis and *H. pylori* detection by 100× immersion microscopy. Endocervical samples were obtained with a brush, and smears were prepared on two separate slides for examination with the same stains.

Statistical analysis was performed by using the Statistics Package for Social Sciences (SPSS 13.0), and $p \leq 0.05$ was accepted as significant.

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Results

The mean age of the patients was 36.54 ± 8.73 years (± SD), and the mean age of marriage was 20.17 ± 2.45 years (± SD). The mean gravida was 2.05 ± 1.16 (± SD) and the mean parity was 1.57 ± 0.91 (± SD).

Twenty-four (66.5%) of the patients were using the following contraceptive methods: 55% were using coitus interruptus; 33%, condoms; 9%, oral contraceptives; 4%, tubal ligation; and 4%, intrauterine devices.

Regarding the cervical smears, 23% were benign; 46%, atypical cells of undetermined significance (ASCUS); 8%, high-grade ASC (ASC-H); 14%, low-grade squamous intraepithelial lesion (LSIL); and 9%, high-grade (H) SIL. Of the histopathological specimens, 23 (66%) were chronic cervicitis; two (6%), cervical intraepithelial neoplasia (CIN) 1; two (6%), CIN 2; two (6%), CIN 2-3; one (3%), invasive squamous carcinoma; and five (13%) had no abnormality.

The distribution of the histopathological results according to the cytology results is shown in Table 1.

Table 1. — *Cytological and histopathological results of the patients.*

Cytology	Histopathology	N	%
Benign	No abnormality	3	37.5
(n = 8)	Chronic cervicitis	5	62.5
ASCUS	No abnormality	2	12.5
(n = 16)	Chronic cervicitis	12	75
	CIN 1	1	6.2
	CIN 2	1	6.2
ASC-H	Chronic cervicitis	2	66.7
(n = 3)	Invasive carcinoma	1	33.3
LSIL	Chronic cervicitis	4	80
(n = 5)	CIN 2	1	20
HSIL	CIN 1	1	33.3
(n = 3)	CIN 2–3	2	66.7

Patients with histopathological diagnoses of CIN 1, CIN 2, and CIN 2-3 were treated with the loop electrosurgical excision procedure (LEEP), and one with the histopathological diagnosis of invasive carcinoma underwent radical hysterectomy. One of the CIN 1 patients who underwent LEEP had the postoperative diagnosis of *in situ* carcinoma with positive margins. She subsequently underwent cold-knife conization, and she was pronounced surgical-margin free. Two other patients who underwent LEEP with the histopathological diagnosis of CIN 2-3 had the same postoperative diagnosis; however, they too needed cold-knife conization to achieve surgical margins free of disease. Three patients were treated with cryotherapy.

The overall *H. pylori* seroprevalence was 65.7%; further, 17.1% of the patients were found to have active infection (Table 2).

Pearson's chi-square test revealed a significant association between the presence of active infection and the IgA antibodies ($p = 0.005$) (Table 3).

H. pylori could not be detected within the cervical smears, histopathological samples, endocervical mucosa, or the cervicovaginal secretions.

Table 2. — *Helicobacter pylori serum antibody and stool antigen test results of the patients.*

Test	N	%
Helicobacter pylori serum		
IgG (+)	23	65.7
IgG (–)	12	34.3
Helicobacter pylori serum		
IgA (+)	12	34.3
IgA (–)	23	65.7
Helicobacter pylori stool		
Antigen (+)	6	17.1
Antigen (–)	29	82.9

Table 3. — *Helicobacter pylori serum IgA and stool antigen test results of the patients.*

	Serum H. pylori IgA (+)	Serum H. pylori IgA (-)	Total
H. pylori stool antigen test (+)	5	1	6
H. pylori stool antigen test (–)	7	22	29
Total	12	23	35

Discussion

In developing countries, *H. pylori* is one of the most common infections. The cervix has a structure similar to that of the stomach with respect to mucinous columnar cells and the surrounding acidic environment. Particularly, the ectopic endocervical columnar epithelium may be a perfect harbor for *H. pylori*.

Another common microorganism, the human papilloma virus (HPV), is known to be transmitted through oral sex and causes esophageal neoplasia and nasopharyngeal papillomatosis [18]. Similar to HPV, *H. pylori* can infect the cervix during oral or anal sex or viral shedding during defecation.

Most of the studies regarding the sexual transmission of *H. pylori* are concentrated on oral-to-oral transmission among homosexual males. Aceti *et al.* has demonstrated that the sexual behavior of homosexual males facilitates the colonization of *H. pylori* and that heterosexual males with similar behaviors are also at increased risk of *H. pylori* infection [19]. It has been demonstrated that the partner of an infected individual has a higher prevalence of *H. pylori* than does the general population [20-23].

H. pylori has been isolated in saliva, dental plaque, feces, and nails [6-9]. Eslick has classified the sexual transmission routes of *H. pylori* as oral-oral, oral-anal, and oral-genital as also through masturbation and the usage of sex toys [24]. Figura *et al.* investigated infertile couples for the presence of *H. pylori* infection [25]. They found a significantly increased prevalence of infection in infertile couples as compared with the control group. Among the infected patients in the study group, all follicular fluid samples and 50% of the sperm samples were positive for *H. pylori* antibodies. Only a minority had such antibodies in vaginal secretions.

Infected sexual partners may create a problem for the fetus during gestation. Previous studies have reported that *H. pylori* can infect the fetus during gestation via vertical transmission [26-28]. Raymond *et al.* isolated *H. pylori* in

a newborn infant suffering from vomiting and weight loss on the sixth day of life [29]. Fijumuro *et al.* detected *H. pylori* DNA in the feces of 30% of a group of 50 3-day-old newborns [30].

In our study, in order to detect the active infection, we used the *H. pylori* stool antigen test, which has a sensitivity and specificity greater than 90% [31]. We detected active infections in 17.1% of our patients. We used brush cytology, which has 98% sensitivity and 96% specificity, to detect *H. pylori* in the endocervical canal [32]; however, there were no positive findings. Although there have been a couple of studies focused on the detection of *H. pylori* in the vagina, none of these have investigated cervical lesions for the presence of the organism. Histopathological methods have been reported as 95-99% specific and 93-99% sensitive for *H. pylori* [33]. In our study, we performed colposcopy-directed biopsies to look for the bacteria in the histopathology of the suspicious areas but detected no *H. pylori* infection.

Conclusion

We were unable to detect *H. pylori* in cervical cytology and histopathological samples. Moreover, the standardized stool *H. pylori* antigen tests for cervicovaginal secretions were negative. We concluded that *H.pylori* does not appear to colonize the cervix with preinvasive lesions.

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Fetuses with single umbilical artery: analysis of 45 cases

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Summary

Objective: The aim of this study was to analyze cases and determine the clinical significance of a prenatally detected single umbilical artery (SUA) in our population. *Materials and Methods:* All second and third trimester sonographic examinations carried out between January 2004 and September 2007 in our perinatology unit were reviewed. The postnatal results of the fetuses with SUA were obtained from the medical records and the patients. *Results:* From a total of 5,620 pregnant patients who were examined by ultrasound (US) scan between 15-36 weeks, a single umbilical artery was found in 45 cases, representing an incidence of 0.8%. Of these, 20 (45%) also presented with other malformations. There were six neonatal deaths, one fetal demise, and six terminations of pregnancy due to severe malformations in this group. Three cases with associated anomalies underwent surgery and one case required intensive care in the neonatal period. The only cytogenetic abnormality was trisomy 18 in one case. Six of 45 fetuses (13%) with single umbilical arteries had abnormal echocardiographic findings. In two of the fetuses associated anomalies (cleft palate and esophageal atresia) were detected after birth. In pregnancies without associated anomalies no aneuploidy was found and they were completely normal at birth and during the neonatal period. *Conclusions:* Scanning the umbilical cord is one of the essential parts of US examination. As the rate of cardiac malformations seen with single umbilical arteries is high, fetal echocardiography should be performed in suspected cases. The newborn should be reexamined immediately after birth due to the possibility of undetected anomalies.

Key words: Single umbilical artery; Prenatal diagnosis; Ultrasound; Congenital malformation; Fetal echocardiography.

Introduction

The umbilical cord normally contains three vessels; two arteries and one vein. The reported incidence of single umbilical artery (SUA) is 1.5% in spontaneous abortuses, and 0.5 to 2.5% of uncomplicated neonates [1]. This is one of the most common congenital malformations with an incidence of approximately 1% of all deliveries [2].

These fetuses have been shown to have a high rate of structural abnormalities, ranging from 18 to 68% [3, 4]. Chromosomal abnormalities are reported in 8-11% of fetuses with SUA, particularly with trisomies 13 and 18, whereas trisomy 21 does not appear to be associated with this anomaly [4, 5]. In a recent study all chromosomally abnormal fetuses with SUA were found to have associated malformations detected by ultrasound (US) [6]. SUA was also reported to be associated with increased risk of fetal growth retardation, prematurity, and increased perinatal mortality rate [7].

We evaluated the associated anomalies and perinatal outcome in fetuses with a SUA detected on US scanning in our clinic.

Materials and Methods

All of the records from detailed sonographic examinations at second and third trimester in fetuses of low- and high-risk pregnant women between January 2004 and July 2007 were reviewed.

We followed by scanning the fetus a standard protocol that included images of the central nervous system, spine, heart, diaphragm, stomach, kidneys, bladder, umbilical cord and cord insertion, and extremities. Cardiac outflow tracts were also routinely imaged and suspected cases were referred to echocardiography. Patients are counseled concerning the findings and offered amniocentesis or cordocentesis to assess fetal karyotype.

The presence of a single umbilical artery was suspected when a cross-sectional image of the umbilical cord demonstrated only two vessels. We used color Doppler US to confirm the diagnosis of a single umbilical artery, with a transverse view of the fetal pelvis showing only one umbilical artery around the fetal bladder.

Fetuses were classified into groups: the first group with an isolated SUA, the second one with a SUA and congenital malformations. Maternal and neonatal data were obtained by a review of the medical records and from the patients. Umbilical artery and associated abnormalities were confirmed after delivery in all cases.

Results

During the study period 5,620 women were examined by US scan between 15-36 weeks of gestation; 45 were found to have a single umbilical artery (0.8%). The mean gestational age at the time of the first examination was 22 weeks/3 days.

In 25 (55%) cases we observed SUA as an isolated finding. One fetus in this group was lost in follow-up and one of the pregnancies is continuing. In the group of 23 fetuses without additional anomalies the mean birthweight was 3,031 ± 477 g and all children were phenotypically healthy at birth. Two cases were under 2,500 g at term.

Twenty of the fetuses were found to have associated anomalies at pre and postnatal examinations. One of these pregnancies is still continuing. Cytogenetic abnor-

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maly (trisomy 18) was diagnosed in one of the 13 (7.7%) fetuses in the group with anomalies. There were six neonatal deaths and six terminations of pregnancy (one fetus with trisomy 18; five fetuses with severe non-chromosomal malformations) in this group. Two cases were operated due to cardiac defects, and one of them died after one year. One case with undetected esophageal atresia was also operated during the neonatal period. Two cases with minor anomalies required intensive care after birth.

Six (42%) of these cases with SUA had abnormal echocardiographic findings. Cleft palate and esophageal atresia were detected after birth in two of the fetuses. Type of anomalies, karyotype and outcomes of the fetuses with associated anomalies are listed in Table 1.

Table 1. — *Outcomes and karyotypes of the cases with associated anomalies.*

GW	Types of anomalies	Outcome	Karyotype
18	VSD, CPC	TOP	Trisomy
24	VSD	Live birth, operated	Normal
34	VSD, Hydrocephaly	Live birth, NND	Normal
20	Atrio ventricular septal defect	Operated, death after 1 year	Normal
33	Single ventricle	Live birth, NND	Normal
19	VSD, Acrania, Spina bifida	TOP	–
22	CPC	Live birth, NICU	Normal
20	CPC, EIF, PE	TOP	Normal
24	CPC	32 weeks of gestation	–
23	DWM, Ventriculomegaly	Live birth, NND	Normal
28	Anencephaly	TOP	–
20	Anencephaly-spina bifida	TOP	–
20	Hydrocephaly	Live birth, NND	–
17	Exstrophy of the bladder	TOP	Normal
20	Mild renal pelviectasy	Live birth	Normal
26	Omphalocele	Live birth, NND	Normal
32	Gastroschisis	Live birth, NND	–
21	Small bowel atresia	Fetal demise	–
24	Esophageal atresia*, short femur	Live birth, operated	Normal
20	Cleft palate*	Live birth, NICU	Normal

GW: gestational week; CPC: Choroid plexus cyst; EIF: Ecogenic intracardiac focus; PE: Pes equinovarus; VSD: Ventricular septal defect; DWM: Dandy Walker Malformation; TOP: Termination of the pregnancy; NND: Neonatal death, NICU: Neonatal intensive care unit; * This anomaly was not detected prenatally.

Discussion

In some pregnancies one of the umbilical arteries is absent due to either primary agenesis, atrophy of one of the arteries or the persistence of the original allantoic artery in the body stalk of the embryo [8]. With the use of US, the presence of a SUA should be detectable in most pregnancies, even as early as 12 weeks of gestation [9]. Despite the methods available for the detection of a SUA [10, 11] and its relatively common occurrence, the antenatal detection rate is reported to be poor with only one-third of the cases identified in previous studies [12]. The availability of color Doppler in routine scanning has improved the detection rate. In a recent study the missing rate of a SUA between cases having sonographic examination was 38% [13].

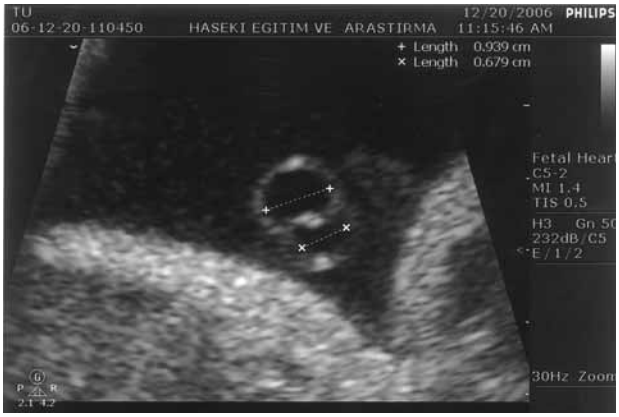

Figure 1. — Umbilical vein/artery < 2.

The reported rate at second trimester scanning is 0.7% [14]. In this study the incidence of a SUA was found to be 0.8% among second and third trimester fetuses at sonografic examination. However the real rate should be higher due to the fact that examination of the cord becomes lower in priority, if the anomalies are noted with US before the presence of a SUA. Therefore the reported rate of associated anomalies in previous studies has varied between 15.4 and 67% [14, 15].

The increase in the diameter of the umbilical artery relative to the umbilical vein has been reported by some authors [16]. They noticed that the diameter of the umbilical artery was larger then 50% of that of the umbilical vein, resulting in a vein to artery (V/A) ratio of < 2. In all of our cases with SUA the V/A ratio was < 2 (Figure 1). We confirmed the presence of SUA by using color Doppler.

According to the reports of several investigators, a variety of congenital anomalies have been associated with SUA, including cardiovascular malformations, central nervous system defects, gastrointestinal or urogenital defects, and musculoskeletal malformations [2, 17, 18].

Some authors have proposed that there should be an association between a SUA and other fetal anomalies. Their findings suggested a possible common underlying vascular pathogenetic factor, which explains the frequent concurrence of a SUA formation, limb reduction defects, atresias, and organ aplasias [19]. The cases with small bowel and esophageal atresia can be examples of this phenomenon.

In a series by Gornall *et al.* there was a preponderance of urogenital anomalies [20]. In our series only two of the fetuses (4%) were found to have anomalies of this system (exstrophy of the bladder and mild renal pelviectasy). In a recent study it was also concluded that it is not necessary to screen for renal anomalies in infants with a SUA without other anomalies seen at physical examination [21].

In our study population, the incidence of cardiac abnormalities among fetuses with a SUA was 13%. In three of these cases the cardiac defect was the only detected asso-

ciated anomaly. The incidence was 10.7% (3 of 28 cases) among an apparently isolated SUA. Budorick *et al.* reported an incidence of 5% and advised fetal echocardiography in all of these cases [22].

However as a result of another study with an incidence of 27.6% of cardiac anomalies among all of the cases with a SUA the authors concluded that fetal echocardiography may not be indicated as a routine part of evaluation of the fetus with an isolated SUA, unless the four-chamber view and outflow tracts are abnormal or cannot be obtained [23]. We also did not perform detailed cardiac examination in cases with normal findings at first scan.

SUAs had an incidence of 3.3% among cytogenetically abnormal pregnancies and was found in 77.8% of trisomy 18 cases [24]. Our single case was also trisomy 18; choroid plexus cysts and ventricular septal defects (VSDs) were additional anomalies. In this case all of these findings were missed at the first trimester scan and nuchal translucency was in normal range.

By second trimester scanning the presence of SUA implied a search for other anomalies. VSD was confirmed with echocardiography at 19 weeks of gestation.

In a review by Pierce *et al.* the rate of aneuploidy for fetuses with an apparent isolated SUA (n: 367) was 0.54%, and this rate increased to 19.9% for fetuses with other identifiable abnormalities (n: 161) [25]. The aneuploidy rate in our series in the group with associated anomalies was 7.7%. In cases with an isolated SUA, most authors do not recommend routine karyotype, as we did in our series.

Although the significantly higher perinatal mortality rate of fetuses with a SUA and no other obvious abnormality has been recognized by some authors [26, 27], prenatal sonography is found to be reliable in the identification of major concurrent anomalies, and no alteration in pregnancy management is recommended whatsoever if no other abnormalities are detected at sonography by others [28]. In two of our cases which required intensive care after birth, cleft palate and esophageal atresia were not detected prenatally and a choroid plexus cyst in the second trimester was the only finding. In two of the cases ventriculomegaly and hydrocephaly were late second and third trimester findings. The cases with an isolated SUA should be reexamined at late gestation and after birth due to the possibility of new occurring anomalies.

In a previous study fetuses with an isolated SUA were found to be at similar risk for being small for gestational age (SGA) compared with fetuses with 3-vessel umbilical cords. It was concluded that antepartum serial US examination does not provide more information for interval fetal growth assessment [29]. In our study the mean birthweight of these fetuses was 3,031 ± 477 g, and only two were SGA (8.6%).

Conclusion

Examination of the umbilical arteries should be a part of first trimester scanning. If a SUA is prenatally detected, a detailed sonographic evaluation is necessary. Fetal echocardiography should also be performed in suspected cases. Although the routine protocols for follow-up should not be altered, all babies with SUA should be examined immediately after birth due to the possibility of undetected life-treating anomalies.

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Is pregnancy over 45 with very high parity related with adverse maternal and fetal outcomes?

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Summary

Objective: To examine whether very high parity and age over 45 years are related with adverse maternal and fetal outcomes. *Study Design:* This study was carried out at the Department of Obstetrics and Gynecology from January 1, 2007 to December 31, 2007. Sixty-one pregnant women were enrolled in this prospective study. Mothers were classified in two groups: the study group (n = 23) included women with very high parity over 45 years of age (age > 45 and ≥ 10 previous live births), and a control group (n = 38) included women with high parity between 40-45 years of age (between 40-45 years and 5-9 previous live births). Hypertensive disorders complicating pregnancy, preterm labor, breech presentation, cesarean section ratio, mean APGAR scores, birthweight, fetal sex, fetal macrosomia, and early neonatal death were compared within groups. *Results:* Six (26%) patients in the study group and 12 (31.5%) patients in the control group had hypertensive disorders of pregnancies ($p > 0.05$). Twelve (52.1%) patients in the study group and 22 (57.8%) patients in the control group had preterm labor ($p > 0.05$). One (4%) patient in the study group and two (5.2%) patients in the control group had breech presentation during delivery ($p > 00.5$). Twelve (52.1%) patients in the study group and 21 (55.2%) patients in the control group had cesarean operations ($p > 0.05$). Mean APGAR scores (at 1 min and 5 min), mean birthweight, fetal sex ratio, fetal macrosomia ratio, and early neonatal death ratio due to prematurity were not statistically significant in the study group as compared with the control group. *Conclusion:* It is generally assumed that women with advanced age have an increased risk for complications during pregnancy. However, prospective population-based studies do not exist and available publications give conflicting views. Based on our results, we hypothesized that cases aged 45 or over with very high parity are not always related with adverse maternal and fetal outcomes.

Key words: High parity; Pregnancy; Age.

Introduction

Pregnant women over 35 years of age are accepted as having advanced age, and being at increased risk of complications during pregnancy and labor such as hypertensive disorders, gestational diabetes, placenta previa or abruptia and cesarean birth [1]. Some pregnancy outcomes in older women may be influenced by parity, Bobrowski *et al.* reviewed the pregnancy outcomes of 9,556 women and found that age and parity influenced the incidence of labor disorders, cesarean operations, gestational diabetes, and macrosomic infants [2].

In contrast, if these women do not have diabetes or hypertension the outcome of pregancy will be comparable with younger-aged pregnant woman. Berkowitz *et al.* studied outcomes of 800 pregnant woman with advanced age and found that slightly increased risks for gestational diabetes, pregnancy-induced hypertension, placenta previa or abruptia and cesarean delivery. The risks of preterm delivery, having an infant who was small for gestational age, or perinatal death did not increase [3].

In our study, we classified mothers into two groups and sought to examine whether very high parity and age over 45 years are related to adverse maternal and fetal outcomes.

Materials and Methods

The study was carried out at the Department of Obstetrics and Gynecology, Dicle University Faculty of Medicine from January 1, 2007 to December 31, 2007. Sixty-one pregnant women were enrolled in this prospective study.

We classified mothers into two groups: the study group (n = 23) included women with very high parity age ≥ 10 previous live births), and age over 45 and the control group (n = 38) included women with high parity (5-9 previous live births) and age between 40-45 years. Hypertensive disorders complicating pregnancy were diagnosed according to the Working Group of the National High Blood Pressure Program [4]. Preterm labor was diagnosed according to the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists [5]. Early neonatal death was defined as death of a live-born infant during the first seven days after birth [6]. Exclusion criteria included patients with pregestational diabetes, chronic hypertension, chronic liver and renal illness. Statistical analysis was performed using the Student's and chi-square tests; a value of $p < 0.05$ was considered statistically significant.

Results

Mean maternal age was 46.43 ± 2.17 in the study group and 41.65 ± 1.79 in the control group ($p < 0.00$). Mean gestational age was 36.43 ± 3.82 in the study group and 36.18 ± 3.25 in the control group ($p > 0.05$). Mean parity was 11.04 ± 1.63 in the study group and 6.15 ± 1.10 in the control group ($p < 0.00$). Six (26%) patients in the

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study group and 12 (31.5%) in the control group had hypertensive disorders of pregnancy (p > 0.05). Twelve (52.1%) patients in the study group and 22 (57.8%) in the control group had preterm labor (p > 0.05). One (4%) patient in the study group and two (5.2%) patients in the control group had breech presentation during delivery (p > 00.5). Twelve (52.1%) patients in the study group and 21 (55.2%) in the control group had cesarean sections (p > 0.05). Mean APGAR score at 1 min was 5.21 ± 1.78 in yhe study group and 5.28 ± 1.85 in the control group (p > 0.05). Mean APGAR score at 5 min was 7.26 ± 2.63 in the study group and 7.63 ± 1.68 in the control group (p > 0.05). Mean birthweight was 2993.47 ± 953.14 g in the study group and 3104.21 ± 920.06 g in the control group. Twelve (52.1%) patients in the study group and 25 (65.7%) in the control group had male fetal sex (p > 0.05), and 11 (47.8%) patients in the study group and 13 (34.2%) in control group had female fetal sex (p > 0.05). Two (8%) patients in the study group and four (10.5%) in the control group had fetal macrosomia (p > 0.05). Two (8%) patients in the study group and three (7.8 %) in the control group had early neonatal death due to prematurity (p > 0.05) (Table 1).

Discussion

It is believed that women over the age of 40 have an increased risk of complications during pregnancy. However, prospective population-based studies do not exist and available publications give conflicting views. Moreower it is diffucult to isolate the effect of parity [1-3].

Studies related to advanced age and preeclampsia show conflicting results. Salihu *et al.* evaluated the outcome of childbearing beyond maternal age 50 and found that the pre-eclampsia rate was 36.4 in 1,000 for the 20-29 age group, but up to 85.3 in 1,000 for the 40-49 age group [7]. Another study found that the relative risk of preclampsia for multiparous women aged 40 or over was 1.96 [8]. Cleary-Goldman *et al.* found the risk for 35-39 year-olds and those over 40 years not to be increased when compared to those younger than 35 years of age [9]. In our study, six (26%) patients in the study group and 12 (31.5%) in the control group had hypertensive disorders of pregnancy (p > 0.05). We found that there was no statistical difference in the incidence of hypertensive disorders of pregnancy in women with very high parity over 45 years of age and women with high parity between 40-45 years of age (p > 0.05).

Maternal age and parity (para > 5) are minor risk factors for spontaneous preterm birth that are important in epidemiological terms [10]. In our study, there was no statistical difference in the incidence of preterm labor between women with very high parity over 45 years of age and women with high parity between 40-45 years of age.

It is known that cesarean delivery non-vertex presentations are more common in older parturients than in women younger than 35, and also it has been demonstrated that the rate of cesarean section increases with age, irrespective of parity [1]. In contrast to the literature data, half of our patients had undergone cesarean sections during delivery but it was not statistically significant between the two groups (p > 0.05). One (4%) patient in the study

Table 1. — *Maternal and infant characteristics of study and control groups.*

Characteristics	Study group (n = 23)	Control group (n = 38)	*p**
Maternal age (years) †	46.43 ± 2.17	41.65 ± 1.79	$p < 0.05$
Gestational age (weeks) †	36.43 ± 3.82	36.18 ± 3.25	NS
Parity †	11.04 ± 1.63	6.15 ± 1.10	$p < 0.05$
Hypertensive disorders of pregnancy (n)	6 (26%)	12 (31.5%)	NS
Preterm labour (n)	12 (52.1%)	22 (57.8%)	NS
Breech presentation (n)	1 (4%)	2 (5.2%)	NS
Cesarean section (n)	12 (52.1%)	21 (55.2%)	NS
APGAR score (at 1 min) †	5.21 ± 1.78	5.28 ± 1.85	NS
APGAR score (at 5 min) †	7.26 ± 2.63	7.63 ± 1.68	NS
Birthweight (g) †	2993.47 ± 953.14	3104.21 ± 920.06	NS
Fetal sex (male) (n)	12 (52.1%)	25 (65.7%)	NS
Fetal sex (female) (n)	11 (47.8%)	13 (34.2%)	NS
Fetal macrosomia (n)	2 (8%)	4 (10.5%)	NS
Early neonatal death (n)	2 (8%)	3 (7.8%)	NS
Cesarean indications	**Study group (n = 12)**	**Control group (n = 21)**	***p*****
Fetal distress	3 (25%)	6 (28.5%)	NS
Fetal macrosomia	2 (16.6%)	4 (19%)	NS
Breech presentation	3 (25%)	1 (4%)	NS
Previous C-section	1 (8.3%)	4 (19%)	NS
Failed induction of labor	1 (8.3%)	3 (14.2%)	NS
Dystocia	1 (8.3%)	2 (9%)	NS
Placenta previa	1 (8.3%)	1 (4%)	NS

*† mean ± standard deviations; * p values were obtained by the chi-square test and t-test; **p values were obtained by the chi-square test; NS = statistically nonsignificant.*

group and two (5.2%) patients in the control group had breech presentation during delivery, but this was not statistically significant.

Chiechi *et al.* retrospectively studied pregnant women over 35 years of age who delivered over a four year period, and the results showed that pregnancy was not a risk for neonatal outcome [11]. In our patients, mean APGAR scores (at 1 min and 5 min), mean birthweights, fetal sex ratio, fetal macrosomia ratio, early neonatal death ratio due to prematurity were not statistically different between groups.

It is generally assumed that women with advanced age have an increased risk for complications during pregnancy. However, most reported age- and parity-related factors are only indirectly related to age and parity, and prospective population-based studies do not exist and available publications give conflicting views. Based on our results, we hypothesized that cases aged 45 or over with very high parity are not always related to adverse maternal and fetal outcomes, as seen in our patients, and antenatal care of older mothers will improve maternal and fetal outcomes.

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Case Reports

Successful long-term management of adenomyosis associated with deep thrombosis by low-dose gonadotropin-releasing hormone agonist therapy

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Summary

We report the case of a patient with adenomyosis complicated by deep vein thrombosis in whom low-dose gonadotropin-releasing hormone agonist (GnRHa) therapy was useful as a uterus-conserving therapeutic option. The patient was a 34-year-old nulliparous woman who presented with edema and pain in the left lower leg. The patient had been treated with four cycles of GnRHa therapy for adenomyosis and repeatedly experienced chronic pelvic pain, dysmenorrhea and anemia due to hypermenorrhea. Leg venography confirmed deep vein thrombosis, and thrombolytic therapy was performed to eliminate symptoms. Because the patient strongly wanted to conserve the uterus, low-dose GnRHa therapy was initiated. The patient is currently taking 450 μg/day buserelin acetate nasally (regular dose: 900 μg/day), and estradiol levels have been maintained at 24-50 pg/ml. Anemia, leg numbness and chronic pelvic pain have dissipated, and the patient has not experienced estrogen deficiency symptoms for more than two years.

Key words: Adenomyosis; Deep thorombosis; GnRH agonist; Estrogen deficiency symptoms.

Introduction

Gonadotropin-releasing hormone agonists (GnRHa) are highly effective against adenomyosis and improve dysmenorrhea, alleviate chronic pelvic pain, and reduce tumor size, but recurrence of adenomyosis and its associated symptoms is common once administration ends, and repeated administration is required in many patients who are followed for long periods of time [1, 2]. Due to the potent suppressive actions on pituitary function, GnRHa markedly lowers estrogen secretion and adverse events, such as estrogen deficiency symptoms and loss of bone mineral density occur, thus limiting the length of administration to six months [3].

In recent years, several investigators have proposed the existence of a therapeutic window [4]. By setting the level of estradiol (E_2) below 50 pg/ml, growth of endometriosis can be suppressed while minimizing hypoestrogenic disorders and loss of bone mineral density (BMD) with E_2 levels above 20 pg/ml.

GnRHa have been shown to suppress pituitary function in a dose-dependent manner [5] and some degree of estrogen secretion can be maintained by lowering the dosage [6]. As a result, low-dose GnRHa therapy (draw-back therapy) has been proposed, and the usefulness of this approach as a treatment for endometriosis with minimal adverse events has been reported [7, 8]. Adenomyosis shares various similarities with endometriosis. Therefore, it is possible that draw-back therapy is effective in management of adenomyosis.

The present report describes a case of successful management of adenomyosis with deep thrombosis with long-term, low-dose gonadotropin-releasing hormone agonist therapy.

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Case Report

A 35-year old primipara woman was admitted to our hospital for the chief complaint of edema and pain of the left lower leg. The symptom appeared a few days before and was getting worse. The patient first visited our department at 28 years of age due to dysmenorrhea, and adenomyosis was identified. Over the next six years, four cycles of standard GnRHa therapy were administered. At the time of presentation, a two-finger-wide abdominal mass was palpable above the navel and magnetic resonance imaging (MRI) findings indicated giant adenomyosis (Figure 1). Uterine volume was calculated at 934.5 cm^3 by using the formula for an ellipsoid (length x width x depth x 0.52). Blood testing showed inflammation (WBC 12,400/μl; CRP 7.58 mg/dl) and anemia (RBC 338 x 10^4/μl; Hgb 9.9 g/dl) and CA125 was markedly elevated at 566.0 IU/ml. Pelvic computed tomography (CT) and leg venography confirmed massive thrombosis from the left popliteal vein to the common iliac vein (Figures 2 and 3).

Based on the above findings, deep vein thrombosis was diagnosed and thrombolytic therapy was initiated using 480,000 units of urokinase, 12,000 units of heparin and 5 mg of warfarin. Clinical symptoms, including left lower leg swelling, disappeared six days after admission.

To prevent recurrence of tumor-induced thrombosis, mass reduction was required, and treatment options such as total hysterectomy were recommended, but the patient insisted on conservation of the uterus and continuation of GnRHa therapy. After obtaining informed consent, 3.75 mg/month of leuprorelin acetate, a more potent GnRHa [9], was administered to induce marked pituitary desensitization. Three months later, the dosage was reduced to 1.88 mg/month. Six months after the start of therapy, CA125 normalized, and then buserelin acetate was administered nasally (regular dose, 900 mg/day). The patient was instructed to visit about twice a month, and at every visit, blood E2 and CA125 were measured. The dosage of buserelin acetate was set between 150 and 750 μg/day so that blood E_2 was between 20 and 50 pg/ml. At present (24 months after starting therapy), the patient is taking 450 μg/day of

Fig. 1

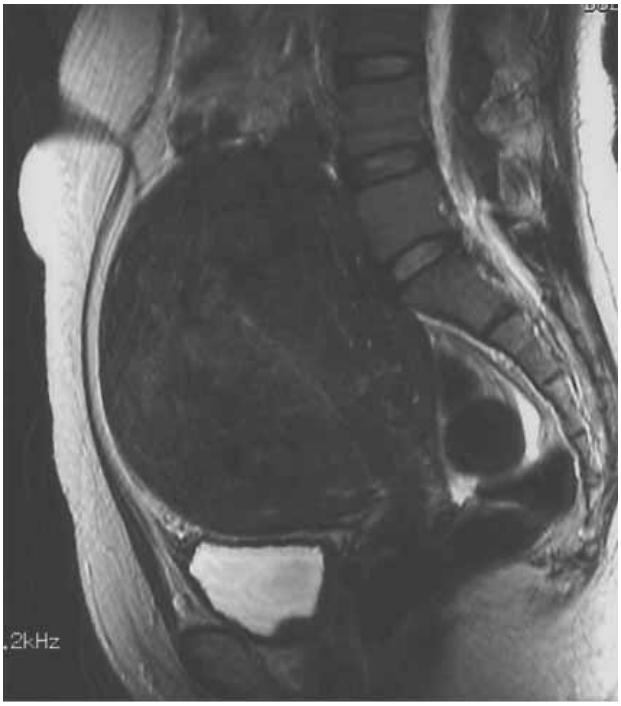

Fig. 2

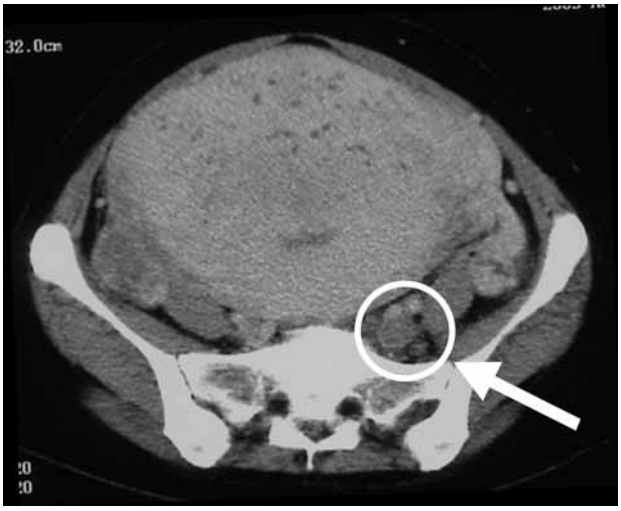

Fig. 3

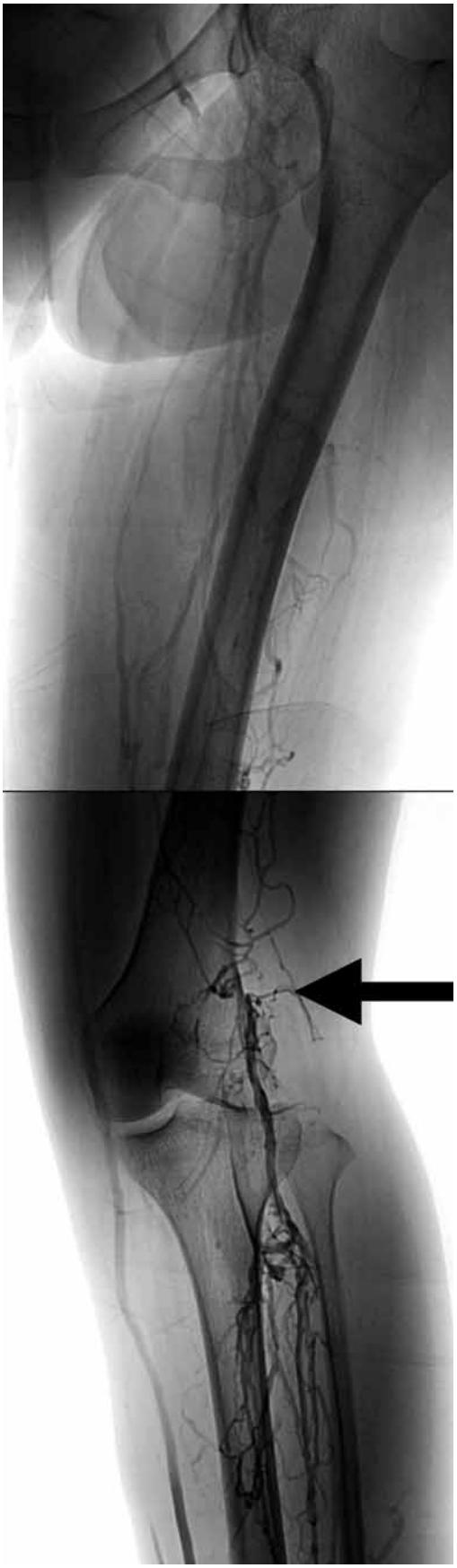

Figure 1. — Pelvic MRI showing a giant adenomyosis.

Figure 2. — Pelvic CT showing thrombosis at the external iliac vein.

Figure 3. — Leg venography showing massive thrombosis from the popliteal vein to the femoral vein.

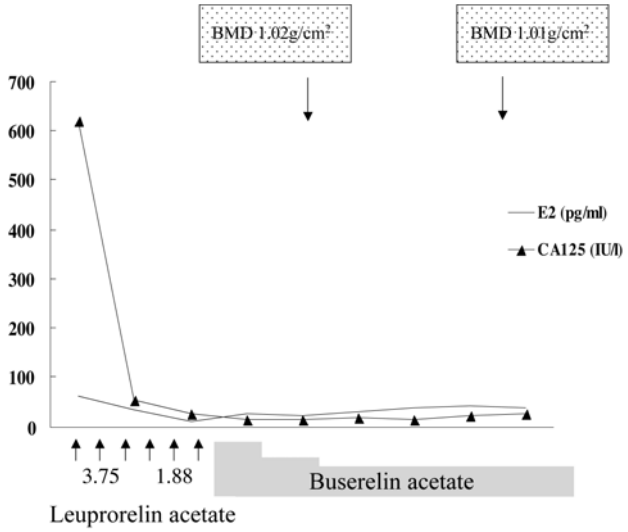

Figure 4. — Clinical course.

buserelin acetate, and E_2 and CA125 have been maintained at 24-50 pg/ml and 13-20 IU/l, respectively (Figure 4). Anemia and chronic pelvic pain have dissipated. Signs of hypoestrogenism, such as hot flushes and vaginal bleeding, are also absent. MRI showed that the uterus has decreased about 40% in size over the 24 month-period. Although BMD, assessed by dual-energy X-ray absorptiometry, was low at 1.027 g/cm^2 at the beginning of low-dose GnRHa therapy, BMD has only decreased by 0.6% per six months.

Discussion

The present study used a buserelin-acetate preparation, for which the number of nasal sprays could be adjusted at six levels to administer tailor-made therapy based on plasma E_2 levels. Tumor size was reduced up to 40% and no sign of recurrence of deep thrombosis has been observed. Regarding the degree of decrease in BMD, the degree of decrease of this case (-0.6% per 6 months) was much less than regular-dose administration methods which is reported to lower BMD by -3.4% per six months [10].

In conclusion, low dose GnRHa therapy (draw-back therapy) using buserelin acetate nasal sprays can maintain therapeutic effectiveness against adenomyosis for a long period, while suppressing the expression of adverse events. Patient quality of life during this therapy is thus high and compliance is also favorable, therefore low-dose GnRHa therapy seems to be one of the alternatives in long-term management of adenomyosis.

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Conservative treatment by endoscopy of a cesarean scar pregnancy: two case reports

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Summary

Background: Cesarean section scar pregnancy is the rarest form of ectopic pregnancy and the most dangerous due to the high risk of uterine rupture and hemorrhage. *Case*: We present two case reports of women diagnosed with an ectopic cesarean scar pregnancy. We performed conservative treatment because both patients desired fertility preservation. The first case was treated with laparoscopy and hysteroscopy simultaneously. For the second case the treatment started with an ultrasound-guided injection of methotrexate. Surgical laparoscopy and hysteroscopy were subsequently performed simultaneously. Four months later, the first woman had a spontaneous singleton pregnancy. An elective cesarean was performed. *Conclusion:* In these two case reports we have presented our experience with endoscopic surgery in the management of two patients who had a cesarean scar pregnancy and desired to preserve their fertility.

Key words: Cesarean scar pregnancy; Laparoscopic surgery; Hysteroscopy surgery; Methotrexate; Transvaginal ultrasound.

Introduction

Cesarean section scar pregnancy is a form of ectopic pregnancy, with a high risk of uterine rupture and hemorrhage, hence the need for termination [1, 2]. It was first described in 1978 by Larsen and Salomón [3], and only a further 19 cases were published in the period up to 2001. However, over the last six years there has been a notable rise in reports of cesarean scar pregnancy (CSP) in the English language literature. Ash *et al.* [4] pointed out that this must be secondary to an increase in the number of cesareans being performed, as well as to improved diagnosis of the condition.

Although the incidence of CSP is actually low (the estimate being 1: 2,226 of all pregnancies), the large rise in the number of cesareans now performed would suggest a concomitant increase in this type of pregnancy [5]. However, the exact incidence of this type of pregnancy is difficult to determine and estimates vary widely: Seow *et al.* [5] reports a rate of 6.1% in patients with a history of ectopic pregnancy and cesarean scar (at least one) or hysterotomy scar, while Wang *et al.* [6] gives a figure of 21.6%.

The etiology of the condition remains unknown, although some hypotheses refer to trophoblast invasion of the myometrium when there is a history of cesarean section (rising by up to 60-70% in the case of multiple cesareans, dilation and curettage or adenomyosis) [2].

The symptoms shown by patients can vary widely and although there is often acute or moderate pain Rotas *et al.* [7] reports a diagnostic rate of 37% in asymptomatic patients under routine ultrasound examination.

The development and use of transvaginal ultrasound (TVS) in the first trimester has aided the diagnosis of this type of pregnancy, and this raises the possibility of conservative treatment [4]. Therapeutic strategies include local (8) or systemic injection of methotrexate (MTX), ultrasound-guided curettage, laparoscopic or laparotomic excision, and hysterectomy, although there is no standard protocol for the diagnosis and treatment [6, 9].

Case Reports

Case 1

The patient was a 38-year-old woman with no history of note except for two previous term cesareans as a result of fetopelvic disproportion and a left salpingo-oophorectomy due to ectopic pregnancy.

She attended our clinic expressing the desire to have a child with her new partner. One month later the patient returned to our clinic complaining of intermittent blood loss and pain in the right iliac fossa. Urine β-hCG was positive and a subsequent TVS revealed an anteverted uterus of 98 x 43 mm with cesarean scar dehiscence (serous) and the presence of a gestational sac with a vitelline vesicle invaginating the scar; both adnexa appeared to be normal with no free fluid in the pouch of Douglas (Figure 1).

Given the suspicion of an ectopic CSP the patient was admitted for treatment. Due to her desire to become pregnant we opted to perform conservative laparoscopic surgery. After opening the cavity we performed uterine scar resection and metroplasty, while simultaneously carrying out hysteroscopic resection of the gestational sac and decidua in the area of the cesarean scar. At the end of the surgery we ensured that the cavity was properly sealed.

After 24 hours the patient was clinically stable and all tests were normal, and she was thus discharged. Follow-up a week later showed β-hCG to be 283 IU/l, and subsequent weekly tests gave values of 26.82 IU/l and 5.48 IU/l, respectively. Analysis of pathological anatomy revealed ovular-decidual remains plus connective tissue with scarce adipose tissue islets and evidence of fibrosis consistent with scar material.

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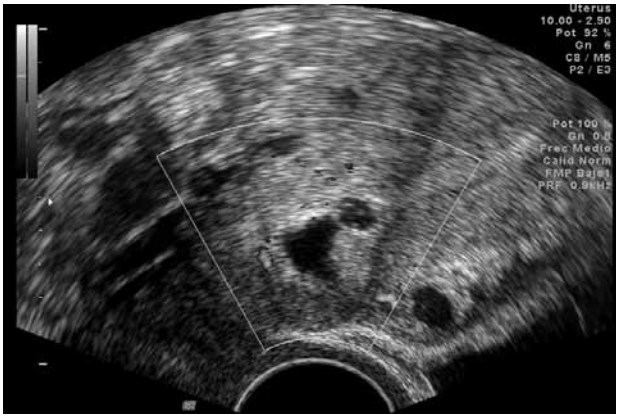

Figure 1. — TVS: cesarean scar dehiscence (serous) with a gestational sac.

Four months later the patient once again attended our clinic due to a spontaneous singleton pregnancy. The pregnancy proceeded without incident and only standard obstetric monitoring was required. An elective cesarean was performed at 37 weeks and a male fetus weighing 3,150 g was delivered. During the cesarean the bladder was observed to be firmly adhered to the uterus, and there was also dehiscence of a previous scar, which was sutured without incident. The patient was discharged five days later and her postoperative evolution was problem-free.

Case 2

The patient was a 36-year-old woman with an unremarkable history except for a previous term cesarean due to fetopelvic disproportion.

She attended our clinic expressing the desire to become pregnant. The case was assessed as infertility secondary to low follicular reserve and male factor, and we thus recommended cycles of IVF-ICSI. Following the first cycle of in vitro fertilization two embryos were transferred on subsequent tests of β-hCG which showed levels of 80.7 and 2,105 five days later. One week later she presented at the emergency department due to heavy blood loss and pain. TVS revealed a retroverted uterus measuring 86 x 59 mm and a blood-filled endometrial cavity. In the area of the previous cesarean scar the echography showed a gestational sac with an embryo and positive fetal heartbeat (Figure 2). The suspected diagnosis was therefore an ectopic

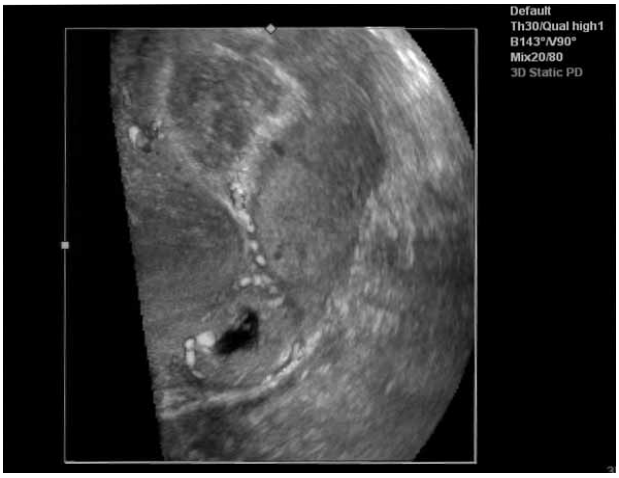

Figure 2. — Echography 3D: gestational sac with an embryo in the area of the previous cesarean scar.

CSP and the patient was admitted for treatment. This began with an ultrasound-guided intrasac injection of 50 mg of MTX to reduce the trophoblastic tissue, the idea being to perform – given the patient's desire to become pregnant – conservative endoscopic surgery 48 hours after medical treatment. Surgical laparoscopy and hysteroscopy were subsequently performed simultaneously. During the laparoscopy we observed bulging of the cesarean scar area and after opening the cavity metroplasty was performed; the opening was then sutured with individual stitches. Hysteroscopy revealed severe hematometra plus dehiscence of the previous cesarean scar, with the abundant presence of necrotized ovular decidual tissue. The abortive remains were then resected and removed via suction curettage. At the end of the intervention we ensured that the cavity was properly sealed.

After 24 hours the patient was clinically stable and all tests were normal, and she was thus discharged. Follow-up a week later showed β-hCG to be 94 IU/l. The analysis of pathological anatomy revealed ovular-decidual remains along with focal adenomyosis.

Discussion

To be able to offer conservative treatment it is necessary to have an early diagnosis, and in the case of ectopic pregnancy this has become possible through the introduction of TVS. The diagnostic ultrasound image shows an empty uterus and cervical canal with a gestational sac in the anterior part of the isthmic portion and reduction of the myometrial wall between the bladder and the gestational sac; this aids differential diagnosis with respect to a cervicoisthmic pregnancy. Cervical ectopic pregnancy is characterized by an empty uterus, a barrel-shaped cervix, a gestational sac present below the level of the uterine arteries, absence of the sliding organ sign and blood flow around the gestational sac on color Doppler. Both Doppler and 3D-ultrasound provide further information for diagnosis and subsequent monitoring. Three-dimensional ultrasonography and its applications allow better images to be obtained thus improving the ability to identify anatomic details that permit a more accurate diagnosis [10, 11].

The differential diagnosis should be made not only with respect to a cervicoisthmic pregnancy but also as regards an incomplete miscarriage that shifts under cervical pressure. The literature does include one case report of a CSP in which an expectant management approach was maintained up to 36 weeks, with the subsequent delivery of a live male fetus [14]. In most cases the evolution of an ectopic cesarean scar pregnancy leads to uterine rupture and consequently, profuse hemorrhaging, thus a termination is required.

Due to the low incidence of this type of pregnancy there are no standard treatment protocols, although proposals for consensus on the diagnosis and treatment of ectopic pregnancies are increasingly to be found in the literature [15].

The approach to this pregnancy can be divided into two broad categories: radical and conservative. Radical hysterectomy is not a therapeutic strategy and is applied only in the case of intractable bleeding and after all conservative methods have failed. Conservative treatment must be

evaluated according to each individual case and the experience of the medical team, the sole objective being to finalize the gestation. Some authors such as Jurkovic *et al.* [16] argue that 44% of pregnancies of this type result in miscarriage. Obviously, the choice of treatment does not depend solely on the patient's desire to have children but also on other factors such as symptomatology, gestational age, the viability of the pregnancy, uteroplacental neovascularization and the patient's preferences.

As regards conservative treatments these can be divided into two types: surgical and medical. Non-surgical treatments offer a range of options, the greatest advantage being that there is no scar resection. Noteworthy among these approaches is conservative systemic treatment with methotrexate, which offers a quick resolution albeit with greater side-effects and the possibility of continued symptoms and the subsequent need to perform surgical laparoscopy [17]. Ultrasound-guided MTX treatment increases concentration in the target area and is associated with fewer side effects. In the series reported by Seow *et al.* [10], 100% of patients were treated successfully, despite the persistence in some cases of post-treatment masses. Another conservative treatment is arterial embolization [18], and some authors combine the two techniques, performing embolization of both uterine arteries and administering an intramuscular injection of MTX [19]. Finally, a little over ten years ago Godin *et al.* [2] reported a case treated successfully with KCl and MTX, while more recently Wang and colleagues [20] have described a case of heterotopic pregnancy in which selective feticide of the CSP was achieved through administration of KCl.

Surgical treatments such as laparotomy are used when the pregnancy has to be localized, is at an advanced stage, or in the event of significant bleeding. However, the lengthy hospital stay and recovery period required following this type of intervention must be taken into account. Laparoscopic treatment can help to localize those gestations that are deeply implanted in the scar and aid not only diagnosis but also metroplasty and repair of the area, as in the cases described here. When there is doubt as to which type of endoscopy to use Wang *et al.* [6] recommend that the decision is made according to the location of the ectopic pregnancy and the medical team's own experience. With respect to pregnancies that are deeply implanted in a cesarean scar, opinion varies: some groups recommend laparoscopy while others prefer hysteroscopic visualization of the uterine cavity or ultrasound-guided curettage [21].

In both cases reported here we opted to combine laparoscopy with hysteroscopy as we believe this aids surgery and makes it easier to assess whether the cavity is properly sealed once the ectopic pregnancy has been removed. As regards the use of suction curettage its efficacy among these patients remains controversial and most groups complement it with other techniques. Finally, and as reported by the group of El-Matary *et al.* [14], it is possible to take an expectant management approach to this type of pregnancy provided that the risks involved are constantly assessed.

Although there is no standard protocol for the follow-up of these patients there is a good degree of consensus regarding the need to monitor blood β-hCG and perform periodic TVS examinations.

In terms of recommendations for future pregnancies Seow *et al.* [8] point out that scar repair does not guarantee the safety of subsequent gestations, and note that reports have described an increased incidence of placenta accreta or percreta following such pregnancies. They also recommend the use of elective cesarean plus blood reserve in any subsequent pregnancies, which could be diagnosed by echography. If placenta acreta or percreta is expected special preparations for surgery can be made. This group also recommend that pregnancy be avoided for between three months and up to 1-2 years [5]. At all events any later pregnancies should be accompanied by detailed ultrasound examinations.

Our experience demonstrates the success of conservative treatment to obtain a term pregnancy. However, in all cases we recommend an elective cesarean due to the risk of scar dehiscence.

Finally, given the increased number of cesarean deliveries today clinicians should be alert to the possibility of encountering a cesarean scar pregnancy.

Acnowledgement

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Ruptured cornual pregnancy: case report

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Summary

Cornual pregnancy is uncommon among ectopic pregnancies. A diagnosis of cornual pregnancy remains challenging, and rupture of a cornual pregnancy causes catastrophic consequence due to massive bleeding. We report a case of a ruptured cornual pregnancy occurring at 12 weeks of gestation. A 34-year-old woman was suspected of having a left cornual pregnancy at 11 weeks of gestation. Transabdominal ultrasound and magnetic resonance imaging revealed an eccentric localization of a gestational sac containing a viable fetus outside the uterine cavity adjacent to the left uterine cornua. The gestational sac was surrounded with a thin myometrial layer. The patient developed a rupture of the left cornual pregnancy with unstable hemodynamics. She underwent emergency laparotomy, which revealed the ruptured left cornual pregnancy with a hemoperitoneum. Cornual resection was performed. The pathological examination confirmed a ruptured cornual pregnancy.

Key words: Cornual pregnancy; Ectopic pregnancy; Magnetic resonance imaging; Ultrasonography.

Introduction

Cornual pregnancy is an uncommon ectopic pregnancy defined by implantation in the uterine cornua [1, 2]. The incidence of cornual pregnancy is approximately 3% of ectopic pregnancies [2] with a mortality rate of 2-2.5% [1]. Cornual pregnancy is sub-classified into angular or interstitial pregnancy [2]. An angular pregnancy implants medially to the insertion of the round ligament, while an interstitial pregnancy implants laterally to the round ligament [2]. Ipsilateral salpingo-oophorectomy, previous ectopic pregnancy, and in vitro fertilization are predisposing factors for interstitial pregnancy [3]. It has been believed that rupture of a cornual pregnancy occurs after 12 weeks due to the thickness of myometrial wall protecting interstitial pregnancy, leading to catastrophic hemorrhage. However, a recent study demonstrated that 14 out of 24 cases ruptured before 12 weeks [3]. A correct diagnosis of cornual pregnancy is pivotal to avoid fatal consequences. Conservative management can be adopted for the treatment of unruptured cornual pregnancy, but cornual resection or hysterectomy remains the mainstay for the treatment of a ruptured cornual pregnancy [4, 5]. Herein, we report an unusual case of ruptured cornual pregnancy occurring at 12 weeks of gestation.

Case Report

The patient was 34-year-old woman, gravida 1, para 1, with no remarkable medical history. She was referred to our hospital at 11 weeks of gestation due to a suspected ectopic pregnancy or pregnancy in the bicornuate uterus. Her vital signs were stable, and the abdomen was not tender. However, a transabdominal ultrasonography revealed an empty uterus and a gestational sac (GS) containing a viable fetus corresponding to 11 weeks located outside the uterine cavity adjacent to the left uterine cornua (Figure 1A). The GS was found to be separated from the empty uterus via the myometrium, and surrounded with a thin myometrial layer (Figure 1A). There was no ascites in the cul-de-sac. Axial T2-weighted magnetic resonance imaging (MRI) of the pelvis clearly displayed that a GS was located at the left lateral uterine cornual region and a thinning of the myometrial layer surrounding the GS (Figure 1B). On the basis of the imaging technologies, a presumptive diagnosis was a left unruptured cornual pregnancy. The patient was informed of the possibility of a cornual pregnancy, a life-threatening complication accompanied by rupture of the cornual pregnancy, the difficulty in the continuity of pregnancy, and the treatment options for cornual pregnancy. She was asymptomatic at that time and discharged home to consult her husband regarding carrying on her pregnancy. Two days later, however, she developed an acute onset of severe abdominal pain at 12 weeks of gestation. On admission, her blood pressure was 117/75 mmHg, and the pulse rate was 88 beats/min. Physical examination showed severe lower abdominal tenderness. The laboratory profile showed that red cell counts were 296×10^4/μl, hemoglobin 8.9 g/dl, hematocrit 26.6%, and platelets 20.8×10^4/μl. Transabdominal ultrasound (TVS) showed the deviation of the GS toward the caudal part compared with a previous image, and the presence of massive fluid retention in the pelvic cavity. Fetal cardiac activity could not be detected. An enhanced computed tomography of the pelvis demonstrated the presence of active bleeding and retention of massive bloody ascites in the pelvic cavity. A diagnosis of the ruptured cornual pregnancy was made. The patient underwent an emergency laparotomy, which revealed a 5×4 cm bulging at the left uterine cornua confirming a left ruptured cornual pregnancy (Figure 2) and a hemoperitoneum containing approximately 2000 ml of fresh blood. An unruptured GS was found in the abdominal cavity, partially adherent to the left cornua. Cornual resection was performed. The patient received six units of packed red blood cells due to the decrease in hemoglobin to 3.9 g/dl postoperation. Pathological examination confirmed a ruptured cornual pregnancy. The patient's postoperative course was uneventful and she was discharged home on the sixth day postoperatively.

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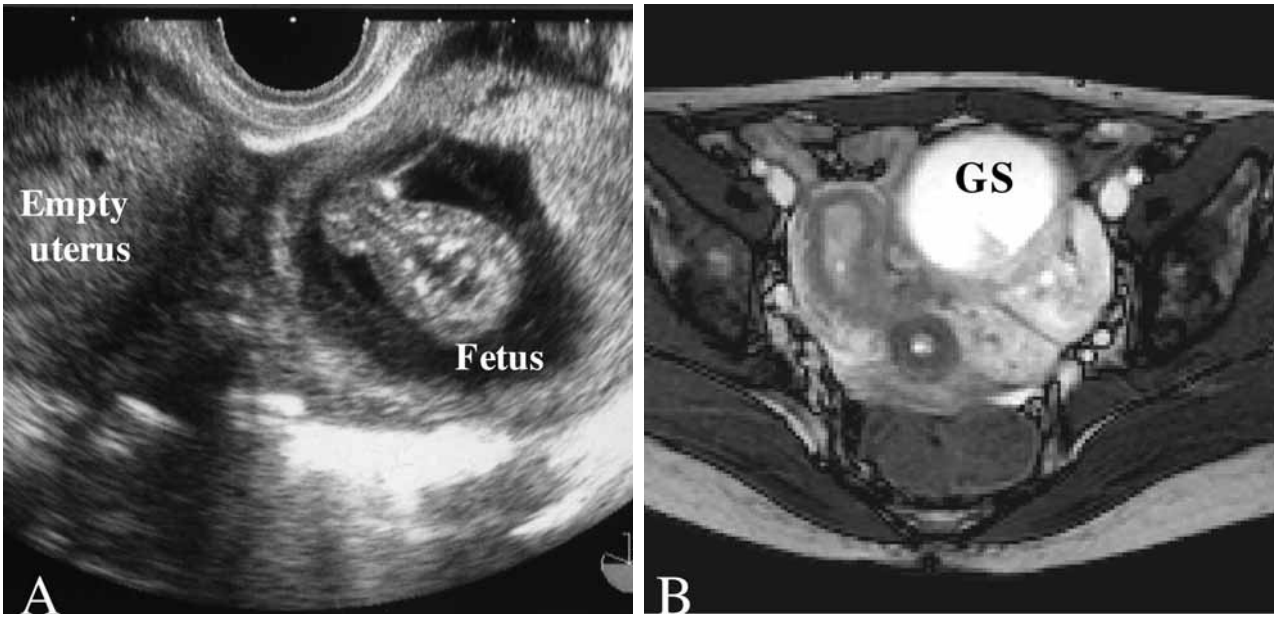

Figure 1. — Transabdominal ultrasonographic view of a left cornual pregnancy (A), and magnetic resonance imaging finding of a left cornual pregnancy on axial T2-weighted images (B).

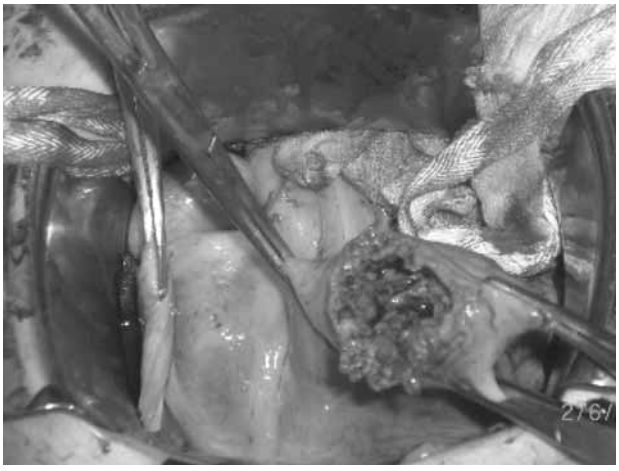

Figure 2. — Ruptured left cornual pregnancy.

Discussion

Cornual pregnancy is a life-threatening disease potentially leading to hypovolemic shock when ruptured as in our case. However, a diagnosis of cornual pregnancy remains clinically challenging. A correct diagnostic rate of cornual pregnancy has been reported to be 55.6% by TVS [6]. Timor-Tritsh *et al.* [7] proposed three ultrasonographic criteria for the diagnosis of interstitial pregnancy: (1) an empty uterine cavity, (2) a gestational sac seen separately and > 1 cm from the most lateral edge of the uterine cavity, and (3) a thin myometrial layer surrounding the gestational sac. The ultrasonographic findings in our case met the above criteria, and a cornual pregnancy was strongly suspected. Furthermore, 4-dimentional transvaginal volume contrast imaging in coronal-plane technology was shown to demonstrate the exact anatomic location of an eccentric gestational sac, thereby improving diagnostic confidence and enabling the differentiation between interstitial pregnancy and unusual forms of intrauterine pregnancy as an angular pregnancy or a pregnancy in the anomalous uterus [8]. We employed MRI as a complementary tool for the diagnosis of cornual pregnancy in addition to the ultrasonography. MRI was found to be useful in evaluating the location of the GS and thickness of the myometrial layer surrounding it. The thinning of the myometrial layer surrounding the GS in our case suggested an impending risk of rupture of the cornual pregnancy, and in fact rupture developed two days later after making a presumptive diagnosis. In the present case, the rupture occurred during the patient's stay at home. Despite her asymptomatic condition at that time, the patient should have been kept in admission with close medical care in order to cope with an emergent situation caused by rupture of the cornual pregnancy.

Treatment for cornual pregnancy includes conservative and surgical management. Several conservative treatment options have been clinically applied, including systemic methotrexate (MTX) [6, 9], transvaginal sonography-guided KCL injection to the amniotic sac [6, 10], hysteroscopic-guided MTX injection to the amniotic sac [6], hysteroscopic suction and evacuation in combination with laparoscopic injection of vasopressin [1], suction and curettage and hysteroscopic resection of the cornual pregnancy [11], and laparoscopic resection of the cornua [6].

In most cases of ruptured cornual pregnancy, laparotomy is undertaken and cornual resection or hysterectomy is necessitated. However, two cases that were successfully treated by laparoscopy in ruptured cornual pregnancies have recently been reported [12, 13].

Although rare, an awareness of cornual pregnancy is

important to prevent delayed diagnosis and determine prompt and adequate treatment options, particularly when the gestational weeks are proceeding to the second trimester.

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A case report supporting the concept that some women have a predisposition for maternal meiosis errors resulting in digyny

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Summary

Purpose: To determine if a primary aborter with recurrent miscarriage times three with her only two fetal products that were evaluated with chromosome analysis demonstrating triploidy in both fetuses could have a predisposition for maternal meiosis errors. *Methods:* In vitro fertilization with intracytoplasmic sperm injection was performed. Embryo biopsy was performed on 3-day old embryos and a single blastomere was evaluated by fluorescent in situ hybridization (FISH). Embryo transfer would be performed on day 5 at the blastocyst stage. *Results:* There were six normal and seven abnormal embryos. One of the seven was a tetraploid embryo (92XXXX). *Conclusions:* The majority of triploidies are related to polyspermy but this factor was excluded by performing ICSI. Thus this woman showed a marked predisposition to digyny. Though the tetraploidy could be explained by fertilization of a digynic egg by a diploid sperm the probability was that in this instance the meiosis error extended back to failure to extrude the first polar body.

Key words:

Introduction

Triploidy in which there are 69 chromosomes in a given cell may be related to fertilization by two sperm (known as dispermy), or by one sperm with two sets of chromosomes, or related to problems of meiosis with the oocyte so that fertilization of a diploid oocyte occurs with a sperm with a single set of chromosomes.

Jacobs et al., found that when dealing with one set of extra chromosomes that 60% of the time the problem is related to fertilization by two sperm (dispermy), 24% due to fertilization with a diploid sperm and only 10% of the time a female factor related to fertilization of a diploid oocyte [1].

A case was reported of recurrent triploidy of maternal origin (2). She not only had two previous spontaneous miscarriages related to triploidy but when they performed in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI), two of the 13 embryos had triploidy [2].

This case exemplifies that some women may have a predilection to meiosis errors resulting in triploidy. By performing ICSI, the embryos with triploidy evaluated by pre-implantation diagnosis had to be of maternal origin.

Triploidy occurs in 2% of all conceptuses [1]. Since maternal origin only occurs in 10% of all triploidy cases, and since triploidy comprises 2% of all conceptuses, it is estimated that one in every 500 oocytes (0.2%) has a failure of maternal meiosis resulting in triploidy [1].

In the case described there were 26.6% (4 of 15) of the embryos showing triploidy (counting the 2 miscarriages) indicating that some women may have a predisposition for recurrent triploidy related to maternal non-dysjunction during oogenesis [2].

Another case is described of maternal meiosis abnormalities leading to retention of extra sets of chromosomes.

Case Report

A 24-year-old woman had a miscarriage at nine weeks. There were no chromosome studies on the fetus performed. She came for investigation to reduce the risk as much as possible of a second miscarriage.

She had a normal hysterosalpingogram. Thyroid studies were normal as was measurement of antiphospholipid antibodies. Also chromosome analyses on both the female and male partner were normal (46XX, 46XY). The patient's dominant follicle was deemed mature (exceeding 18 mm average diameter with a minimum serum estradiol > 200 pg/ml) so she was treated in the luteal phase with 200 mg twice daily progesterone vaginal suppositories which was increased to 400 mg twice daily after she quickly conceived. When she reached six weeks of gestation there was no heart beat. Nine days later a heart beat was detected but it was only 100 per minute and the crown rump length was behind by one week. The following week it was no longer viable. A D&E was performed and chromosome analysis revealed triploidy.

A few months later she conceived again just taking progesterone in the luteal phase and the first trimester. A single viable fetus was seen at 6.5 weeks' gestation but the sac size and crown rump length was one week behind the fetus. Viability was still seen at 8.7 weeks but the crown rump length was 19 mm consistent with 8.3 weeks but the sac average was 21mm consistent with 6.95 weeks. The sac fell behind a full two weeks by 9.5 weeks of gestation. The fetus remained alive though

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through the first trimester and died shortly after the second trimester. Chromosome analysis again showed triploidy.

It was decided, based on three consecutive losses in such a young woman with triploidy in her last two (the chromosome constitution of the first miscarriage was unknown), to do in vitro fertilization-embryo transfer (IVF-ET) with preimplantation genetic diagnosis (PGD). There were 13 embryos biopsied on day 3 and they were evaluated by FISH analysis for chromosomes 13, 18, 21 X and Y. There were six "normal" embryos and five grew to blastocysts. There were two transferred and three cryopreserved. She achieved a pregnancy and successfully completed her first trimester with a completely normal rise in the serum beta-hCG level and appropriate growth of the fetus.

There were no trisomies among the seven abnormal embryos but interestingly there was one tetraploidy (92XXXX).

Discussion

One of the most common mechanisms involved in triploidy of maternal origin is non-extrusion of the second polar body [3]. This state of digynic triploidy was described by Grossman *et al.* in 1997 after performing PGD on eggs fertilized by ICSI [4].

The fact that ICSI was performed eliminates the possibility of the most common etiology for an additional set of chromosomes which is the penetration of the oocyte by two sperm [1].

One possible explanation for a tetraploid embryo was the combination of two digynic (female) pronuclei and two diandric (male) pronuclei.

Of the 42 documented cases of diandric trisomy [4]: 37 were related to dispermy and five (12%) were related to fertilization by diploid sperm.

Failure of maternal meiosis leading to an extra set of chromosomes has been estimated to occur in 1 of 500 conceptuses (0.2% [2]. Thus the chance that two consecutive miscarriages would be trisomies is 1:40,000.

Though diploid sperm are considered to occur in at least 1.9% of the sperm in an infertile population, they are rarely present in noromozoospermic males [5, 6]. The male partner in this study was considered to be normozoospermic and thus the likelihood of diploid sperm would be unlikely.

Nevertheless even if in this couple the tetraploid embryo was fertilized by a diploid sperm this would account only for an embryo with triploidy. Thus, if there was diandry present, there would also have to be a maternal meiosis error, i.e., digyny with failure to extrude the second polar body.

With the 92XXXX karyotype another possibility which seems more probable was that since the one-cell zygote is tetraploid after DNA replication, the tetraploidy may have resulted from failure to extrude even the first polar body.

With 1:500 odds of any given embryo having triploidy (or tetraploidy) related to a maternal meiosis disorder, it is highly unlikely that one of 13 embryos tested by PGD would be either a triploid or tetraploid embryo. Thus this case clearly shows that some women may have a tendency for failure to extrude the polar bodies.

Since only one in seven eggs that had the potential to result in a pregnancy was digynic, the odds of trisomy occurring in two pregnancies considering an equal chance of any egg being the one fertilized would be about one in 50. Thus this case suggests that possibly there is some property concerning a digynic egg that makes it more likely to result in attaining the status of a dominant follicle.

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Mucocele of the appendix mimicking an adnexal mass: a case report

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Summary

Mucocele of the appendix is a rare entity usually mimicking an adnexal tumour. There is no specific imaging or screening method to determine the diagnosis with certainty preoperatively. Appendiceal malignancy can be the underlying cause, although it is not common. We present a case of an appendiceal mucocele mimicking an ovarian tumour by both clinical and imaging (TVS and MRI) methods. This pathological condition should be considered by all the gynaecologists in the differential diagnosis of a right-sided pelvic mass.

Key words: Adnexal mass; Appendix; Cystadenoma; Mucocele.

Introduction

Mucocele of the appendix is a rare entity, infrequently diagnosed prior to surgery or autopsy. It is characterised by cystic dilatation of the appendiceal lumen due to abnormal accumulation of mucus. Its incidence ranges between 0.2% and 0.3% of all appendicectomy specimens [1], with a higher frequency in females (4:1) over the age of 50 years. Mucocele of the appendix is often asymptomatic. Due to the rarity of this entity and the lack of specific symptoms this condition is often not considered when women complaining of lower quadrant pain present to the gynaecologist.

We present a case of a 63-year-old woman with a right-sided pelvic mass misdiagnosed to be of ovarian origin.

Case Report

A 63-year-old postmenopausal woman was referred to our department with the diagnosis of an asymptomatic right-sided pelvic mass. On admission a mobile, cystic, painless mass, about 7 x 3 cm in size was palpated in the lower right abdomen.

Transvaginal ultrasound (TVS) scan revealed a multilocular oval cyst measuring 7.5 x 3.5 x 3.0 cm, that was thought to be of ovarian origin. The uterus and left adnexa were normal. Magnetic resonance imaging (MRI) examination revealed a cystic oval mass, 7.9 x 2.8 x 3.2 cm in size, in the right lower abdomen with clear margins, fluid content and rim enhancement (Figure 1).

Laboratory tests, including AFP, CA 15-3, CA 19-9 and CA 125 were unremarkable, while CEA was slightly elevated measuring 11.2 ng/ml (normal values < 5 ng/ml).

An exploratory laparotomy was performed. The uterus and both adnexa were macroscopically normal. A mass 8 x 3 x 3 cm in size was found arising from the appendix. The mass was mobile, in close relation to the right ovary, and without any adhesions in the area (Figure 2).

An appendicectomy was performed. The appendiceal tumour was removed intact. Frozen section revealed mucocele of the appendix with no evidence of malignancy. The final pathologic diagnosis was mucocele caused by mucinous cystadenoma with slight cellular dysplasia. A colonoscopy was performed three months later and no abnormality was found.

Discussion

Appendiceal mucocele is a general term applied for dilatation of the lumen of the appendix due to mucinous secretions, regardless of the underlying disease.The pathogenesis could be neoplastic or non-neoplastic. Simple (non-neoplastic) mucoceles are usually the result of obstruction of the appendiceal outflow by postinflammatory fibrosis or fecolith while the epithelium may be normal or just hyperplastic. Mucinous cystadenoma is the most common subgroup (63-84%), its pathogenesis is neoplastic, but histological examination does not reveal any malignant cells [2]. Finally, mucinous cystadenocarcinoma, applicable for 11-20% of all cases, is the malignant form of mucocele. Intraperitoneal spread of the neoplastic cells and formation of the adhesive, semi-solid mucin results in a condition called pseudomyxoma peritonei [2].

A correct preoperative diagnosis of appendiceal mucocele is extremely difficult and rarely made. Usually it is considered as an adnexal mass. There is no specific imaging or screening method that could diagnose this condition with certainty preoperatively [3, 4], except for a slight elevation of serum CEA which should always alert the physician as CEA rises in mucinous tumors [5]. CEA was also elevated in our case, but it was estimated wrongfully to be of ovarian origin. Diagnosis is confirmed only during surgery, as also occurred in our case. Rare complications include intestinal obstruction, intestinal bleeding and pseudomyxoma peritonei [6].

The differential diagnosis mainly includes cystic tumours of the right adnexa as well as mesenteric and omental cysts, lymphocele, mesenteric and retroperi-

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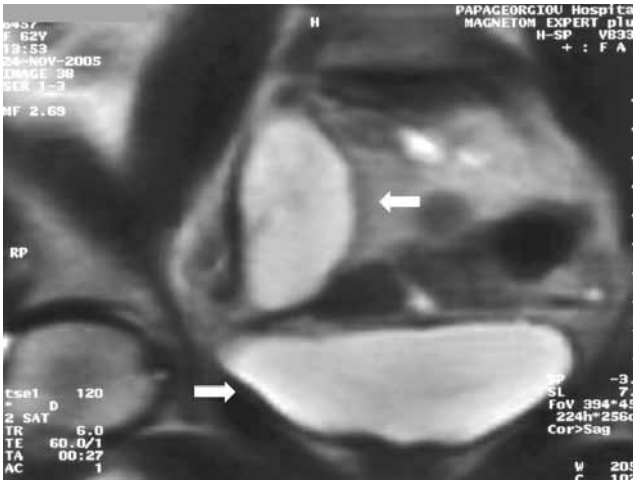

Figure 1. — MRI examination showing the cystic mass (top arrow: cystic mass; bottom arrow: urinary bladder).

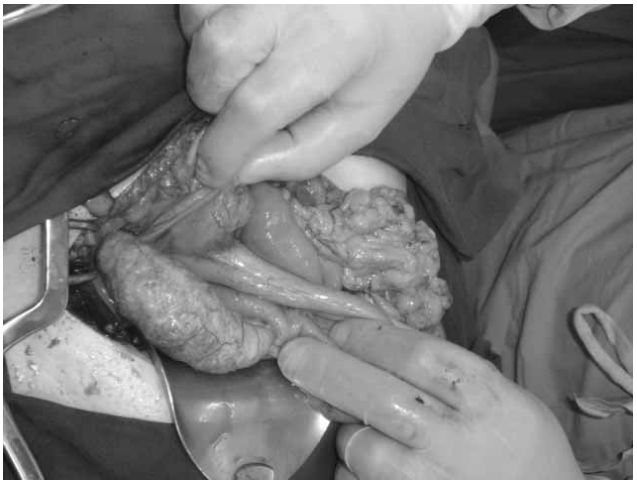

Figure 2. — Appendiceal tumour during the operation.

toneal haematoma or tumours and abdominal or retroperitoneal abscess [4].

Simple appendicectomy is curative in uncomplicated unruptured cases. When surgical exploration reveals a ruptured mucocele, then removal of all gross implants should follow the primary resection. Surgical excision of mucocele can be done either by laparotomy or laparoscopy. The endoscopic approach provides the advantages of good exposure and exploration of the abdominal cavity as well as rapid recovery postoperatively [7, 8].

The prognosis of patients with simple or benign mucoceles is excellent with a 5-year survival rate of 91-100%. Patients with cystadenocarcinomas, however, have a markedly diminished 5-year survival rate of 25%, mainly due to the complications of pseudomyxoma peritonei [6]. Postoperative evaluation by a gastroenterologist is always suggested, as cases with adenocarcinoma of the appendix have been missed by the pathologist or pseudomyxoma peritonei has been reported without intraoperative rupture of the mucocele [9]. In our case a colonoscopy was performed three months after the operation and no obvious pathology was detected.

Conclusion

Appendiceal mucoceles are extremely rare. They should always be considered in the differential diagnosis in women presenting with a right-sided pelvic mass, especially when clinical features are not indicative of gynaecological pathology. A correct preoperative diagnosis, though quite difficult, could be helpful in avoiding iatrogenic rupture as well as to plan surgery.

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